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An Ultrastructural Study in Various Meningioma Types

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Summary

Four psammomatous, three meningothelial, one fibromatous and one angiomatous meningiomas which were diagnosed by light microscope were also studied by electron microscopy. Their structural differences were characterized. The origin of the meningioma cell, psammoma body characteristics, nuclear inclusion bodies and etiopathogenesis of meningiomas were studied.

Key Words: Meningioma, ultrastructure, nuclear body, gap junction, desmosome, hemidesmosome, cillium, microfilaments, inclusion.

Introduction

Light and electron microscopic observations show some similarities and differences in various meningiomas.[1-3] Etiopathogenesis of meningiomas and virutic theory is still controversial.[1-8] Ultrastructural studies in this area are cumulated on these topics. We studied electron microscopic samples of human meningiomas to test the viral etiology and the importance of inclusion bodies in viral pathogenesis.

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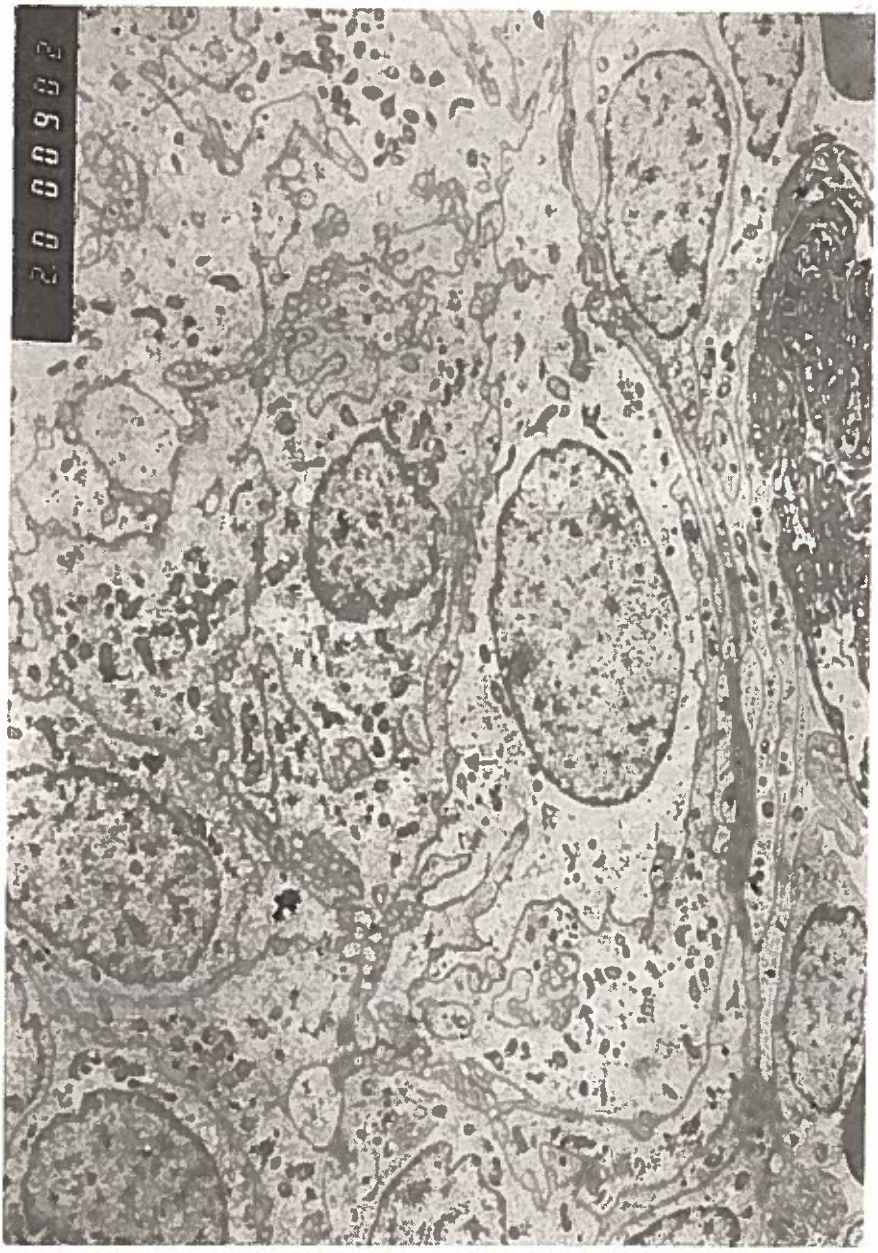

Figure 1

Delicate interdigitations (arrows) are between meningioma cells. Organelles are few in number in cytoplasms. N: Nucleus, X 2000.

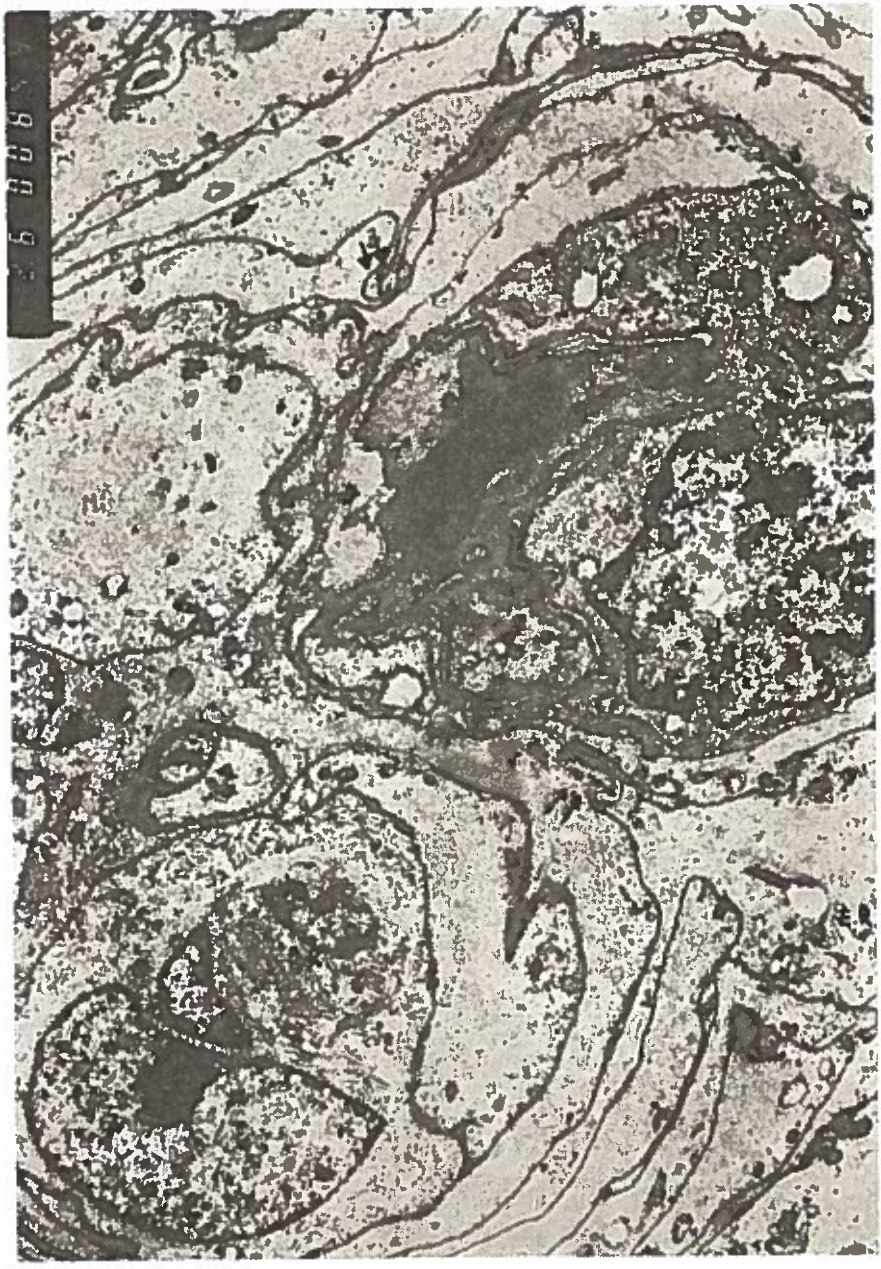

Figure 2

Close relationship between meningioma cell and capillary (arrow) is seen. Microfilaments (Mi), interdigitations (double arrow), granular endoplasmic (ER) are the main ultrastructural elements of the tumor cells, X 2600.

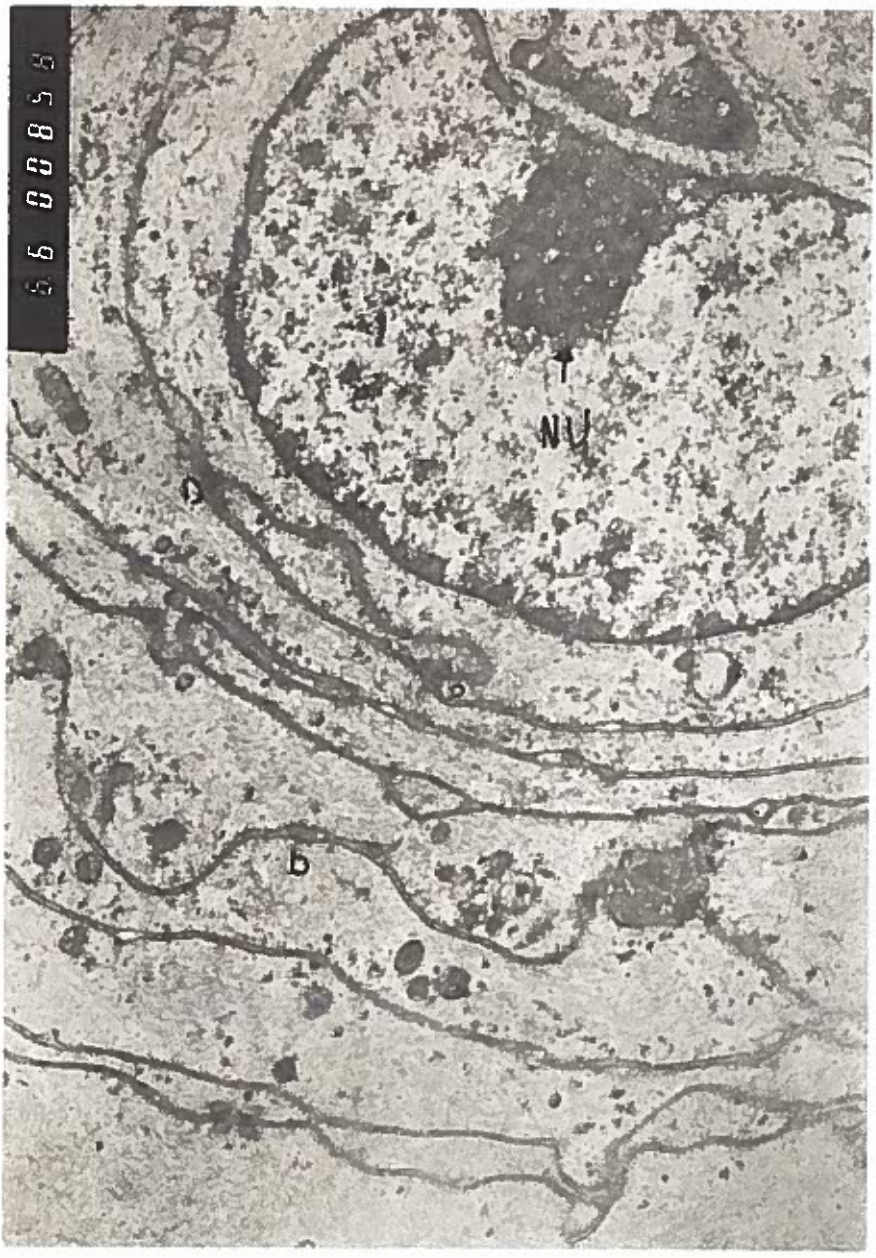

Figure 3

Cytoplasmic details of the innermost cell in one of psommoma bodies conspicious nucleoli (NU) in the euchromatic nucleus desmosomes (D), microfilaments take place in the cytoplasmic processes of the meningioma cells, X 6600.

Figure 4

At this high power electrone micrograph cytoplasmic invagination (arrow) a simple (1) and a complex (2) intranuclear body are seen in one of meningioma cell nuclei. Microfilaments (Mi) and glycogen (G) vary in number in the cytoplasm. X 10000.

Material and Methods

Four pasmmomatous, three meningothelial, one fibromatous and one angiomatous meningiomas which had been diagnosed by light microscope were studied by electron microscopy.

Phosphate buffered 2,5 % glutaraldeyhde and 1 % osmium tetroxide fixed biopsies were embedded in epoxy resin. After cutting thick sections from electron microscopic blocks for light microscopy, ultrathin sections were obtained in a Reichert ultratome III. Reynolds'lead citrate and uranyl acetate double contrasted ultrathin sections were studied in Jeol 100 - C electron microscope.

Results

Electron microscopic observations of nine meningioma cases showed fibroblastic, meningothelial cell types and transitional forms between the two. In psammomatous meningiomas psammoma bodies were seen (Figure 1-3). Only a few of them contained vascular structures (Figure 2). Usually they had microfilaments containing inner-most cells at the center (Figure 1,3). Meningioma cells had apparent membrane-bounded central nuclei (Figure 1,2). Most of them had various types of nuclear bodies (Figure 4), cytoplasmic invaginations (Figure 3), nucleolei (Figure 2,3), and thin dispersed chromatine (Figure 4). In one case, local chromatolysis near the nuclear body was recognised (Figure 4). In one case cilia were observed. Especially in psammomatous meningiomas, complex interdigitations and intercellular junctions were the main intercellular junctions (Figure 3). Fibroblastic cells contained more microfilaments than meningothelial cells.

Glycogen granules were placed within microfilaments (Figure 4). Pinocytotic vesicules and granular endoplasmic reticulum (Figure 2), dense bodies (Figure 4) and lipid inclusions were seen mostly in meningothelial cells.

Discussion

The classification of meningiomas according to the cell type, vascular component, or cytoplasmic appearence was not correlated.[1-21] All different types may be seen in the same tumour. To classify the meningiomas such as fibroblastic, meningothelial, etc., is also not of value from a prognostic standpoint.[1-5]

The origin of the meningiomas are still unknown. Dural vascular endothelia were suspected at first and these tumours were called "dural endotheliomas". This may be true for angioblastic meningioma.[14] By

accepting arachnoidal cell islands as a source of meningioma in 1922, Cushing used the term "meningioma".[9, 14] Arachnoid fibroblasts were also accused and they were celled "arachnoid fibroblastoma" or "meningeal fibroblastoma".[2] Observations of microfilament bundles crossing thin contents of the meningioma cells at the ultrastructural level gave rise to this concept.[12] Interdigitations, desmosomes, gap junctions, and tight junctions are seen in meningiomas.[3, 11, 12] Copeland found hemidesmosome-like intercellular specializations in human meningiomas and this type of junctional complexes is seen in arachnoidal cells, but not in other meninges. This finding supported the endothelial origin of the meningiomas.[6, 18] Another indication of this origin of the meningiomas are gap-junctions.[19] There are numerous gap-junctions in arachnoid.[19] This complexis also seen in neural crest-originated skin melanocytes.[8] It indicates extrameningeal source of meningiomas.[8, 18] Gap-junction is responsible for molecular transport controling development and differantiation.[19] Desmosomes, gap-junctions and tight junction are intracellular specifications which have been described in human meningiomas. The fine structure of human meningiomas has been well studied by a number investigators. Much attention has been directed to the types of intracellular junctions in this tumor. Three types have been identified; 1) tight junctions in which the outer leaflet of apposed plasma membranes fuse, 2) gap or nexus junctions in which the outer leaflets are separated by a 2-3 nm. and, 3) intercellular space and classic desmosomes as seen in our cases.[8] "Intermediate sized (6-11 nm.) filaments occur in most, if not all vertebrate cells. The major classes include tonofilaments, neurofilaments, glial filaments and vimentin filaments.[21] In our electron-microscopic study microfilamentous structures and their close relationship to junctional complex as support the idea of the epithelial origin of meningiomas. Multipotent cells may show diverse differentiation into epithelial and fibroblastic meningioma types.[12] Moreover, transient forms between these two cell types occur.[12] Fine structural details of meningioma cell correlate these findings.[9] Fibroblastic and meningothelial cells are two basic components of the tumor.[9] Ultrastructural studis of individual tumors may help to classify them according to the proportion of these two cell types.[9] Mitochondria, lipid droplets, glycogen granules, cilia, interdigitations, other intracellular organels were also found in our study.[4, 9-12] The existence of these organels is not an important finding in the pathogenesis of meningiomas, because they may also occur in many kinds of tissue cells.

Psammoma bodies were seen in four cases in this study. The earliest morphologic observations of meningiomas suggested that blind-ended

vascular buds are responsible for the formation of psammoma bodies.[2] The capillary obstruction due to deposition of tumor cells, hyalinisation, calcification of stroma and necrotic areas are suspected by light microscopists.[2] Intracytoplasmic, thin, long and homogenous fibrillar material that are parallel to one another is found in electron microscopic studies. This is the new concept which supports the source of psammoma bodies. This proteinous fibrillar material is concentrically wrinkled and consequently causes the cell to bend to its' axis.[2] This cell is the nuclei of a psammoma body and is called "inned most cell".[12] Neighboring cells become curved at its' periphery and psammoma bodies are formed.[2] The reason for fibrillar curving is still controversial.[12] Changes in the nature of semiliquid content of the cytoplasm may lead to fibrillar curving.[12] If the medium does not change for a long time whorl pattern and psammoma body formations take place in cultured meningiomas.[3] This finding indicates its' degenerative characteristic.[3] Our ultrastructural findings such as curved intracytoplasmic microfilaments, concentric arrangements in psammoma bodies support the hypothesis mentioned above. Meningioma cells usually show sharpedged, membrane-surrounded nuclei.[12] Their thin, dispersed chromatines cause their vesicular appearance.[12] Intranuclear inclusions take place in brain tumor.[20] Eosinophilic inclusions which were seen by light microscope are observed as intranuclear cytoplasmic invaginations in electron microscopy. This finding reveals that the presence of intranuclear eosinophilic inclusions does not confirm virutic etiology of meningiomas. The intranuclear filamentous, granular bodies, and chromatolysis which were seen in our study are also described by others.[3, 5, 13, 20] The resolving power of the light microscope is inadequate for these kind of findings.[6-8] Inclusion bodies are also seen in viral diseases and in diseases such as Hodgkin's and gliomas.[4, 13] Their meaning and functions ara not clear.[13] Furthermore, they are found in lymphocytes, plasmocytes, mesanchymal reticular cells, fibroblasts and endothelial nuclei a even in cultured leptomeningeal cells.[10, 17] Their appearance may be strong indication of nuclear activity.[10] Nuclear bodies usually are divided into simple and complex types.[10] Morphologically they are fibrous in nature, are seen as a dense chromatin mass or make whorls. Various nuclear body types may be related to their maturation.[5, 13] Herpes simplex viral antigens were isolated in some meningioma cell cultures.[6] This finding supported the morphology. It was also shown that numerous increases in the number of gap junctions caused by viruses lead to excessive development of meningiomas.[19] In conclusion, intranuclear body existence and the richness of gap junction in meningioma cells in this study support the hypothesis of virutic etiology and its' pathogenetic mechanisms of meningiomas.

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A New Statistical Approach for Analyzing Left Ventricular Wall Motion

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Summary

A new statistical approach is proposed for evaluating the left ventricular wall motion characteristics of patients in four different pacing modes. This method is applicable to finding the relative position of subjects within a group or subject's location compared to a specific group, with a general computer program already available for such cases.

Key Words: Classification, Ranking, Digital Subtraction Angiography, Left ventricular Wall Motion.

Introduction

Analysis of left ventricular wall motion is a very important aspect of the left ventriculogram analysis, because it shows how the left ventricle contracts. The evaluation of the left ventricular wall motion may be made subjectively, estimating it on the basis of the personal experience of the physician who reports the ventriculogram. Since this is subject to multiple variants of interpretation, it is convenient to objectify it in a simple and quick manner.[1]

In addition to conventional contrast angiography, radionuclide angiography, echocardiography and digital subtraction angiography have been used to evaluate abnormal motion of the left ventricular

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wall.[2] Digital subtraction ventriculography is well suited to rapid and routine quantitative analysis of segmental function that can reduce intra-observer and interobserver error. This is of particular importance if interventions are to be accurately studied.[3]

Despite the widespread application of left ventricular function analysis, relatively few studies have used quantitative methods to evaluate regional left ventricular wall motion.

In this paper we present our data on the effect of various artificial cardiac pacing modes on left ventricular regional wall motion analysed by a statistical method which is suggested to be a new approach for evaluation.

Materials and Methods

The study group comprised of 16 patients who underwent diagnostic cardiac catheterization and coronary angiography at The Cardiac Catheterization Laboratory of Hacettepe University School of Medicine, Department of Cardiology, Ankara, Turkey. Five patients were excluded because of the inadequency of the obtained ventriculograms for evaluation. The ventriculograms from 11 female patients aged 31-64 (mean = 43) were considered for evaluation.

Each patient underwent digital subtraction left ventriculography at 30 degree RAO position by direct injection of the contrast material (Urographin, 25 ml of 1/2 diluted my NaCl solution) during different pacing modes which were choosen randomly as well as at the patient's own rhythm. Digital subtraction ventriculography was performed by Philip b DVI-CV x-ray unit by means of TID system.

Technically adequate studies were evaluated by DVI-CV system computer. Left ventricular regional wall motion was measured by the same microprocessor controlled automated system as percent radius shortening. By this approach the change of the distance of each left ventricular segment to the gravity centre of the heart during the cardiac cycle is defined as the ratio of this shortening to diastolic distance and expressed as percentage. In this study, we evaluated the left ventricular wall motion by dividing the left ventricular silhouette into six segments.

Details of Statistical Analysis: In each pacing mode and for each segment the percent radial shortening values of 11 patients were plotted (Figure 1a-1d). For plotting we marked off the percent radius shortening values on the vertical axis and the segments on the horizontal axis.

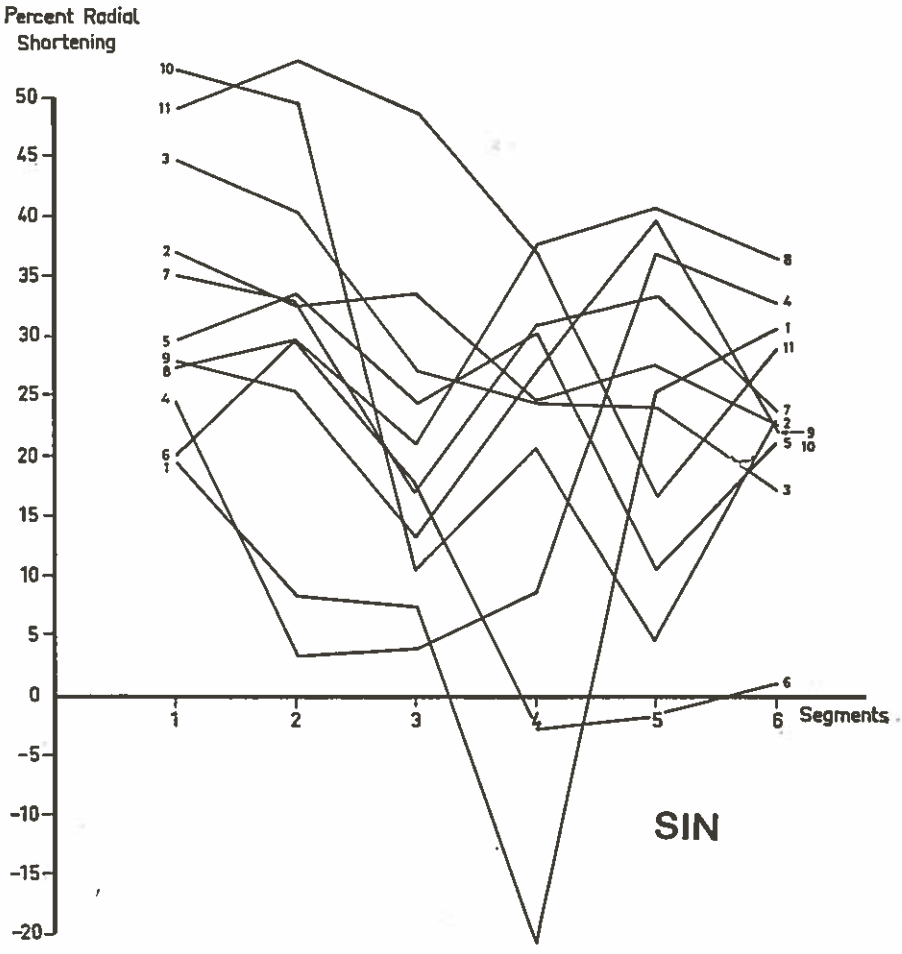

Figure 1a

Percent radial shortening values of 11 patients in SIN mode.

Our hypothesis was "each patient's left ventricular wall motion characteristics, namely percent radial shortening values, should not be changed in various pacing modes". That is to say, the position of a patient's line among other subjects' should be nearly constant during different stimulation modes.

Having plotted the percent shortening values of patients one can not discriminate between the patients, because the lines intersect each other at many points, and it is almost impossible to follow the lines. Therefore, it is necessary to introduce some mathematical definitions.

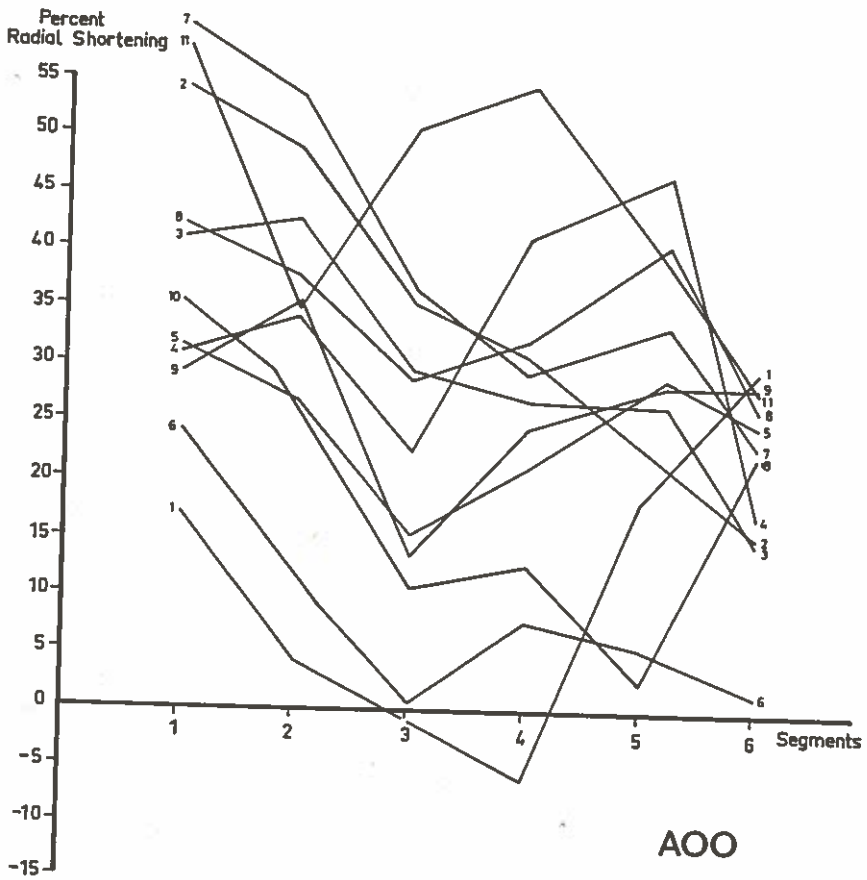

Figure 1b
Percent radial shortening values of 11 patients in AOO mode.

A patient's position can be identified by the sum of his distance from the members of the group in each segment. At a specific segment the distance between two subjects can be defined as follows:

$$d_{ij.s} = (x_{i.s.} - x_{j.s.}) \qquad s = 1,2,\ldots,6 \qquad i,j = 1,2,\ldots,11 \qquad (1)$$

Here the subscripts s is used to denote the segment. In order to define the position of any subject relative to the others, the lines have to be ranked. If two lines do not cross then one may be said to be higher than other. But if they do cross, which one is higher? To prevent ambiguity the distance formula defined in (1) has to be squared, so that the distan-

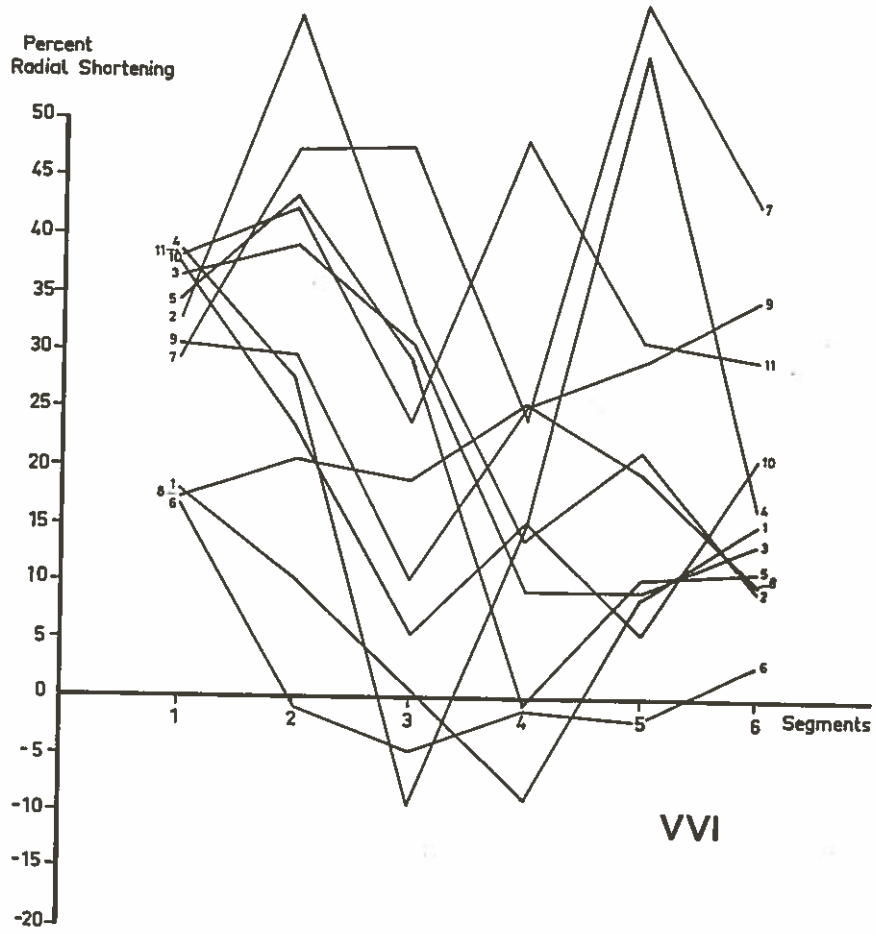

Figure 1c
Percent radial shortening values of 11 patients in VVI mode.

ces between various points of two intersecting lines will not compensate each other. Therefore, the squared distances can be defined as

$$d^2_{ij,s} = (x_{i,s} - x_{j,s})^2 \qquad \begin{matrix} s = 1,2,\ldots,6 \\ i,j = 1,2,\ldots,11 \end{matrix} \qquad (2)$$

A measure of the i th patient's location relative to the other patients at a specific pacing mode is his total distance or sum of distances from the other members of the group in each segment.

$$d_i = \sum_{s=1}^{6} \sum_{j=1}^{11} d^2_{ij,s} \qquad i = 1,2,\ldots,11 \qquad (3)$$

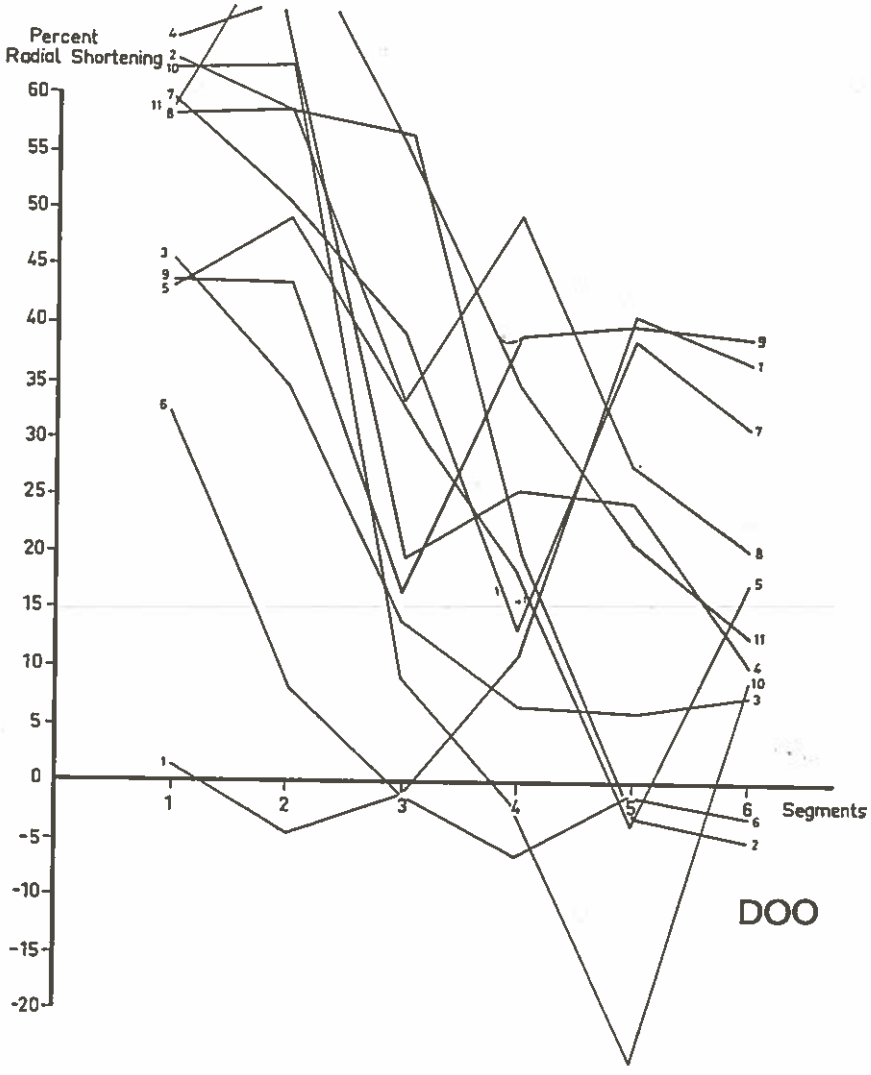

Figure 1d
Percent radial shortening values of 11 patients in DOO mode.

Our hypothesis can be tested by this statistic. The smaller d_i the more central line is within the group.[4] The median line at a specific pacing mode is that line which minimizes d_i,i.e., if

$$d_k = \min_{i = 1,n} (d_i)$$

then the median subject at a specific pacing mode is the k th subject.

Results

We calculated the sum of squared distances of each patient's segmental radial shortening values to others in sinus rhythm, ventricular, atrial, and sequential antrioventricular pacing. We obtained each subject's position by considering the left ventricular wall motion as a whole. For example, for sinus rhythm the sum of distances of one subject from the other subjects in six segments gives the particular subject's total relative distances which can be used for ranking.

In sinus rhythm, for each patient the total distances are given in Table I.

TABLE I

THE SUM OF i th PATİENT'S DISTANCES FROM THE MEMBERS OF THE GROUP IN SINUS RHYTHM

Patient No (i)	Total Distance (d_i)
1	45632
2	15321
3	16272
4	45513
5	15162
6	35722
7	14548
8	20165
9	14990
10	25437
11	31829

The subject with the smallest total distance is considered as the most central, or the median subject. In sinus rhythm the 7th patient is the median subject, and the first patient is the most extreme one.

The total distances can be put in increasing order to give an idea about the position of each subject. The ordering or patients, or ordered distances are given in Table II for all pacing modes.

TABLE II

THE ORDERING OF PATIENTS ACCORDING TO THEIR TOTAL DISTANCES IN EACH PACING MODE

Pacing Mode	Patient No (i)										
SIN Patient's own sinus rhythm	7	9	5	2	3	8	10	11	6	4	1
AOO Artrial pacing	5	3	9	8	4	2	10	7	11	6	1
VVI Ventricular pacing	9	8	10	3	2	5	11	1	4	6	7
DOO Atrioventricular pacing	5	3	4	7	9	8	11	2	10	6	1

Data in Table II suggests some correlation between stimulation modalities with respect to the ordering of patients. Such a correlation can be detected by Sperman's Rank Correlation. In order to use this method each patient must have a rank number, and these rank numbers will serve as the variable in the analysis.[5] The most central subject will be assigned a rank number of 1, and the most extreme subject a rank number of 11. For example in Sinus rhythm if the total distances are put in increasing order the 1st patient will have a rank number of 11 since he is the most extreme subject; the second patient will have a rank number of 4; ...; the 7th patient will have a rank number of 1, since he is the most central subject and the 11th patient will have a rank number of 8 in the sequence. Table III gives the rank numbers of each patient in each pacing mode.

TABLE III
RANKING OF PATIENTS

Pacing Mode	Rank No (R_i) Patient No (i)										
	1	2	3	4	5	6	7	8	9	10	11
SIN	11	4	5	10	3	9	1	6	2	7	8
AOO	11	6	2	5	1	10	8	4	3	7	9
VVI	8	5	4	9	6	10	11	2	1	3	7
DOO	11	8	2	3	1	10	4	6	5	9	7

Sperman's rank correlations between pairs of pacing modes can be demonstrated by the correlation matrix given below.

		Pacing Modes			
		SIN	AOO	VVI	DOO
	SIN	1	0.56*	0.29	0.54*
Pacing	AOO		1	0.58*	0.83**
Mades	VVI			1	0.09
	DOO				1

* shows significance at 0.05 level.
** shows significance at 0.005 level.

Sperman's Rank Correlation gives the strength of relationship between two rankings. If there are more than two rankings, as is the case, in order to specify the magnitude of relationship between those rankings Concordance Coefficient can be calculated.[6] For our problem the amount of relationship between the rankings of subjects in all pacing modes is 0.65 ($P < 0.01$), which is highly significant.

Discussion

We hypothesized that each patient's radial shortening values should not be changed in various pacing modes. The proposed technique seems sufficient for confirming our hypothesis.

We calculated the patient's left ventricular contraction as a whole by analyzing the relative position in the study group in different conditions. Our findings have shown that the relative location of patients, which are evaluated by ranking them, are highly correlated in all pacing modes.

In this paper we propose a new statistical approach for ranking and finding the relative position of subjects in a study group. We believe that this method can also be used and will be very useful for classifying subjects as "normal" or "not normal" or like. For subjects who are known to be completely normal or healthy, total distances can be calculated; and for any subject the total distance can be compared with those of the normal group. This statistic can then be used as a decision maker for the classification of that subject as "healthy" for "not healthy".

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Fine Needle Aspiration Biopsy of Thyroid Nodules

Report of 319 Cases

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Summary

The results of thyroid scanning, ultrasound and fine-needle aspiration cytology were compared in 319 patients who had aspiration biopsies at Hacettepe University Hospitals. Of these 319 patients, 104 had surgical pathology results available. It was shown that the sensitivity of scintigrams, ultrasound, and fine-needle aspiration biopsies were 71.4 %, 50 %, and 69 %, respectively. Specifity was 34.8 %, 47.7 %, and 93.3 %, in the same order. Positive predictive values were 14.7 %, 11.1 %, and 64.7 %. It was concluded that fine-needle aspiration is the best procedure in the diagnosis of thyroid nodules at Hacettepe University Hospitals.

Key Words: Thyroid scanning, thyroid ultrasound, fine-needle aspiration biopsy of the thyroid.

Introduction

Thyroid nodules are a source of concern to patients, and their management remains a controversy among physicians. Several diagnostic techniques are available to distinguish frequently encountered benign

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nodules from rare malignant lesions. The past decade has witnessed widespread application of fine-needle aspiration biopsy, new radionuclide scanning agents, and sensitive ultrasonography for diagnosing nodular thyroid diseases. These diagnostic techniques vary in reliability.

Fine-needle aspiration (FNA) has proven to be the single most sensitive, specific, and cost effective diagnostic test to determine if a nodule is malignant or benign.[1-4] As the skills of the physicians that obtain the material and the histological analysis by cytologists that interpret the microscopic findings continue to improve, the accuracy of this technique increases. Consequently, every institution should analyze its data, determine the reliability of FNA results and manage patients accordingly.

The purpose of this study was to evaluate the results of FNA biopsies done at Hacettepe University Hospitals, to compare them with the results of other diagnostic techniques, and to determine their management value at this institution.

Materials and Methods

During an 18-month period between July 1984 and December 1985, 399 fine-needle aspiration biopsies were performed on patients with benign or malignant, nodular or diffuse enlargements of the thyroid or metastases from thyroid tumors at the endocrinology clinic of Hacettepe University Hospitals. Of these 399 patients, medical records of 319 were found and included in the study. Of the study group, 104 patients subsequently underwent thyroidectomies.

Thyroid scanning was performed with (^{99m}Tc) pertechnetate using PhoGamma 5 Camera (Picket).[5] All patients except six, had a thyroid scan that were categorized as single nonfunctioning "cold" nodule, single hyperfunctioning ."hot" nodule, single "warm" nodule, multinodular goiter, and suppressed scan. Six patients were not scanned either because of pregnancy (one patient) or thyroid hormone suppression therapy.

Thyroid ultrasonography was available in 114 patients with conventional B-mode ultrasound. The results were categorized as solid, cystic, mixed lesion, and multinodular goiter.

Fine-needle aspiration biopsy of the thyroid was performed according to the method described by Miller.[6] The cytologic diagnostic categories were: insufficient material, benign cells consistent with nodular goiter, cells consistent with lymphocytic or granulomatous thyroiditis, cells suspicious for cancer, and malignant cells.

Statistical analysis: Sensitivity and specifity of the techniques used in the evaluation of thyroid nodules can be defined in various ways. Sensitivity measures the fraction of patients with disease that will be detected by the diagnostic test in question.[7] Specifity measures the fraction of patients correctly identified as having no disease. Positive predictive value was calculated according to the following equation:

Positive predictive value= (true positives / true positives + false positives) x 100.

Chi square, Mann-Whitney U, and McNamara tests were used to determine statistical significance.[8]

Results

A total of 319 patients were studied, with a female to male ratio of 5.9 (273 women, 46 men), and mean age of 40.2 $\pm$ 12.5 years (range 17-77).

A final diagnosis was reached in 104 patients after thyroidectomy. Among these patients, most (91.5 %) had a single nodule by palpation, and 8.5 % had multinodular goiters. Patients with diffuse goiters presented with clinical thyroiditis and did not have subsequent thyroidectomies; therefore these patients were not included in the group with definitive diagnosis. In patients with single nodules, 15.5 % had carcinoma and the majority (84.5 %) had benign thyroid disease. Multinodular goiters were also mostly benign (88.9 %), and 11.1 % harbored a malignancy. These percentages were not statistically significant ($p > 0.05$).

Thyroid scanning results: Of 319 patients, six individuals did not have a scan taken and four had suppressed thyroid scans (Table I). The majority of patients (59.3 %) had "cold" nodules, with multinodular goiters, "hot" and "warm" nodules being seen in decreasing order of frequency.

TABLE I

RESULTS OF THYROID SCINTIGRAMS

Classification	Number of Patients (%)
Cold nodule	189 (59.3)
Hot nodule	32 (10.0)
Warm nodule	17 (5.3)
Multinodular goiter	71 (22.3)
Suppressed scintigram	4 (1.2)
Not scanned	6 (1.9)
Total	319 (100.0)

When scintigraphy results were evaluated in the light of surgical pathology, among patients with a "cold" nodule, 85.3 % had a nodular goiter and/or thyroiditis and 14.7 % had carcinoma. "Hot" nodules and multinodular goiters were mostly benign (85.7 % and 90.5 %, respectively). If a "cold" nodule was accepted as the criterion for malignancy, sensitivity of this test was 71.4 %, with a specificity of 34.8 % and a positive predictive value of 14.7 %. The information obtained from thyroid scanning was statistically significant from that obtained from surgical pathology ($X^2 = 47.03$, $p < 0.001$ McNamara test).

Ultrasound results: Ultrasound results were available in 114 patients. Table II shows that most patients had a single solid nodule (44.7 %); solid-cystic mixed lesions and multinodular goiters were seen in decreasing order of frequency.

TABLE II
RESULTS OF THYROID ULTRASOUND

Classification	Number of Patients (%)
Solid	51 (44.7)
Cystic	20 (17.5)
Mixed	28 (24.6)
Multinodular goiter	15 (13.2)
Total	114 (100.0)

Ultrasound results were also evaluated in comparison to surgical pathology in a smaller group of patients. Ultrasonographically, solid nodules and mixed nodules were mostly benign (94.7 % and 88.9 %, respectively), while no malignancies were detected in ultrasonographically cystic nodules and multinodular goiters. If both solid and mixed nodules are accepted as a sign of malignancy, the sensitivity of this test is 100 %, but specificity is 23.5 %, and positive predictive value is 7.1 %. If only solid nodules are accepted as suspicious for malignancy, then sensitivity drops to 50 %, but specificity rises to 47.6 % and positive predictive value to 11.1 %. The information gathered from ultrasound is statistically significant from the results of surgical pathology ($x^2 = 26$, $p < 0.001$).

Thyroid aspiration cytology results: Adequate material for cytologic analysis was obtained from 98.7 % of 319 patients who had FNA. A large majority of these patients had benign cytologic findings (92.5 %), while only 6.2 % had cells either suspicious or diagnostic for malignancy.

Other than a very small hematoma that regressed quickly in one patient, no complications were encountered.

A comparison of cytologic findings and definitive diagnosis is shown in Table III. In 7 patients with a final diagnosis of thyroiditis, only 14.3 % had a correct cytologic diagnosis. The remaining patients either had benign cells in their aspirates (57.1 %) or cells suspicious for malignancy (28.6 %).

TABLE III

A COMPARISON OF FINE NEEDLE ASPIRATION CYTOLOGY (FNA) AND SURGICAL PATHOLOGY

	FNA Cytology (%)			
Surgical Pathology	Benign cells	Thyroiditis	Suspicious cells	Malig. cells
Benign nodular goiter	74 (89.1)	5 (6.0)	1 (1.3)	3 (3.6)
Thyroiditis	4 (57.1)	1 (14.3)	2 (28.6)	–
Papillary carcinoma	1 (25.0)	–	–	3 (75.0)
Follicular carcinoma	4 (57.1)	–	–	3 (42.9)
Medullary carcinoma	–	–	–	1
Anaplastic carcinoma	–	–	–	1
Metastatic carcinoma	–	–	–	3 (100)
Total	83 (78.3)	6 (5.7)	3 (2.8)	14 (13.2)

Thyroid aspiration biopsy gave false negative results in 5 patients (4.7 %), all with single thyroid nodules. One of these patients had papillary, another had follicular-papillary mixed, and the remaining 3 had follicular thyroid carcinoma. In one patient with follicular carcinoma, the malignant focus was outside the nodule that was biopsied. Mean nodule diameter of these five patients was 2.5 cm, and this was smaller than the nodule sizes of patients with false positive (4.8 cm.), true positive (4.8 cm), and true negative (3.5 cm) results.

Six patients (5.7 %) had false positive thyroid aspiration cytology results. Of these six, three were reported as suspicious for malignancy, and had a final diagnosis of nodular goiter (one patient) or thyroiditis (two patients). In the remaining three patients with cytologic diagnosis of malignancy, two were found to have a follicular adenoma and the third one a Hurthle cell adenoma after surgery.

The sensitivity of FNA biopsy was calculated to be 69 %, with a specificity of 93.3 %, and positive predictive value of 64.7 %. The information that FNA biopsy provides is not statistically significant from that provided by thyroid surgery ($x^2 = 0.09$, $p > 0.05$).

Discussion

The major problem in the diagnosis of nodular disorders is to find a highly specific test that will differentiate benign nodules from malignant ones. The ideal test would provide 100 % sensitivity and 100 % specificity. When sensitivity is reduced, the frequency of missed malignancies increases; and if specificity is poor, many patients with benign lesions are referred for surgery. Most techniques used in the diagnosis of the nodular thyroid disorders have acceptable sensitivity but poor specificity.[1]

This study shows that 15.5 % of the patients with a single nodule and 11.1 % of those with multinodular goiters had malignancies. On the other hand, a previous study by Sayek *et. al.* from the same institution, documented a malignancy frequency of 11.5 % in single nodules and 3.7 % in multinodular goiters.[9] Although the rate of malignancy in multinodular goiters is lower in the literature[10, 11], this study did not find any difference in the risk of malignancy between single nodules and multinodular goiters. This may be due to the fact that patients with multinodular goiters were selected to be biopsied because of highly suspicious lesions.

Thyroid carcinomas were observed in 14.7 % of scintigraphically "cold" nodules, in 14.3 % of "hot" nodules, and in 9.5 % of multinodular goiters. Sayek *et. al.* found malignancies in 8.5 % of cold, and 1.5 % of hot nodules.[9] In the literature these percentages are 16 % for cold and 4 % for hot nodules.[12, 13] Consequently, "cold" or "hot" nodules do not exclude the probability of malignancy. The sensitivity (71.4 %), specificity (34.8 %), and positive predictive value (14.7 %) of thyroid scanning that this study documented, and the sensitivity (88 %), and specificity (8 %) reported in the literature[12, 13], suggest that thyroid scanning does not have a substantial impact on the management of thyroid nodules.

In our study, ultrasound showed 44.7 % of the nodules to be solid, 24.6 % to be solid-cystic mixed, and 17.5 % to be cystic. In Ashcraft's and Van Herle's survey of 16 series, these values were 69 %, 12 %, and 19 %, respectively[12, 13], and were not much different from our results. In the same series, 21 % of solid, 12 % of mixed lesions, and 7 % of cystic lesions were malignant.[12, 13] In the present study, 5.3 % of solid lesions, and 11.1 % of mixed lesions were malignant, with no malignancies in the pure cystic lesions. This difference may be due to the fact that ultrasound results were available in only a small group of patients.

If ultrasonographically solid and mixed nodules are accepted as criteria of malignancy, this test is found to be 100 % sensitive, and 23.5 % spectific in our current study, and 96.6 % sensitive, 18 % specific in the

above mentioned studies.[12, 13] If only solid lesions are considered, the calculated sensitivity is 50 %, and specificity is 47 % in our series, with 85 % sensitivity, and 33 % specificity in other series.[1] Consequently, ultrasound does not allow for high accuracy in the diagnosis of thyroid cancer.

As experience with thyroid aspiration biopsy increases, the problem of obtaining insufficient tissue for diagnosis decreases, and satisfactory aspirates may be obtained in 94 to 97 % of nodules.[14, 15] Similarly, we have procured adequate specimens in 98.7 % of the patients.

In this study aspiration cytology was not successful in diagnosing thyroiditis. The causes of diagnostic error have been identified as similarity of epithelial cell nuclear atypia in lymphocytic thyroiditis and cellular neoplasm, inexperience, and inadequate sampling.[16] It is expected that diagnostic error will decrease with more experience.

In various thyroid aspiration biopsy series, reported false-positive and false negative rates range between 1-20 %.[2, 3, 17] In the present study this rate is around 5 %. Since the mean nodule diameter was not below 1 cm or above 4 cm, nodule dimensions cannot be a source of this inaccuracy.

In most patients with a false-negative result, a follicular thyroid carcinoma was discovered. Even experienced cytologists agree that it is very difficult to differentiate the benign Hurthle cell and follicular neoplasms from their malignant forms.[18, 19] One patient with papillary thyroid carcinoma also had a false-negative FNA. Papillary thyroid carcinoma rarely causes an error in cytologic diagnosis; it occurs only when cellularity is reduced or only when intranuclear cytoplasmic inclusions or psammoma bodies are present.[20] Although the risk of obtaining a false negative result is higher in patients with multinodular goiters, we did not have any false negative biopsy results in patients with multinodular goiter.

In three patients with a false-positive diagnosis, this was not avoidable because they had follicular and Hurthle cell adenomas. Further, it was not unusual to find thyroiditis in two patients with suspicious cytology; both subacute granulomatous thyroditis and lymphocytic thyroiditis may present with nuclear atypia and may be reported as "possible malignancy".

In this study, the sensitivity (69 %), specificity (93.3 %) and positive predictive value (64.7 %) of FNA cytology were found to be similar to the results in the previous studies (73-86 %, 80-99 %, and 71 %, respectively).[1] These data show that, compared to thyroid scintigrams and

ultrasound, FNA is the most accurate method for selecting patients with a thyroid nodule for surgery at this institution. In addition, it is safe and inexpensive. As the skills of the physicians who obtain the material and the cytologists who interpret the microscopic studies improve, the accuracy of aspiration cytology will increase.

To avoid the risks of false-negative results, those who do not undergo surgery should be rebiopsied after 6-8 months of suppressive therapy. If there is a clinical suspicion of malignancy, surgery should be done despite negative cytologic findings.[1]

In summary, although FNA is the best procedure in the diagnosis of thyroid nodules at this institution, it is not flawless. As experience with it increases, the diagnostic yield may get better. Thyroid scanning and ultrasound define different aspects of thyroid nodules. While scanning shows the activity of the nodule, ultrasound describes the structure, and both are complimentary to thyroid aspiration biopsy in the management of nodular thyroid disease.

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Encopresis

Treatment Outcome of 24 non-Constipated Encopretic Children

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Summary

Behavioral management of twenty four non-constipated encopretic children are presented. The treatment programme consisted of four stages. Stage I and II involved simple positive reinforcement. With these two initial stages of treatment twelve patients recovered and did not require further stages of the treatment program. In stages III and IV where glycerin suppositories and enemas were used, six patients recovered. This paper reviews the management of encopresis and emphasizes the success of behavior therapy in non-constipated encopretic children.

Key Words: Encopresis, Children, Behavior Therapy.

Introduction

Encopresis is defined as the repeated, involuntary passage of stool into clothing without the presence of any organic cause to explain the symptom.[1] Soiling past the age of four is generally accepted as pathological.[2] Some authors lower the age limit to 2-3 years with the understanding that toilet training should have been accomplished by that time.[1,3]

Encopresis can be with or without constipation. Mechanisms associated with these two types of encopresis appear to be quite different. Accordingly, treatment programmes and outcomes also vary. A classification which implies this distinction was suggested by Easson.[4] He subdivided encopresis into:

1. Primary infantile encopresis (never toilet trained, not constipated)
2. Primary reactive encopresis (never toilet trained, constipated with overflow).

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3. Secondary infantile encopresis (toilet trained, regression to incontinence without constipation).
4. Secondary reactive encopresis (toilet trained, incontinence with constipation).

A number of modes of treatment have been recommended for encopresis.[5, 6, 7] Most of the literature regarding treatment have been on encopresis with constipation.[2, 8] Here we report the results of our behavior therapy approach to non-constipated children with encopresis (secondary infantile encopresis).

Material and Method

All children over four years of age with fecal incontinence seen as outpatients in the Department of Psychiatry within a period of two years were included in this study. Patients who had constipation, an organic basis for their problem, and patients with mental retardation were excluded. The study group consisted of 24 encopretic children without constipation who had soiling several times a day.

Diagnostic studies included physical and rectal digital examination, plain abdominal X-ray, stool and urine analysis. In some of the cases additional studies such as IVP, barium enema and I.Q. tests were performed.

The treatment program applied to the study group was as follows:

Stage I: It was explained to the parents and the child that encopresis is a common problem which other children also have and that it is not the fault of the child if s/he is encopretic. After informing them about the treatment program, they were given a chart to document the soiling and the use of the toilet for a period of seven days. The child was instructed to put a sticker on the chart beside the related day for defecation into the toilet and a minus (-) sign if soiling occurred. The mother was to help the child with the charting when necessary. The family was supplied with enough colorful stickers for the seven day period. They were then asked to come back in one week and to bring the chart with them.

Stage II: In stage II, in addition to the stickers, other positive reinforcers were given for the use of the toilet. Types of reinforcers to be given were discussed with the parents and the child. The child was asked to choose three of the options in order of preference. Doing things such as playing games or going on rides were recommended rather than material reinforcers. The rewarding was done during the weekend. If the child had not soiled at all during the week, preference 1; and if soiling had occurred one or two times, preference 2 and 3 were given respectively.

Stage III: Children who had soiled more than twice in one week in stage II were given stage III treatment. This is as follows:

1. The child was awakened at least one hour before he had to leave the house if he was going to the nursery or to school.

2. After breakfast, s/he was instructed to sit on the toilet for 5 minutes and to try to defecate.

3. If s/he was unable to defecate, a glycerin suppository was inserted.

4. After half an hour s/he was asked to sit on the toilet for another 5 minutes.

With stage III treatment if the child had soiled less than 3 times in one week, s/he was considered to have improved and rewarded as in stage II.

Stage IV: If there was not a satisfactory response to stage III treatment program (soiling more than 2 times in one week), stage IV program was applied. Stage IV treatment is similar to that of stage III except that the child was given an enema instead of glycerin suppository if s/he was unable to defecate after breakfast. The use of suppository and enema was mostly for the purpose of conditioning rather than evacuation.

If the result was satisfactory (soiling less than 3 times in one week), in stage I the charting and in stage II the rewarding was continued up to a month. In stage III the suppository and in stage IV the enema was discontinued after one soiling-free week. In all stages, after satisfactory results had been achieved, patients were asked to come for monthly follow-ups for six months.

Results

There were a total of 24 children included in the treatment program. Four patients were lost to follow-up.

The mean age of the encopretic children was 7.13 $\pm$ 0.38 years, the range being 5-11 years. Nineteen of the 24 cases were male (79.17 %) and five were female (20.83 %). The mean duration of encopresis at the start of the treatment was 1.41 $\pm$ 0.27 years. Enuresis at the time of the initial visit had a prevalance of 8.3 %. The summary data of the cases studied are given in Table I.

At the end of three months, of the 24 children who received stage I treatment, 8 showed positive response (stopped soiling). The patients (16 cases) who did not respond to stage I treatment were given stage II treatment. In stage II, four patients stopped soiling. The remaining 12 non-responders were passed on to stage III. Of these 12 patients,

TABLO I

SUMMARY DATA OF 24 NON-CONSTIPATED ENCOPRETIC CHILDREN

Case Number	Age (Year)	Sex	Duration of Symptoms (Year)	Associated features	Applied treatments					Response to treatment
					Stage I	Stage II	Stage III	Stage IV	Psycho-therapy	
1	7	M	2	–	+					Cured in stage I
2	9	M	1	–	+	+	+			" " " III
3	5	M	9/12	–	+	+	+			" " " III
4	7	M	7/12	Enuresis	+					" " " I
5	6	M	1/12	–	+					" " " I
6	6	F	1	–	+	+				" " II
7	7	M	2	–	+	+				" " " II
8	6	M	1	–	+					" " " I
9	5	F	6/12	–	+	+				" " " II
10	9	M	2	–	+					" " " I
11	9	M	4	–	+					" " " I
12	5	M	5/12	–	+					" " " II
13	8	F	2/12	–	+	+				" " " III
14	7	M	2	–	+	+	+			Lost to followup
15	5	M	1/12	–	+	+	+			" "
16	6	M	1	Enuresis	+	+	+			" "
17	7	M	1/12	–	+	+	+			Cured in Stage I
18	5	M	8/12	–	+					Lost to followup
19	7	M	2	–	+	+	+			Cured in Stage III
20	9	M	1	–	+	+	+			No change
21	9	F	4	–	+	+	+	+	+	Cured in Stage IV
22	5	M	6/12	–	+	+	+	+		Cured in Stage IV
23	11	F	2	–	+	+	+	+		Improved with
24	11	M	5	–	+	+	+	+	+	Psychotherapy

4 were cured (positive response), 4 did not respond so that they were passed on to stage IV and the rest of these patients did not return for follow-up. There were only 4 patients to whom stage IV treatment was applied (stage III non-responders). Two patients stopped soiling at this stage. Two patients who did not respond at all were started on psychotherapy. With psychotherapy one of the patients improved (soiling reduced to less then three times a week) but the other one showed no change. These results remained the same till the end of the study.

Discussion

Treatment of encopresis is still a problem despite the diversity of available treatment programs. Most of the cases studied by various authors involved patients who had constipation and impaction. Eighty and 95 % percent of the patients reported by Levine[2] and Fitzgerald[8], respectively, had impaction. Accordingly, the treatment programs suggested by these authors are for constipated encopretic children. Treatment approaches differ according to different disciplines. Enemas, suppositories, diet controls, stool softeners, bulk producers and laxatives are generally recommended by pediatricians. The preferred treatment by psychiatrists is psychotherapy. In recent years behavior therapy has found a wider application in the management of encopretic children.[7] Some authors are in favor of using a multidisciplinary approach for the treatment of encopresis.[9] The treatment we applied to our study group is similar to the conditional programme described by Wright[10], with some modifications. We started all of our patients on a very simple reward system and used, on a gradual basis, more aggressive methods only with non-responders.

The mean age and the sex ratio of our study group are in compliance with the literature.[11, 12, 13]

In our study the prevalence of enuresis as an associated feature is 8.3 %, which is somewhat lower than the percentages reported in other studies.[11, 13]

Some authors report that children with minimal accumulation of stool in the bowel have poor outcomes from the use of treatment programs that decrease constipation.[14, 15] In our study of non-constipated encopretic children, with simple charting and using a sticker as a positive reinforcer for defecation into the toilet, 30 % of the patients recovered (stage I). In stage II, 25 % of the children who received this treatment, simply a weekly reward system, stopped soiling. The recovery rate in stage I and II, where only positive reinforcers were used, was 50 %.

The cure rates reported in encopresis vary from 60-87 %.[9] These rates are achieved mostly in the treatment of constipated children. Only in Taitz's group were non-constipated soilers included. In our study group, which consists of only non-constipated children, the total cure rate was 75 %. We are unable to compare our cure rate with other studies due to differences in treatment methods and the choice of patients.

Encopresis, a common pediatric problem with a diversity of treatments, is still a challenge to the physician due to its multifactorial nature.

Before determining a management program, subdividing the patients into constipated and non-constipated groups appears to be most useful. Although children with little or no stool retention are usually considered to be at the greatest risk for treatment failures and are referred to a child psychiatrist, we have had a fairly high success rate with this group through behavior therapy. This type of treatment has the advantage of being less time consuming, easier to apply, and less costly compared to psychotherapy. We acknowledge that stage III and IV of our treatment programme are rather aggressive, but 50 % of the cases did not require these stages and were treated successfully in the first two stages without being traumatized.

Here we would like to emphasize that a physician should be the one to treat encopresis, as a non-physician cannot distinguish between the constipated and non-constipated types.

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Hepatitis B Surface Antigen Associated Exfoliative Erythroderma

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Summary

A variety of skin findings, which are considered to result from immune complex deposition, can be seen during the clinical course of hepatitis B surface antigenemia. In this paper, a case of exfoliative erythroderma associated with hepatitis B surface antigen (HB_sAg) positive-chronic liver disease is described. HB_sAg was detected in the lysate of exfoliated epidermal cells by radioimmunoassay. Immunofluorescent and immunoperoxidase studies showed HB_sAg deposition in epidermal cells and the liver.

Key Words: Hepatitis B Surface Antigen, Exfoliative Erythroderma.

Introduction

Several extrahepatic clinical syndromes have been described with hepatitis B surface antigenemia. These include infantile papular acrodermatitis (PAC), serum sickness-like syndrome, glomerulonephritis, polyarteritis nodosa, essential mixed cryoglobulinemia and polymyalgia rheumatica.[1,2] In these diseases skin, joints, arterioles, small arteries and renal glomeruli were involved. Although Gianotti-Crosti syndrome (PAC) was first[3] described in 1955 and its association with hepatitis B surface antigen (HB_sAg) was demonstrated in 1970[4,5] the nature of the skin lesions and location of the antigen were not well-understood.

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HB_sAg was detected in sweat[6] as well as other body fluids and secretions,[7-11] however the mechanism of transmission into sweat has not been elucidated.

We report herein a case of exfoliative erythroderma associated with HB_sAg positive chronic liver disease. In this study, HB_sAg, has been demonstrated in the epidermal cells for the first time.

Case Report

A twenty-six year old male presented to Hacettepe University Hospital with major complaints of progressive pruritic erythroderma and weakness of six months duration. Previous medical history was not remarkable except for transient icterus which appeared 6 months ago. He denied having had a previous history of atopic dermatitis. Upon physical examination generalized desquamative erythroderma and, in the axillary regions, hyperpigmented skin folds were found. The spleen was 5 cm below the costal margin. Laboratory investigations showed a hemoglobin level of 9,10 gm/dL, platelets 60.000/cumm, a leucocyte count of 3.000/cumm with 53 % segmented neutrophiles, 33 % lymphocytes, 6 % monocytes, 5 % eosinophiles and 3 % Sezáry-like cells. Other laboratory data included blood urea nitrogen 21 mg/dL, serum creatinine 1,5 mg/dL, alkaline phosphatase 30 King Armstrong Unit, SGOT 87 units (normal, 10-40 U/L) SGPT 77 units (normal 10-40 U/L), total bilirubin 1.8 mg/dL (direct bilirubin 0.6 mg/dL), albumin 3.0 gm/dL, globulin 2.8 gm/dL, prothrombin time 16s (control 12S). Serum protein electrophoresis was within normal limits. Antinuclear antibody, LE cell and VDRL were negative. HB_sAg was positive in the serum by radioimmunoassay. The lysate of exfoliated epidermal was also found to be positive for HB_sAg on solid phase radioimmunoassay (see "special techniques").

The patient was treated with oral (60 mg/day, prednisolone) and topical steroids, but the exfoliative erythroderma did not respond to therapy. He developed progressive hepatic encephalopathy following a massive upper gastrointestinal hemorrhage and died on the 29th day of admission.

Postmortem Findigs :

An autopsy examination disclosed a nodular liver of 1360 g. Microscopically there was piece-meal necrosis, focal bile duct proliferation, portal lymphocytic infiltration and occasional hepatocytes with ground-glass cytoplasm and hyperchromatic nuclei were observed. Sections of the kidneys showed diffuse, mild epithelial cell proliferation and mesangial thickening of the glomeruli. Numerous sections of the skin as well as a

review of 3 antemortem skin biopsies all revealed irregular acanthosis, para-and hyperkeratosis of the epidermis. A mononuclear inflammatory cell infiltrate was noted perivascularly in the dermis. Other significant findings included congestive splenomegaly (700 g), esophageal varices and massive gastrointestinal hemorrhage.

Special Techniques

Demonstration of HB_sAg in Exfoliated Cell Lysates: Exfoliated epidermal cells of the patient, were collected in distilled water, and disrupted by 3 cycles of freezing and thawing. The resulting cell lysate was centrifuged and the supernatant was used for detection of HB_sAg. HB_sAg was assayed by commercial radioimmunoassay kits (Abbott Laboratories, North Chicago, IL).

Immunofluorescence Studies : Tissues (skin, liver, kidney) obtained at autopsy were frozen and sectioned at 6 microns in a cryostat. Sections were stained for IgG, IgA, C_3 and HB_sAg by fluorescein isothiocyanate (FTIC)-labelled specific antihuman antisera (Behring Diagnostics). They were examined under a Leitz fluorescence microscope. Appropriate controls to assure the specificity of the staining by immunofluorescence included specific absorption of anti-HB_s activity with purified HB_sAg.

Immunoperoxidase Studies : To investigate HB_sAg in the skin and liver, sections of formalin-fixed, paraffin embedded tissues were stained by an indirect technique utilizing rabbit anti-HB_s serum (Behring Diagnostics) and peroxidase labelled goat anti-rabbit antiserum (Behring Diagnostics). Peroxidase activity was revealed by incubating the slides with hydrogen-peroxide and 3,3'-diaminobenzidine. Specificity control of immunoperoxidase staining for HB_sAg was carried out using normal rabbit serum.

Results

Hepatitis B surface antigen (HB_sAg) was positive in the patient's serum and in the epidermal cells'lysate as detected by the radioimmunoassay.

Immunofluorescence staining (not shown) showed deposition of HB_sAg in the cytoplasm of some hepatocytes and superficial epidermal cells and in glomerular basal membranes in a granular fashion. Deposition of IgG, IgM and C_3 were found in the glomeruli, IgM and C_3 in dermal blood vessel walls. IgA was negative in all parts.

Immunoperoxidase staining for HB_sAg showed liver cell dysplasia[12] and confirmed the presence of the antigen in some hepatocytes (Figure 1), as well as vacuolated superficial epidermal cells (Figure 2A and 2B).

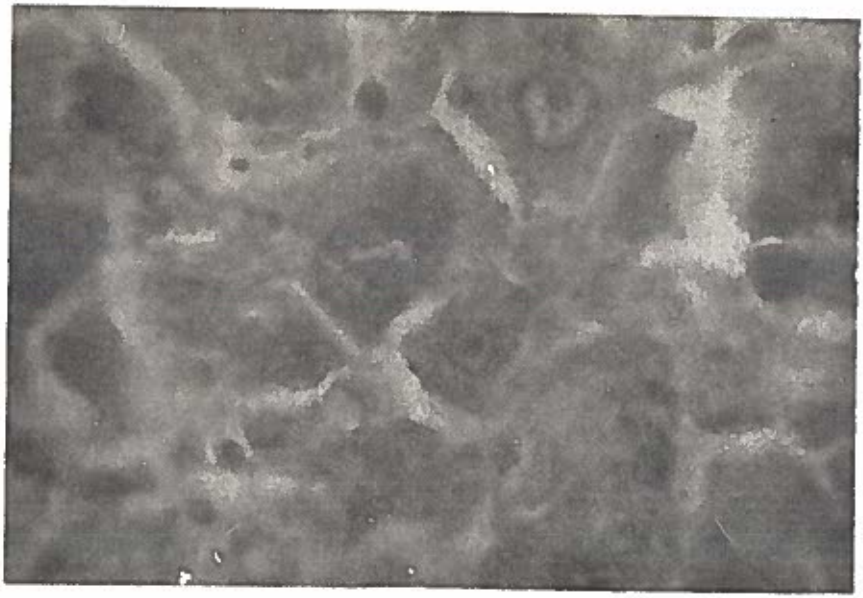
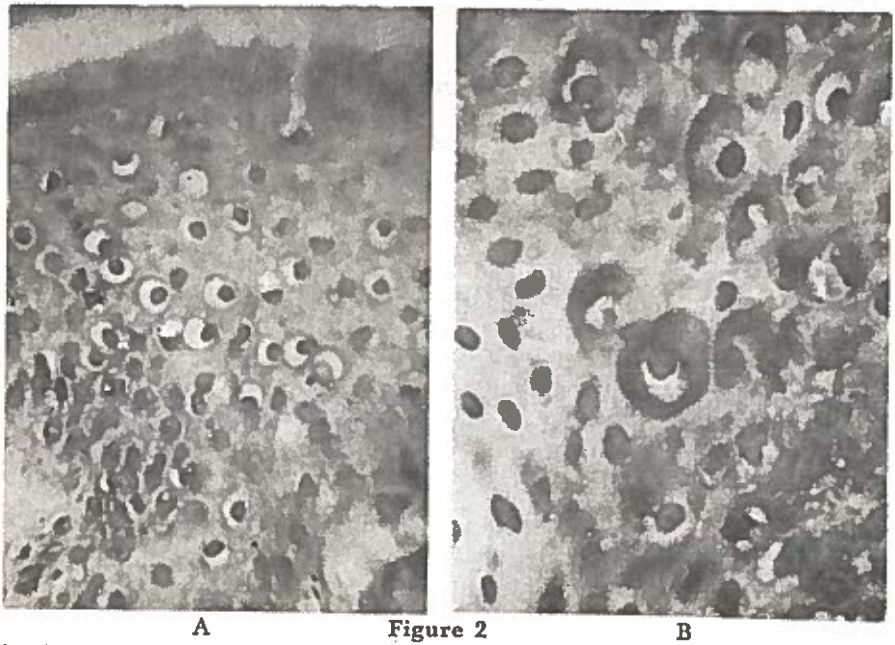


Figure 1

Section of liver showing HB_sAg localized in the cytoplasm of a hepatocyte which has enlarged and dysplastic nucleus. Indirect immunoperoxidase for HB_sAg (x 600).



A B

Figure 2

Section of skin showing intracytoplasmic localization of HB_sAg within vacuolated epidermal cells. A) Control section without HB_sAg-specific antiserum. Indirect immunoperoxidase (x 250). B) Section of HB_sAg-specific antiserum. Indirect immunoperoxidase (x 400).

Discussion

Extrahepatic manifestations of hepatitis B virus infection is not rare. Among these, a serum sickness-like syndrome of varying findings is the most frequently encountered clinical entity. There is a growing list of skin lesions including maculopapular eruptions,[13] macular rashes,[14] urticaria,[15] erythematous plaques,[15] erythematous nodules,[16] scarlatiniform rash,[17] petechiae[18] and purpura.[19, 20] Antigen-antibody-complement complexes are believed to play an important role in the pathogenesis of most of these conditions.[1, 15, 20] Circulating HB_sAg-anti HB_s-complement complexes have been detected in patients' sera and deposition of HB_sAg, anti-HB_s, complement, immunoglobulins and fibrin shown in extrahepatic vessel walls of these patients.[15, 20, 21, 22] Moreover, extravascular immunoglobulins, complement and fibrinogen were demonstrated in skin lesions due to HB_sAg.[15] Although HB_sAg was shown in skin vascular endothelium, as far as we know, it has not previously been found in the epidermal cells. In this case, we demonstrated HB_sAg in the epidermal cells using immunoperoxidase and immunofluorescence staining. In addition, HB_sAg was detected in the lysate of desquamated epidermal cells. In agreement with previous reports,[15, 20] in our case HBsAg, IgM and C_3 were found to be positive in dermal blood vessel walls. This supports the diagnosis of immune complex disease. However, there was no evidence showing HB_sAg in the sweat glands. Therefore we consider that passive diffusion and exfoliation of HB_sAg positive epithelial cells may be probable mechanisms in the transmission of HB_sAg into sweat.

A concomitant finding in this case was the presence of Sezáry-like cells. These cells had cerebriform nuclei, ranged from 2 % to 5 % with a mean 3 % in the differential leucocyte count, and never exceeded 5 % at any time during the clinical course. They were periodic acid schiff-negative. In fact, Sezáry-like cells have also been seen in peripheral blood smears in chronic generalized inflammatory dermatoses such as atopic dermatitis, contact dermatitis, and prosiasis.[22, 24] Further investigations are needed to elucidate the effect of HB_sAg on T cells and the occurrence of Sezáry-like cells.

In conclusion, exfoliative erythroderma is added to the expanding spectrum of hepatitis B surface antigen associated skin lesions. The presence of HB_sAg in epidermal cells, in addition to skin vessel walls, may provide new evidence for the causal relationship between hepatitis B surface antigenemia and associated skin findings.

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Leiomyosarcomas of the Urinary Bladder

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Summary

Two cases of leiomyosarcoma involving the urinary bladder are reported. Tumors were excised by transurethral resection and partial cystectomy. Early diagnosis and agressive surgical treatment are necessary to assure survival of patients with this rare but lethal tumor.

Key Words: Leiomyosarcoma, urinary bladder.

Introduction

Leiomyosarcomas of he urinary bladder are rare in adults and children. The incidence of these tumors have been estimated to be 0.38 and 0.67 percent of all bladder neoplasms.[1] Leiomyosarcomas are mesodermal in origin and undergo variable degrees of differantiation into striated, smooth or connective tissues and may occur anywhere in the bladder. The well differentiated tumors may be initially observed as intramural nodules beneath the normal epithelium.[2] Histologically they are composed of spindly, elongated tumor cells, often arranged in parallel bundles. Nuclear abnormalities are of paramount importance in ascertaining the diagnosis.[3,4]

Our purpose here is to present two cases of leiomyosarcoma involving the urinary bladder and to give a summary of related literature.

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Case Reports

Case 1: A forty-year-old female presented with ta three-month history of painless gross hematuria. Results of physical examination were unremarkable. Excretory urography showed normal upper tract with a vesical filling defect on the left (Figure 1). Cystoscopy revealed papillary tumoral lesions arising from the anterior and the left lateral wall of the bladder. The patient underwent partial cystectomy. Histopathologic examination of the excised specimen showed leiomyosarcoma of the bladder (Figure 2). One year after the operation, cystoscopic examination showed no recurrence of the disease.

Case 2: A sixty seven-year-old male presented with a two-month history of painless gross hematuria. Physical examination showed no abnormalities. Excretory urography showed a vesical filling defect on the left (Figure 3). Cystoscopy revealed a pedinculated bulky tumor mass arising from the left lateral wall of the urinary bladder. This tumor was excised transurethrally. Histopathologic examination showed leiomyosarcoma of the urinary bladder (Figure 4). Six months after the transurethral resection, cystoscopic examination showed no recurrence of the tumor.

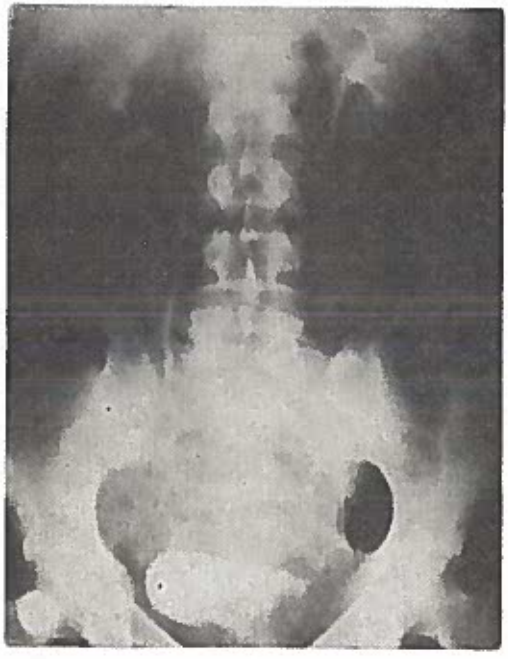

Figure 1
Excretory urography shows normal upper tract with vesical filling defect on the left.

Figure 2

The spindle cell sarcoma, presumably a leiomyosarcoma, grade III was removed from a 40 year-old woman. Note the loss of orderly arrangement of spindly tumor cells which show marked nuclear abnormalities. (hematoxylin and eosin: Original magnification x 250).

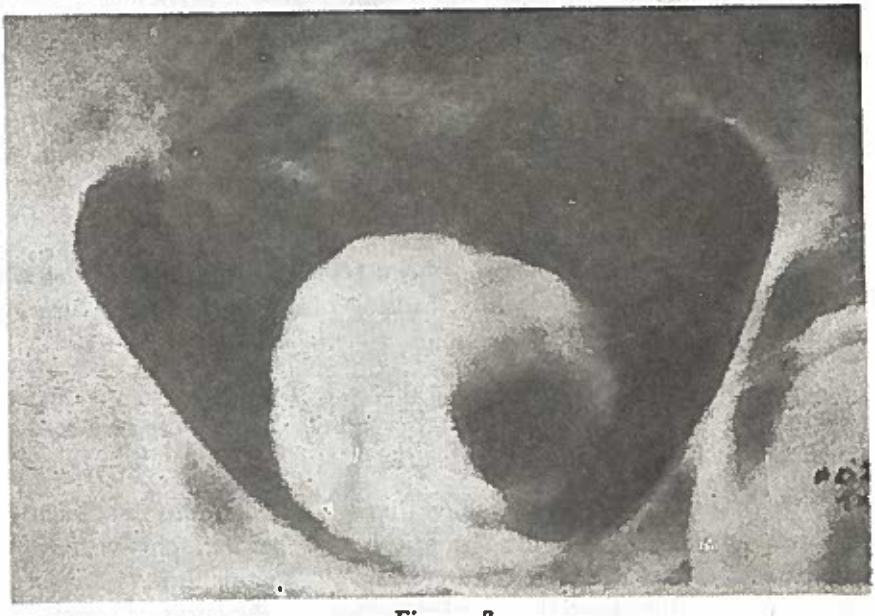

Figure 3

Cystogram shows vesical filling defect on the left.

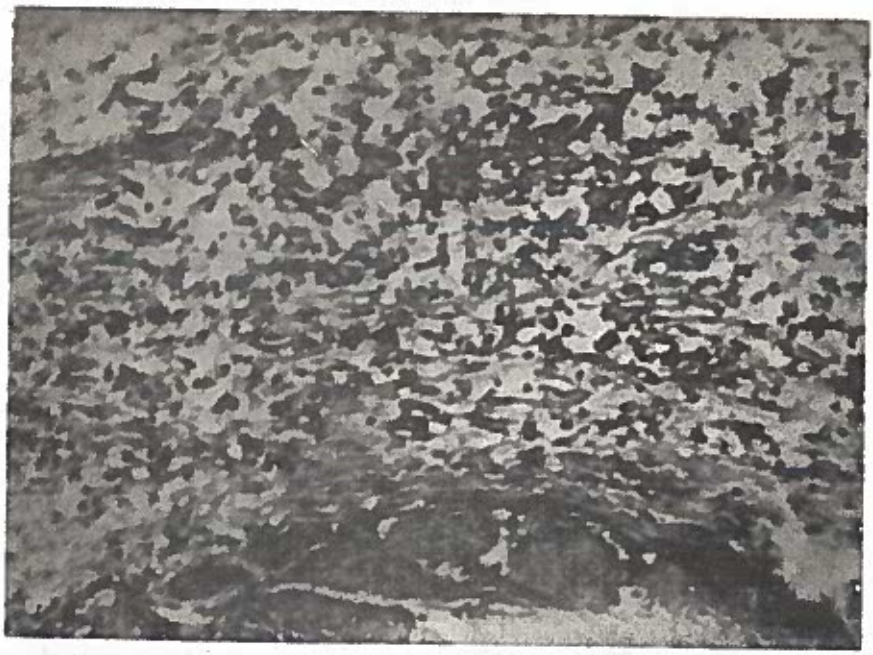

Figure 4

The histologic pattern of leiomyosarcoma which was removed from a 67 year-old male. The spindle cell tumor cells show nuclear abnormalities and cytoplasmic striations could not be identified (Hematoxylin and eosin: original magnification x 250).

Discussion

Before 1983, 70 cases of leiomyosarcoma of the urinary bladder were reported.[5] Males were affected twice as often as females and tumor occured in patients at any age but are more common during their fifties and sixties.[6]

The origin of these tumors has been reviewed by various authors.[6,7] The tumor tends to invade locally, causing obstruction of the ureteral orifices.[8]

The prognosis is poor. A two-year survival rate has been reported in a number of patients. By 1973, only eight patients were reported to have survived for more than five years.[1]

Different modes of therapy have been used, including radioactive seed implantation, radium implantation, cryotherapy, chemotherapy, external radiation and pelvic exanteration.[9,10] At this time, it appears proper staging, either partial or total cystectomy is the curative procedure that with of choice.[11]

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Primary Intrafollicular Ovarian Pregnancy

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Summary

Primary ovarian pregnancy is a rare form of ectopic pregnancies. Intrafollicular pregnancy which accounts for 15 percent of ovarian pregnancies is more infrequent.

A case of primary intrafollicular ovarian pregnancy which was diagnosed and treated in our department in 1986 is presented and the literature is revieved. This was the third ovarian pregnancy case that we encountered since 1974.

Key Words : Primary Ovarian Pregnancy, Intrafollicular.

Introduction

Ovarian pregnancy is seen once in every 25 000-40 000 pregnancies but in recent years reports claim that the incidence is four times higher.[1] Ovarian pregnancy is still rare and only 15 percent of these develop in the follicule. In 1878 Spiegelberg defined four criteria for diagnosing pirimary ovarian pregnancy. These criteria are as follows :

1. Gestational sac should be in the ovary,
2. The tube on the affected side must be intact,
3. The ovary must be connected to the uterus by the ovarian ligament,
4. Ovarian tissue should definitely be found in the sac wall.

The case presented here is the third case since 1974 in our department and it fulfills all the criteria.[2,3]

Case Report

N.S. a 30 year old female gravida: 2 para: 2 was admitted on September 30, 1986 because of pelvic pain. It was noted in her history that

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20 days before she had had curettage for abortion twice (the sac was thought to be intact after the first curettage), because of 6 weeks of pregnancy. She had not been using an intrauterine device (IUD). Her past medical and family history were uneventful. The physical findings were normal. The gynecological examination revealed external genitalia, cervix and uterus as normal, but there was a tender mass of 6x8 cm. in the left adnexal area. Ultrasonographic examination showed the uterus to be 44x66 mm. and regular, while the left ovary was 7x7 cm. and contained a 2 cm. diameter cystic mass. Laboratory findings were: Hgb: 10.8 gr; Hct: 35 %; WBC: 4000; Bleeding time: 1 min. 20 sec.; Coagulation time: 5 min. 30 sec.; Thrombocyte count: 199 000; Glucose: 140 mg/100 cc.; Urea: 41 mg/100 cc. and a pregnancy test was positive. The culdocentesis was positive for blood, so prompt laparatomy was decided. On exploration, the left ovary was 5x5x6 cm, cystic, adherent to the left tube and bleeding from the lateral side. Left salpingooferectomy was applied and the patient was discharged on the 8th postoperative day.

On macroscopic examination of the ovary, the amniotic sac (2,5x2,5 cm), and a 0,5 cm embrio in the sac was seen (Figure 1). Microscopically (B-3503-1986) normal ovarian tissue and corpus luteum adjacent fo chorionic villi and membranes were detected (Figure 2). Upon these findings the Pathology department reported the case as an intrafollicular primary ovarian pregnancy.

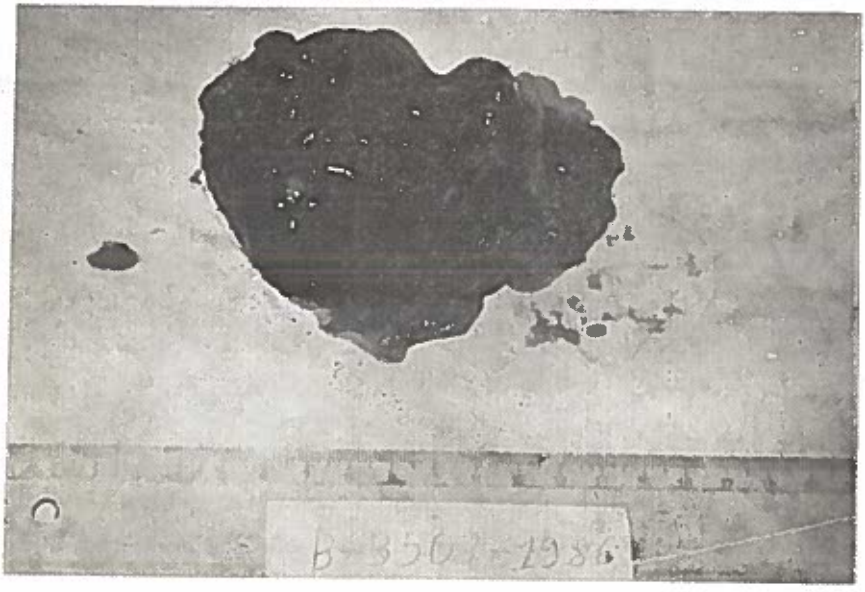

Figure 1
The microscopic appearence of the left ovary. Gestational sac can be seen.

Figure 2

The Microscopic appearence of the left ovary. Chorionic villi are adjacent to the corpus luteum.

Discussion

Under normal conditions the ovum cannot be fertilized in the ovary; fertilization occurs in the tube after maturation of the ovum. Grimes *et al.* reported that there are 300 cases of ovarian pregnancies in the literature since the first one presented by Saint Maurice in 1862. In their review of five different reports, they concluded that the incidence of primary ovarian pregnancy is one in 6970-8263 deliveries and approximately one in 34-42 ectopic pregnancies.[1]

The zygote may implant into the ovarian cortex or less frequently into the follicule because of factors such as Iffy's menstruel reflux theory or reverse tubal motility depending on the changes in prostaglandin secretion.[2, 4, 5]

The increased incidence of ovarian pregnancy has been attributed to the widespread use of intrauterine devices. Gray and Ruffalo reported 4 patients with ovarian pregnansies who were using Cu-7 IUD.[4] The ratio of IUD users with ovarian pregnancy has been reported as 17-24 percent.[6, 7]

Ovarian pregnancies generally result in rupture as in the case presented, or less frequently, it form a cystic mass filled with blood and placental tissue within the ovarian capsule. In spite of this, Williams *et al.* reported a term pregnancy that was delivered by caeserian section, which was an ovarian pregnancy.[8]

The presented case is the third case in our department since 1974, among 16 500 deliveries, yet only one of the three was using an IUD.

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The Rehabilitation of Three Cases of Neurogenic Arthrogryposis Multiplex Congenita and a Review of the Literature

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Summary

In this article three cases of Arthrogryposis Multiplex Congenita(AMC) are presented. The diagnostic categorisation of the three patients are neurogenic type of arthrogryposis. The first one is a typical example of AMC with extension contractures, the others are examples of AMC with flexion contractures. As a result of surgical treatment, intensive physiotherapy, comprehensive rehabilitation programme and correct bracing, the extent of the handicap is minimized, and the three children are able to function independently.

Key Words : Rehabilitation, Neurogenic Arthrogryposis Multiplex Congenita.

Introduction

AMC is a syndrome characterized by congenital, painless, static multiple contractures, fixed between extreme flexion and extension. This condition is not a clinical entity but a syndrome residual of a variety of prenatal etiologic factors, most of which are but incompletely known. In some cases there is a defect in the central nervous system with failure of innervation of skeletal muscle. In others, muscle dystrophy, with or without a known genetic basis, has been thought to be the reason[1, 2]

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Further, there are various theories of intrauterine mechanical circulatory or toxic etiology (that limit fetal movements during or shortly after the formation of appropriate joints) which may be responsible.[1, 2]

Case Reports

Case 1 : E.Y. was the second child of a 25 year-old mother and a 25 year-old father and there was consanguinity between them. As it was the second pregnancy of the mother, decrease in fetal movements were definetely detected as compared with her previous pregnancy. The gestation was of 40 weeks duration by six weeks delay and terminated in normal labor, but the delivery was from breech presentation.

The child was first examined by us when he was 5 years old. Symptomatology revealed motor retardation. There was a generalized decrease in his muscle mass and there were contractures in extension in his knees and elbows, and flexion in his wrists. There was painless but severe limitation in all directions. He was walking tiptoed because of his pes equinus deformities. His head circumference was less than the second percentile for his age. The skull showed flattening of the occiput. The thorax was bell shaped, both scapulae were prominent and elevated. The heart and lungs appeared normal. Both testes and the scrotum were normal. He was able to move his shoulders up to 15 degrees in all directions, but his elbows were straight (contractures in extension). The lower extremities revealed deformities of hips, knees and ankles. His knees were both straight and in extension contractures. There were severe equinus deformities of the feet. Neurologic examination revealed normal cranial nerve functions and the absence of deep tendon reflexes in the upper and in the lower extremities. Analysis of muscle strength showed the shoulder girdle muscles to be symmetrical and rated at a fair grade whereas the muscles of the pelvic girdle were rated poor. The proximal muscles of the lower extremities were also graded as fair while the distal muscle groups were bilaterally at good level. A series of roentgenograms of the skull and a general bone survey showed the following: Extension deformities of the feet, knees, elbows and flexion contractures of the wrists and generalized demineralization surrounding the affected joints with obvious soft tissue contractures. A bone age of 4 years was determined by the Greulich and Pyle method (Figure 1).

EMG demonstrated potentials characteristic for neurogenic muscle weakness (in quadriceps, anterior tibial muscle), conduction velocities of the peroneal and ulnar nerves were well within normal limits. An ECG was interpreted to be within normal limits. Results of the complete blood cell count and urinalysis were within normal limits. Urine culture was

Figure 1
The photograph of the first case after he was rehabilitated.

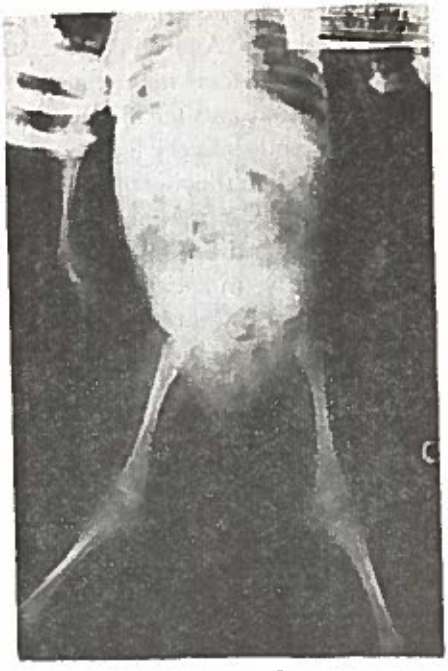

Figure 2
The X ray showing the kness in extension contractures

negative but serum chemical values were within normal limits. Chromosomal karyotype was 46.XY. Intelligence assesment placed him in the normal range (Stanford-Binet IQ 90 examiner's estimate). After he was treated with an intensive and regular rehabilitation programme for 74 days, it was seen that the treatment in the knees was successful as measured by goniometric means, but the contractures of the elbows and ankles remained unchanged. As the contractures of the ankle had become more firmly fixed with the passage of time, it was concluded that surgical procedures were essential. Later, achilloplasty was performed and as a result of intensive physiotherapy and a comprehensive rehabilitation programme, the child began to walk freely, with his feet completely touching the ground. Only his right lower extremity was externally rotating during his gait and this was corrected by means of an elastic twister (Figure 2- The photograph of the reported child after he was treated).

Case 2 and 3: I.A. and S.A. were the twins of a 24 year old mother and a 26 year old father. The first child of the couple (a male 2 years older than our patient of 9 years of age) was reportedly normal but was not examined by us. The second child had died of measles when he was one year old. The third and fourth children were our patients.

There was no consanguinity, but the couple originated from the same district in Anatolia. The pregnancy was uncomplicated with no abnormality of fetal movements being noticed. The gestation was of 40 weeks duration and terminated in a normal labor at home with the help of a nurse and delivery from vertex presentation. The patients were first seen by us when they were 5 years old. They appeared younger than their stated age. Examination at that time in the outpatient department showed a generalized mild decrease in muscle mass. They were able to sit independently, move their upper extremities within normal limits. But the flexion contractures of the hips and knees were obvious. The hips and knees showed 10° and 35° flexion contractures respectively. Their right feet were in pes equino varus position (Figure 3,4).

Figure 3

The photograph of the twins, with Arthrogryposis Multiplex Congenita. (Left: The second case, elder brother, right side, the third case; The younger brother. Both after rehabilitation, and they are dressed, as the parents didn't let us take the naked photographs of their children.

Fine motor, social and language milestones were all within normal limits. The chests were bell-shaped, the pectoral muscles could not be palpated and mild axillary webbing was seen bilaterally. Hearts and lungs appeared normal. Both testes and scrotum were normal. First, they were treated surgically; supracondylar osteotomy was performed bilaterally in order to correct the flexion contractures at the knees. After the operation, although they were not able to walk, with assistance, they were able to stand. Their contractures had decreased remarkably.

Neurological examination revealed normal cranial nerve function and normal deep tendon reflexes in the upper extremities, but these were absent in the lower extremities. The muscles of the shoulder girdle were bilaterally generally good when graded. All other upper extremity muscles were good to normal. The gluteus maximus and tensor fascia lata had fair strength, the other muscles of the lower extremities were symmetrically good.

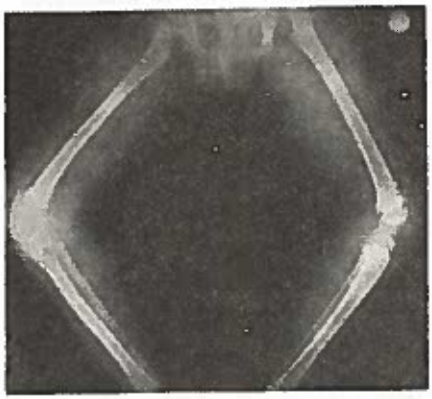

Figure 4
The X ray showing the abduction deformities of hips, flexion contractures of knees. (Before treatment, second case).

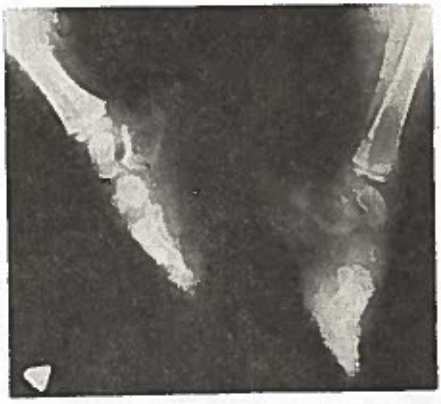

Figure 5
The X ray showing the pes equino varus deformities, at the feet. (Before treatment third case).

Roentgenograms of the skull showed flattening of the occiput. There were generalized demineralization of bone surrounding affected joints as well as soft tissue shortening and contractures (hips, knees, and ankles). Skeletal maturation of the twins were approximately 6 years of age, as determined by the Greulich and Pyle method with a chronological age of 7. This was considered below average, but within normal range. The femoral necks appeared retroverted. Intravenous pyelogram and cystourethrogram were considered normal. E.M.G. demonstrated potential characteristics of neurogenic muscle weakness and it was very difficult to find M.U.P. as most of the muscles were atrophic and were replaced by fibrous tissue, this included involved tendons and ligaments. According to these findings, it was noted that there was a serious involvement of the anterior horn cells. Nerve conduction velocities were normal. An ECG was interpreted to be within normal limits. Results of complete blood cell count and urinalysis were within normal limits. Urine cultures were negative. Serum chemical values were within normal limits. Chromosomal karyotypes without banding were 46 XY. Hearing evaluation indicated normal auditory sensitivity. Intelligence assessment placed them in the normal range. They were treated for 78 days by means of physical medicine and rehabilitation.

Following an intensive and regular rehabilitation programme, they began to walk through the parallel bars by wearing bilateral long leg walking braces. Later they were able to walk and climb up and down the stairs independently by means of two crutches and long leg braces (Figure 5). They were allowed to go home, but were advised to be controlled with our department every 3 months.

Discussion

AMC is not a clinical entity but a syndrome residual of a variety of prenatal etiologic factors most of which are incompletely known. It is characterised by congenital, painless static multiple contractures of extension and flexion.[3] Although an accurate indcidence of AMC is unknown, it has been estimated as occurring in 0.31/1000 live births. Both the involvement of the upper and lower extremities are observed (46 %) but it may involve either the lower extremities (43 %) or the upper extremities (11 %).[4, 5] Some authors, especially Mc Kusick, were impressed with the thickening of the articular capsule of the joints and, therefore, felt that the primary pathology resides in the connective tissue.[3] Myopathic changes and sometimes no specific changes have been revealed by EMG or muscle biopsy, but in the majority of patients potential characteristics for neurogenic muscle weakness were found.[5, 6] There are many theories about the etiology of the AMC, but according to most of the theories this condition is thought to be the end result of a number of pathologic processes that cause limitation of fetal movements during or shortly after the formation of appropriate joints.[2,7] The most common hypothesis is the mechanical restriction such as leakage of amniotic fluid, amniotic bands, an abnormal uterus, small pelvis presence of a twin, or environmentally related teratogens such as drugs, intrauterine infections, alcohol, hypertermia and trauma.[3, 7, 8] In such children congenital contractures are often present and transverse lie or breech presentation is frequent. As a few twins have been reported with only one of each pair being affected, mechanical theories seem to be losing their credibility. According to some authors the neurogenic disorder is related to autosomal dominant inheritance and myopathic disorder with autosomal recessive.[8] However, in these cases a specific pattern of associated congenital malformations are not seen.[8] Various anomalies of the musculoskeletal, genitourinary, cardiovascular, gastrointestinal, and central nervous systems have been reported in association with AMC.[8] Disorders encountered in patients with AMC have been classified by Gericke, *et al.* as arthrogryposis due to nervous system disorders, abnormalities of joints and contiguous tissue (20 %) distal arthrogryposis syndromes (19 %), skeletal disorders 10 %, pterygium syndromes (8 %) intrauterine maternal factors (7%), myopathies (1 %) and miscellaneous group(3 %).[9]

Gericke *et al* reported a large series of patients with AMC, all of who had pterygio formation.[9] Norum *et al* described 3 children from eastern Kentucky having AMC who presented with a webbing across multiple joint and the neck, rocker bottom feet and dystophia conthorum.[10]

Aarskog discovered a patient exhibiting AMC who had a with webbed neck, multiple pterygia of fingers with camptodactyly like contractures, dystophia canthorum, fusion of several cervical and thoracic vertebrae and hypoplasia of the labia minora.[11-14] Krieger and Espiruti studied patients with (PAMC) pterygoarthromyodysplasia.[1, 13] Scott reported a patient who had AMC with pteygium colli and cervical spine fusions.[15-18] The results of the authors mentioned above and our results conform with each other concerning the presence of similar neurologic and arthrogrypotic changes in the musculoskeletal systems. The difference between our and their cases concern the presence of pterygium syndromes, genitourinary, cardiovascular and gastrointestinal anomalies and the absence of twins having similar musculoskeletal deformities. A careful examination, documentation and investigation is imperative in the diagnosis of AMC. Therefore, according to the physical examination at birth; with presentation of congenital contractures since delivery and with the help of further examinations, investigations and documentations, these three cases were specific examples of Arthrogryposis Multiplex Congenita. Moreover, the electromyograms were diagnostic and they classified the patients in the neurogenic type of arthrogryposis.

Subsequently as these were milder cases without tne presence of any congenital anomalies, intensive physiotherapy and a comprehensive rehabilitation programme minimized the extent of the handicap.

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Blind Orotracheal Intubation in A Patient with Rheumatoid Arthritis

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Summary

Patients who have flexion deformities of the cervical spine and ankylosis of temporo-mandibular joint due to rheumatoid arthritis present difficulties in endotracheal intubation.

In this paper, a different intubation technique which was applied in such a case is presented and discussed.

Key Words: Rheumatoid arthritis, intubation, endotracheal.

Introduction

Patients who have flexion deformities of the cervical spine due to rheumatoid arthritis often present difficulties during endotrocheal intubation.

The head and neck should be manipulated with great care during positioning or intubation, other wise cervical vertebral erosion and subluxation may occur, especially at the atlantooccipital joint. Further, temporomandibular problems are common, and certain arthritic patients have an acquired laryngeal deviation due to the abnormal position of the larynx.

Many clinicians prefer regional anesthesia or awake intubation in such patients.

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If the mouth cannot be opened, nasal intubation or tracheotomy must be performed for maintenance of the airway during anesthesia.

Application of the flexible fiberoptic bronchoscope may reduce the risk of general anesthesia in these patients. The trachea can be intubated prior to induction of anesthesia.

Case Report

A 31- year-old man had flexion deformity of the cervical spine which was associated with rheumatoid arthritis involving the hands, hips and vertebrae. Physical examination revealed an immobile neck. His mouth movements were limited by rheumatoid arthritis.

Roentgenographic examination revealed a smooth cervical spine (Figure 1). The larynx was displaced anteriorly.

Preoperative blood tests, urinalysis, electrocardiogram and chest roentgenogram were normal.

The patient had undergone three replacement procedures (right hip replacement, 10 years ago: left hip replacement, two years ago and knee replacement, 18 month ago). This time he was admitted to the hospital for right hip replacement.

Preoperative medication was given with Atropine (0.50 mg), Meperidine (50 mg) and Haloperidole (5 mg) im. before induction of anesthesia.

In the operating room, venous cannula was placed percutaneusly and 5 % Dextrose solution was infused.

Epidural anesthesia could not be performed because of the deformities in the spine.

Diazepam (10 mg) and Ketamine (1.5 mg/kg) were given intravenously. The patient was ventilated with nitrous oxide, oxygen (50/50 % inspired concentration) and Halothane (0.5%) in a semiclosed system via a face mask. Then, sodium thiopental (2 mg/kg) was given intravenously without depressing his respiration.

Orotracheal intubation was impossible because the laryngoscope could not be manipulated inside the mouth.

An 10 mm endotracheal tube was inserted into the mouth while holding the tube between the index and middle finger of the anesthesiologist's hand. The tip of tube was advanced above the vocal cords, while the patient's breath was reflected to reservoir bag (Figure 2). The baloon was inflated and a sponge was wrapped around the tube.

Figure 1

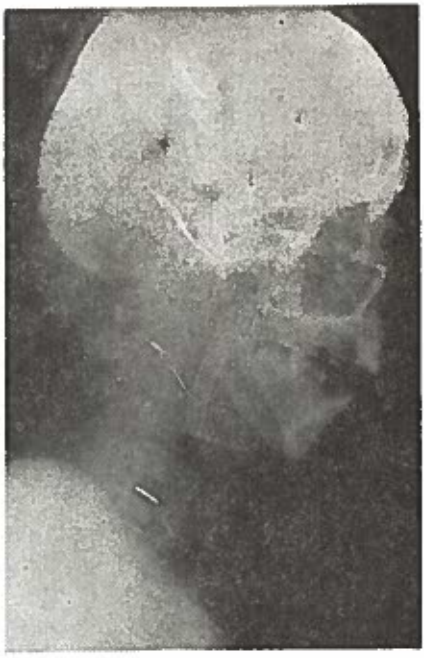

Figure 2

Anesthesia was maintained with nitrous oxide 50 % in oxygen (5/5 L/min) and Halothane (0.50 %). The patient was anesthetised throughout the procedure and showed no signs of discomfort. His vital signs were stable throughout the operation period (approximately 5 hours).

Monitoring included continuous arterial blood pressure, pulse, and electrocardiogram. These parameters were regular and smooth during surgical intervention (Blood pressure 120 torr to 80 torr, pulse 80-110 per minute).

At the end of the surgery, anesthesia was terminated. When the patient was fully awake, extubation was performed.

Discussion

Simmons[1] corrected flexion deformities of the cervical spine using local anesthesia which was supllemented with nitrous oxide or narcotic analgesia. Munson *at al*[2] reported a cervical osteotomy, in a similar case, using nasotracheal general anesthesia. Intubation of the trachea was achieved with the help of the wine hook described by Bearman.[3] Fiberoptic endotracheal intubation is very effective in patients with rheumatoid arthritis and ankylosing spondylitis.[4, 5] The lack of mobility of the head

and neck increase the difficulty of adequate exposure of the larynx with rigid laryngoscopy. Blind nasotracheal intubation would be impossible, while the stimulation of the larynx could cause laryngospasm in patients who are not sedated and have no topical anesthesia. Moreover, repeated attempts at tracheal intubation could increase the likelihood of trauma and laryngeal edema.

We performed general anesthesia without muscle relaxants, placing the tube above the vocal chords in our patient. We believe that this method can be utilized when other methods cannot be applied.

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Real Time B-Mode Carotid Ultrasonography in the Diagnosis of Carotid Artery Disease

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Introduction

Atherosclerosis of the cerebral vessels was once thought to be the major cause of strokes. However, after the introduction of cerebral angiography, diseases of the extracranial portions of the cerebral arteries gradually commenced to be the focus of attention. Although occlusive disease of the middle cerebral artery seems to appear once again among the Japanese and the blacks, especially in younger and female subjects, the association between extracranial atherosclerosis and focal neurologic dysfunction is well established.

The mechanism by which carotid atheroma produces neurologic deficits has been explained by the arterial stenotic and artery-to-artery embolic theories. Significant clinical disease is associated with > 50-60 % stenosis or occlusion of the carotid arteries.[1, 2] Surgical intervention may be considered in these circumstances, although there is still plenty of debate about whether to operate on the totally occluded vessel. Surgeons are generally apprehensive of dislodging a clot during operative manipulations and causing an embolic stroke. Yet there still remains the option of performing extracranial-intracranial or carotid-carotid bypass surgery in these cases.[3, 4]

The ulcerated carotid artery plaque is a well known source in the pathogenesis of ischemic cerebrovascular disease. Embolization to the brain from mural thrombus on a carotid bifurcation atheroma was demonstrated as early as 1905 by Chiari.[5] The exposed intima of the ulcerat-

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ed lesion stimulates clumping of the platelets, which may then become dislodged and occlude the cerebral vessels. The histologic structure of the plaque, as well as the plaque surface may play a significant role in the production of symptoms of cerebral ischemia. There are recent reports implicating intraplaque hemorrhage as an etiologic factor in symptomatic carotid artery disease.[6,7]

As extracranial vascular disease may be successfully treated by medical and surgical measures, the major problem remains as how to investigate the patients, with minimal discomfort.

Diagnostic Tests

There is a battery of noninvasive diagnostic techniques available, in the assessment of carotid atherosclerosis. Indirect tests e.g. oculoplethysmography, photoplethysmography, periorbital doppler imaging, are sensitive to highly stenotic unilateral lesions; while bilateral symmetric disease and ulcerated plaques unaccompanied by hemodynamic compromise are not given proper attention.[2] In addition to the fact that a 40 to 50 % diameter reduction stenosis is necessary to yield positive results with these methods, the estimation of the localization of the detected abnormality is inaccurate. It may lie somewhere between the aortic arch and the orbit of the eye.[8] In one study, utilizing conventional Doppler examination, the specifity was 95 % and the sensitivity 60 % for stenoses greater than 70%.[8] In another, ipsilateral carotid abnormalities were present in 44 % of patients with stroke and 26 % of patients with transient ischemic attacks, while they had angiographically proven 89 % and 71 % abnormalities, respectively.[9] It is estimated that this technique overlooks aproximately 70 % of surgically correctible carotid lesions.[9]

Direct carotid imaging techniques have potential advantages over other non-invasive tests, in that they may yield direct structural information. It is universally accepted that angiography remains the most accurate imaging technique for evaluation of the carotid arteries. Areas of stenosis, ulceration, intraluminal thrombus and extracranial or intracranial vessel occlusion can be accurately detected by this method, although it, too, has significant limitations.

In one study, ulceration was found at surgery in 20 out of 50 carotid arteries (40 %). However, only 12 of these 20 ulcers (60 %) were demonstrated angiographically. 50% of the ulcers not demonstrated at angiography occured as benign appearing plaques, while a very high degree of stenosis ($>$ 90 %) also led to inaccurate results. Of the 30 carotid rateries, which were free of ulceration at surgery, 17 (34 %) had false

positive results, incorrectly diagnosed as typical ulcer niches. Most of these patients were thought to have intramural hemorrhage and subintimal hematomas.[6, 10] In addition, because the procedure carries certain risks and expense, non-invasive direct imaging techniques were developed. Real-time B-mode ultrasonic imaging provides structural detail of the vessel wall and the atherosclerotic plaque.

Carotid Ultrasonography

Hobson *et al* compared ultrasonographic findings in 84 arteries with contrast angiograms. Ultrasonography (US) accurately detected normal arteries in 81 % of the cases; where by 78 % of patients showing high degree of stenosis (>50 %) were also recognized. However, US failed to identify carotid occlusion in the majority of the cases (18 % accuracy).[1] The specificity, that is the ability to correctly identify the normal state, was 86 % with vessels having less than 40 % stenosis.[8] 40-70 % stenosis were demonstrated with this method in 75 % of the cases, and the sensitivity of the correctly identified advanced disease (stenosis greater than 70 %) was 44 %. The overall accuracy of the B-mode scan was 78 %. The scan identified 38 % of the totally occluded vessels.[8] The lower accuracy rate of the scan in higher grades of disease is by no means surprising, in view of the fact that poorly defined tissue interfaces will give a poor image and calcific plaque material will not allow visualization of deeper structures. It has been suggested that the nonvisualized scan is an indirect evidence of significant atherosclerotic disease of the carotid artery.[8] However, the inability to identify total occlusion is sometimes considered as a major limitation, because the patient may be deemed inoperable. On the other hand, some surgeons adopt different techniques to circumvent this problem, but the patient must be referred to angiography for a detailed preoperative assessment.

Anderson *et al.*, found similar sensitivity of 79 % with US for surgically important disease, while complete occlusion was demonstrated in 25 % of the cases. However, the prevalence of a surgically important disease disclosed by angiography in carotid systems with normal US findings was 19 %.[11] Other series revealed even higher rates of specificity and sensitivity (around 90 %);[12] whereas some recent reports give lower rates of specificity and sensitivity (75.2 % and 77.7 % respectively) on patients with vague cerebral symptoms.[13] The variation in results between different investigators is most likely due to the experience level of the technician, diverse technical difficulties encountered in carrying out the test, and to changes in the sensitivity of the equipment.

In another study conducted by this author to assess the prevalence of atherosclerotic changes in the carotid systems of patient with ischemic strokes in the territory of these vessels, 26 patients having 29 cerebral infarcts were investigated by US. 41. 4 % ipsilateral and 26 % contralateral carotid abnormalities were demonstrated; this appears to be a high proportion in consideration of the method's non-invasive character. Most of the abnormalities detected were irregularities of the vessel wall. Clearly demonstrated atheromatous plaques consisted of 13 % ipsilateral versus 8.7 % contralateral carotid abnormalities. The statistical difference between the ipsilateral and contralateral abnormalities, as related to the site of the brain infarct, was not significant.[14] However, the study was carried out with low-power probes which failed to demonstarate any occluded vessel and which missed several morphological details including ulcerations; this reflects the technical inferiority of the devices still in use in this country.

Ulceration of the atheromatous plaque is important from the pathophysiological point of view, and US is an important diagnostic tool because angiography misses ulcers in a significant number of cases.[15] It has been suggested that US is unreliable in detecting ulceration,[11] and 34 ulcerated plaques in 27 patients were missed by US in one series.[16] On the other hand, several investigators claim that US is superior to angiography in detecting hemorrhage into the plaque, which is closely related to stroke. Such lesions yield heterogenous US patterns which can be picked up with 10 MHz transducers.[7] US is more satisfactory in such circumstances as intraplaque hemorrhage is a mural phenomenon. Recently, some investigators report that the capacity of US in discovering ulceration is better than angiography, but this technique may miss ulcerations which are only 2 mm deep. Of 29 ulcerated arteries, the lesion was predicted by US in 20.7 % and by carotid angiography in 13.8 % of the arteries.[13]

The Technique

The examination is carried out with the patient supine and head resting on a thin support. Starting low in the neck, the common carotid artery is visualized along its longitudinal axis. The transducer is moved, slowly toward the carotid bulb, which is easily identified because of the relative widening at this point. The internal and external carotid arteries at their origin are then viewed.

The structures are recognized by sound wave reflections from the interfaces of tissues. Different acoustic impedance provids true anatomic detail. The normal carotid artery displays an echo free lumen bounded

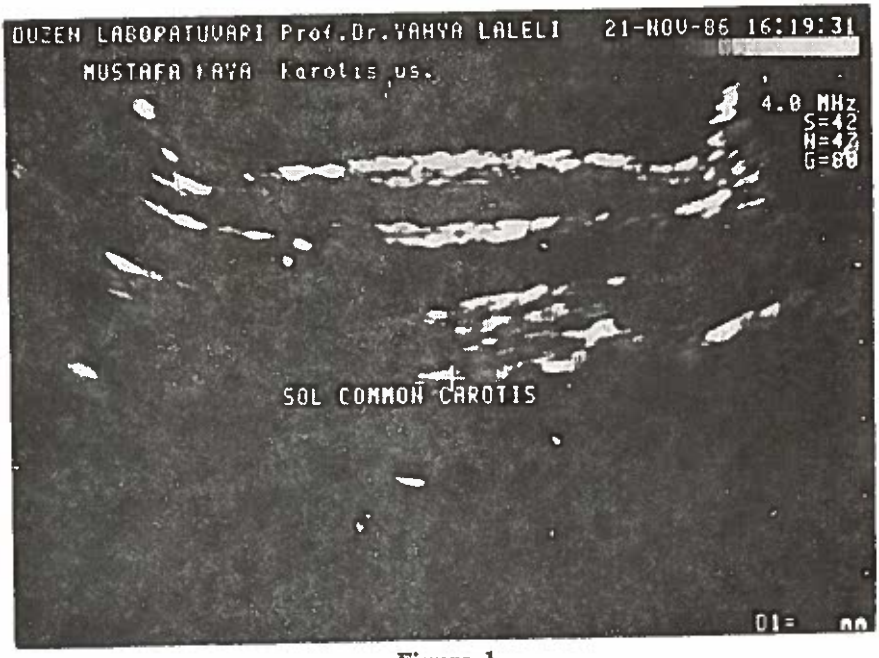

Figure 1
Normal scan

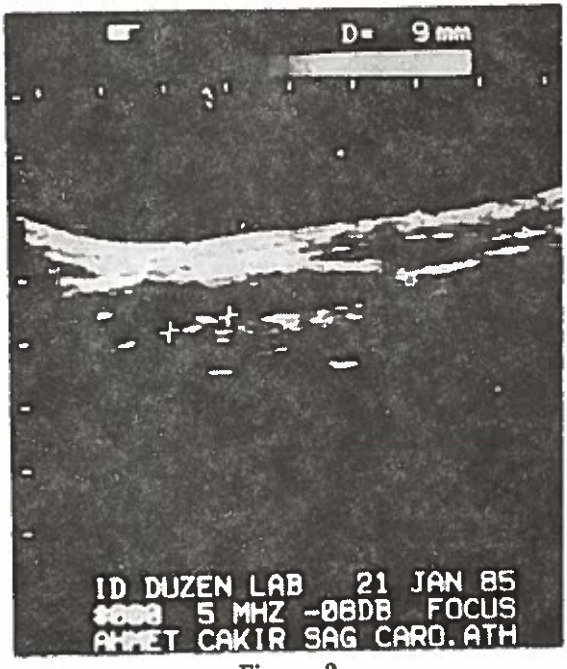

Figure 2
Carotid bifurcation plaque identified by ultrasonography.

by a wall(Figure 1).Atheromatous plaques appear as echo densities within the lumen (Figure 2). Excavations in the carotid wall are interpreted as evidence of ulceration. Several angles of insonation may be used to ensure optimum results.

The Problem of False Positives and False Negatives

The rate of false positive and false negative results is also important for a noninvasive technique, and in this sense false positives seem to outweigh false negatives. In one study utilizing duplex scanning (B-mode-Doppler), the rate of false positives were 63.5 % and false negatives 2.7 %.[17] In another study of 1044 patients, there were 23 false positives and 27 false negatives.[8] The false positives seem to be due to better identification of low grade stenoses with this method. The false negative group generally encompassed poor quality scans; this leads to the underestimation of stenosis. On the other hand, the quality of false positive scans were good. James *et al* reported a false negative rate of 63 %. These authors also contend that abnormal US studies in the face of normal angiograms reflect the sensitivity of the former method in detecting early atheromatous disease.[16]

Combination of US With Other Physiologic Studies

Combination of US with other techniques may give rise to improved results.[2,17,18] In one study real time B-Mode imaging combined with pulsed doppler (Duplex scanning) correctly identified the presence of athero. matous disease in 97 % (17), in another 94 % (2) of the cases. Most of the authors share the view that, the diagnostic accuracy of the physiologic studies such as pulsed doppler, oculoplethysmography and carotid phonoangiography is higher in detecting lesions causing greater than 50 % stenosis. However, when these investigations fail to demonstrate an abnormality, one cannot conclude that the vessel is normal. Real time B-Mode US is much more sensitive to early atheromatous disease.[8, 13] These studies are therefore complementary.

At the same time, some authors advocate the view that these physiologic studies add nothing to the diagnostic accuracy of US.[13]

Conclusions

As surgical measures directed to correct the atheromatous abnormalities in the cervical vessels gain in popularity, the demand for noninvasive methods as well as angiography is expected to increase.

Real time B-Mode carotid ultrasonography can adequately be performed in private laboratories in larger cities in Turkey. The procedure

is carried out by physicians educated in diagnostic radiology, or other medical specialties. This is in contrast to the Western countries where the technique is mainly conducted by skillful technicians.

The main problem here is that the transducers used in the procedure are of low power, mainly 3.5 and 5 MHz; whereas more powerful probes of 7.5 and 10 MHz are necessary in order to obtain scans of high quality. With the low-power probes much detail is lost and the degree of stenosis cannot be defined easily. Moreover, ulcerations may be missed. Wider use of the technique possibly in combination with other noninvasive procedures will no doubt lead to improvements in this field in the coming years.

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Platelet Activation in Chronic Granulocytic Leukemia

In Relation to Proliferative Retinopathy

Rauf Haznedar, M.D.* / Mehmet Levent Alkan, M.D. / Berati Hasanreisoğlu, M.D.*** / Meral Or, M.D.**** / Leyla Suna Atmaca, M.D.*******

Summary

This study was designed to investigate platelet activation in chronic granulocytic leukemia (CGL) and the relationship of platelet-specific proteins, beta-thromboglobulin and platelet factor 4, with the proliferative retinopathy seen in CGL. 12 patients were entered to this study and 19 healthy subjects served as controls. βTG and PF4 were determined by radioimmunoassay. Retinal damage in three patients have been confirmed by fluoroscein fundus angiography. βTG values and the βTG/PF4 ratio were found to be increased. However there was no correlation between platelet-specific proteins and proliferative retinopathy.

Key Words : Chronic granulocytic leukemia, beta-thromboglobulin, platelet factor 4, proliferative retinopathy.

Introduction

Thrombotic and hemorrhagic episodes are not uncommon in myeloproliferative disorders.[1] Platelet involvement is one of the common features in this group of disorders. Platelets may be responsible for the evolution of these complications.[2] However, thrombohemorrhagic events occur infrequetly in chronic granulocytic leukemia (CGL) in comparison to polycythemia wera and essential thrombocythemia.[3,4] The reasons for this infrequency are unclear.

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In the recent decade beta-thromboglobulin (βTG) and platelet factor 4 (PF4), both platelet α-granule release products, were used in assessing platelet activation and participation in thrombus formation.[5-7] In addition to other prethrombotic states, βTG and PF4 were also found to be increased in myeloproliferative disorders, including CGL.[4, 8, 9] Otherwise arguments still continue on the assessment and significance of elevated βTG and PF4 levels in clinical conditions with thrombotic tendency.

On the other hand, abnormalities of optic fundi may be seen along the course of CGL.[10] The current study was designed to investigate platelet activation in CGL in relation to retinopathy.

Materials and Methods

Twelve patients with chronic myelocytic leukemia were entered to this study. Data on age, sex, blood counts, βTG, PF4, spleen size, reticulin content of bone marrow, and retinal findings are presented in Table I. The fundoscopic examinations of all patients were performed by one of the ophtalmologist authors. In three of the patients having proliferative retinopathy, a fluoroscein angiography of fundus was performed. In addition, sickling test, hemoglobin electrophoresis, protein electrophoresis, fasting blood glucose level and glucose tolerance test were performed to rule out other diseases which can cause proliferative retinopathy. The platelet count was between 444.000 and 150.000 per cu mm in all patients. None of the patients had clinical or laboratory evidence for thromboembolism and renal faliure.None of the patients were receiving medication except for allopurinol at the time of investigation.

The mean plasma βTG and PF4 were determined in 19 healthy subjects ranging in age from 25 to 54.

Blood was obtained from an antecubital vein using a 19-gauge butterfly needle with minimal venous occlusion. For β-TG assay, 2.5 ml blood was collected in tubes containing liquid ethylene-diaminetetraacetic acid (EDTA) and theophylline. For the PF4 assay blood was collected in liquid EDTA, 2-chloroadenosine and procaine-HCl. Following collection, the tubes were immediately kept on crushed ice, and then spun at 4°C at 1800 g for 30 minutes. Aliquots of platelet-poor plasma were seperated and frozen at -70°C until assayed. Radioimmunoassay kits were supplied by Radiochemical Centre (Amersham, England) and Abbott Laboratories (North Chicago, Ill) for βTG and PF4 determinations, respectively.

Results were examined for significance using Student's "t" test.

TABLE I

CLINICAL AND HEMATOLOGICAL FEATURES OF THE PATIENTS

Patient	Age	Sex	Hb (% gm)	Leucocyte (cu mm)	Platelet (cu mm)	βTG ng/ml	PF4 ng/ml	Spleen size below costal margin	Bone Marrow Biopsy	Optic fundus
6482, R.D.	53	M	11.7	320.000	376.000	57	8.0	17 cm	Not done	Normal
						49	9.0			
73179, E.S.	37	M	8.5	138.400	150.000	112	11.0	6 cm	Reticulin (–)	Normal
						117	8.0			
102 , L.Ö.	48	F	9.7	138.600	444.000	101	12.0	6 cm	Reticulin (–)	Proliferative retinopathy
						106	16.0			
53057, A.D.	45	F	9.5	67.400	272.000	70	10.0	3 cm	Reticulin (+)	Normal
						65	6.0			
61686, F.Ç.	26	F	11	76.000	432.000	65	5.0	20 cm	Not done	Normal
						62	13.0			
50417, İ.Ö.	52	M	10.5	64.000	214.000	104	20.0	13 cm	Not done	Proliferative retinopathy optic disc neovascularization
						116	16.0			
98521, M.K.	28	M	10	100.000	156.000	71	7.0	7 cm	Not done	Normal
						66	9.0			
36948, M.A.	43	M	9.8	95.000	330.000	22	6.0	16 cm	Not done	Normal
						24	7.0			
56405, K.B.	52	M	11.5	99.000	196.000	76	8.0	15 cm	Reticulin (–)	Proliferative retinopathy, optic disc neovascularization
						81	16.0			
717 , F.B. 2	56	F	8.8	122.000	430.000	89	12.0	13 cm	Not done	Normal
						80	9.0			
0875 , S.K.	61	F	12	10.400	240.000	26	7.0	6 cm	Not done	Normal
						27	5.0			
7674 , K.K.	60	M	11.2	25.000	216.000	85	7.0	3 cm	Not done	Normal
						75	8.0			

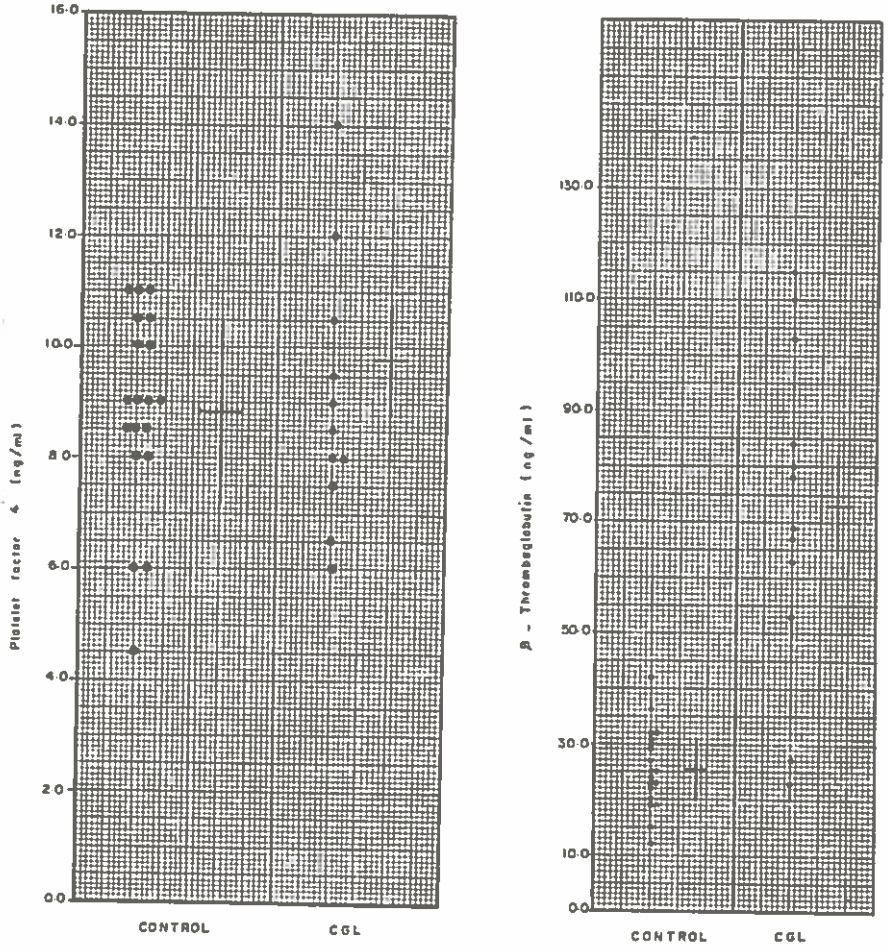

Figure 1

B-thromboglobulin and platelet factor 4 distribution in CGL and in the control group. Horizontal bar represents the mean.

Results

In 19 healthy controls, plasma βTG levels ranged from 12 to 42 ng/ml with a mean 25.5±7.56 ng/ml and a median of 26.0 ng/ml. PF4 levels changed between 4.5 ng/ml and 11.0 ng/ml. Mean PF4 level was 8.84±1.57 and the median was 8.5 ng/ml. βTG/PF4 ratio was found to be 2.9±0.55. These findings are in accord with the literature.

In ten of 12 patients, plasma βTG levels were found to be increased. Only one patient showed increased PF4 level. The mean concentration of plasma βTG and βTG/PF4 ratio were both significantly higher in the patient group than in control group ($p < 0.001$). Albeit slightly increased PF4 values were obtained, this was found to be not significant ($p > 0.01$). (Table II and Figure 1)There were no significant correlations between the platelet count and the βTG and PF4 levels.

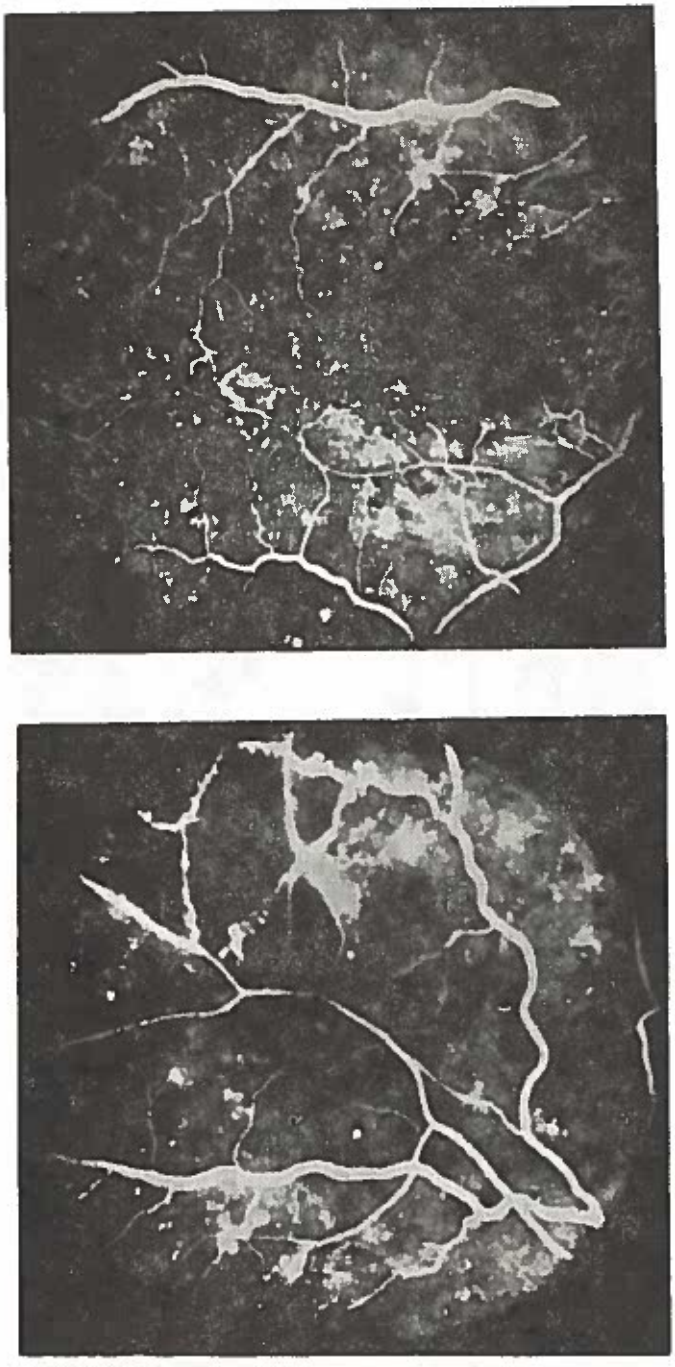

Figure 2

Case I, right eye. Top, fluorescein angiogram in the late arterio-venous phase shows microaneurysms. Bottom, hypoxic areas are seen.

Three patients had findings of proliferative retinopathy. Two of them also demonstrated optic disc neovascularization on FFA (Figures 2,3,4 and 5).

Figure 3

Case I, left eye. Fluorescein angiogram in the late arteriovenous phase. Note the microaneurysms, hypoxic areas, and fluorescein leakage from epipapiller optic disc neovascularization.

Figure 4

Case I, Right eye. Postoperative view of vitreoretinal proliferation is demonstrated.

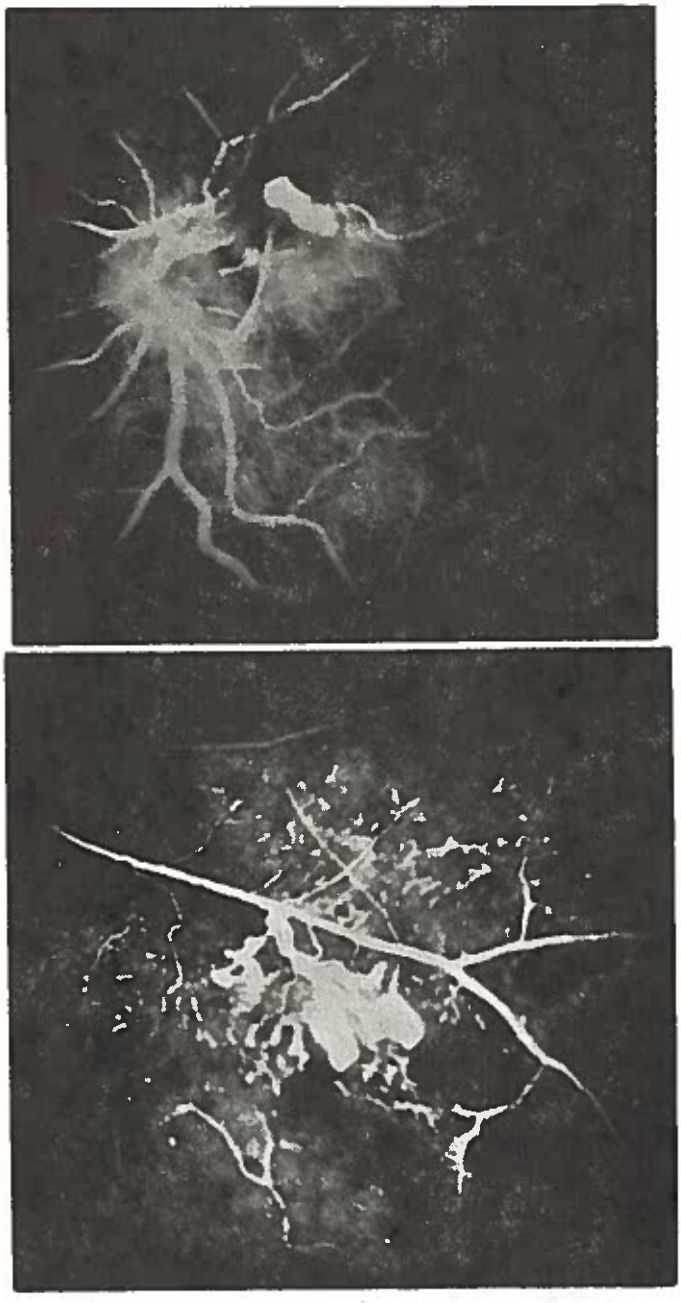

Figure 5

Case 2, left eye. Top, fluorescein angiogram in the arterio-venous phase. Note optic disc neovascularization. Bottom, retinal neovascularization. microaneurysms and hypoxic areas are demonstrated.

TABLE II

PLASMA βTG, PF4 AND βTG/PF4 RATIO IN PATIENTS WITH CGL AND IN THE CONTROL GROUP*

	βTG ng/ml	PF4 ng/ml	βTG/PF4 ratio
Chronic granulocytic leukemia (n= 12)	72.75± 8.46 (23.0 –114.5)	9.75± 1.00 (6.0 –18.0)	7.4±0.67
Control group (n= 19)	25.5 ± 7.56 (12.0 –42.0)	8.84± 1.57 (4.5 –11.0)	2.9±0.55

* Resuls are shown as the mean and range (in parentheses)

A sickling test using sodium bisulfide was negative; and hemoglobin electrophoresis demonstrated an AA hemoglobin pattern in these three patients. Albumin and globulin levels and their ratios and the protein electrophoresis pattern were all found to be normal. Fasting blood glucose level and glucose tolerance test were normal in two cases(number 3 and 6). The third patient (number 9) had a fasting blood glucose level of between 98 mg/dl and 120 mg/dl and a postprandial glucose level of 160 mg/dl.

Discussion

Plasma βTG values were observed to be increased in 10 of 12 patients. This result is in accord with previous studies.[4, 8, 9, 11] PF4 levels the other platelet-spesific protein, were normal except for case 6. These findings seem to be different from the results of Cortelazzo and Viero.[8, 12]

On the assessment of the platelet release-reaction, not only absolute plasma levels of βTG and PF4, but platelet count should be taken into account.[13] Distinction of *in-vivo* release from *in vitro* release is very important. According to Kaplan and Owen, a high plasma βTG and normal or slightly increased PF4 level may denote *in vivo* platelet activation.[6] Since platelet factor 4 has a very short half-life in plasma, due its rapid binding to endothelial cells, its plasma level is within normal limits (except for heparin administration and in vitro release).[6] Several investigations revealed increased plasma βTG levels and a βTG/platelet count ratio in myeloproliferative disoders.[4, 8, 9] Cortelazzo and Viero detected elevated PF4 levels as well.[8]

However the subjects studied previously were heteregenous and gave different results. Ireland *et al* found elevated plasma βTG together with high platelet count in essential thrombocythemia; they denoted each platelet secreted a subnormal amount of βTG. Contrary to this finding, they demonstrated high βTG: platelet count ratios in myelofibrosis and polycythemia vera, which indicate a releasing defect in platelets.[9] On the

other hand, Viero, Cortelazzo and Barbui, using a βTG/PF4 ratio in their cases, documented insignificant changes in this ratio together with elevated both βTG and PF4 values;they concluded that platelet membrane alterations might be responsible for the results.[12] Our findings differ from the study of Viero *et al.* Except for case 6, plasma PF4 levels were all normal. In this study, βTG/PF4 ratio can be considered in favor of increased *in vivo* platelet activation. The exact meaning of elevated βTG levels and elevated βTG/PF4 ratio still remain unclear, in view of the lack of thrombotic events. On the other hand, Fabris *et al* studied intraplatelet βTG together with plazma βTG in patients with thrombocytosis. They showed that patients having increased plasma βTG and decreased platelet content developed thrombosis most frequently.[14] Finally it has been suggested that the measurements of arterial βTG may reflect *in vivo* platelet secretion more accurately than venous βTG.[15] It remains to be determined whether arterial βTG values and/or βTG content of platelets, in comparison to plasma βTG can elucidate the matter in patients with CGL.

Among leukemias, CGL is most freguently associated with abnormalities of optic fundus.[16] Retinal abnormalities were found to be 11.4 per cent of 179 cases with CGL.[17] Although peripheral retinal neovascularization is seen relatively common, optic disc neovascularization occurs very infrequently.[18, 19, 20] Only three leukemic cases with optic dise neovascularization have been described in the literature, and one of them was CGL.[21, 22, 23] Three patients of 12 (25 %) in our series showed proliferative retinopathy. This seems to be a very high rate when compared with literature. However the longstanding course of case 9, and development of blastic transformation in case 6, might be important in the pathogenesis of retinopathy. Case 9, who was also found to be diabetic, needed no additional treatment to lower his glycemia except his diabetic diet. He never experienced any diabetic complications such as nephropathy, neuropathy or microangiopathy. Hence retinopathy is not necessarily a result of diabetes mellitus and may well be due to chronic granulocytic leukemia. On retrospective evaluation, the onset of retinopathy seems to correspond to the time of the development of weakness and easy bruising. Similarly, in a case of erythroleukemia associated with optic disc neovascularization, Sunakouva reported that his case also had diabetes mellitus; however neovascularization appeared in association with leukemia. No difference was detected in the βTG/PF4 ratio in these 3 cases from those of cases without retinopathy. Therefore, a platelet participation in the evolution of proliferative retinopathy seems to lack present support. However, platelet participation cannot totaly be excluded. Most importantly, platelet-derived growth factor (PDGF), also

an α-granule release product, is considered to have a role in the development of atherosclerosis.[24, 25, 26] PDGF may also play a role in the formation of proliferative retinopathy. On the other hand, leucocyte count, leucocyte volume, leucocyte deformability, platelet count and hematocrit are thought to contribute to the formation of vascular stagnation which lead to retinal ischemic damage.[10]

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Correlation of Estrogen Receptors with Prognostic Factors in Endometrial Carcinoma

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Summary

Fourteen cases of primary endometrial carcinoma were assayed for the presence or absence of cytoplasmic estrogen receptors by the immunflorescent technique. The clinical stage, histologic type and grade, myometrial invasion and lymph node involvement were compared to the status of estrogen receptors. No statistical correlation was found between the presence of estrogen receptors and the other recognized parameters in this study.

Key Words: Estrogen receptor, Histologic grade, Myometrial invasion, Endometrial carcinoma, Clinical stage.

Introduction

Endometrial carcinomas are derived from sex steroid target tissue.[1] Several clinicopathologic associations have been made for steroid receptor content of endometrial carcinomas.[2, 3] Receptor content appears to correlate with histological differentiation in that well-differentiated lesions have higher mean levels of receptor"positive"states than do poorly differentiated lesions.[4, 5] Furhermore, receptor levels and status appear to correlate with prognosis of primary endometrial carcinomas and response to hormonal therapy.[5, 6, 7]

The purpose of this study is to evaluate the presence of the cytoplasmic estrogen receptor in primary endometrial cancer and to compare the clinical and histopathological parameters.

Materials and Methods

The study includes 14 patients who underwent radical hysterectomy, bilateral salpingo-oopherectomy, pelvic and paraaortic lymphadenectomy and peritoneal cytology for early primary endometrial carcinoma. Data were obtained from patients' files. The median age was 56 years of age (range 52 to 70). Of these 14 patients, 10 were Stage I and 4 were Stage II according to the FIGO classification system. During the surgical procedure, an excisional biopsy was performed from every patient's tumor. The material was sent to the laboratory and was quickly frozen and stored in liquid nitrogen until assayed.

The tissue was later homogenized in Tris-HCL buffer containing 20 mm. of sodium molybdate and the homogenate was centrifuged at 105.000xg for 60 minutes to yield the cytosol. Radioligand 17-β-estradiol (135 Ci/mmol) was used for cytoplasmic estrogen. Receptor assays were conducted according to a published method.[6] All specimens were pathologically reevaluated and are listed in Table I.

TABLE I

ASSOCIATION OF ER STATUS WITH CLINICOPATHOLOGIC FACTORS IN 14 PATIENTS WITH EARLY ENDOMETRIAL CARCINOMA

	ER positive (n: 4)	ER negative (n: 10)	Total (n: 14)	
Histologic differentiation				
well (grade 1)	2	7	9	x^2: 1.478
moderately (grade 2)	2	2	4	$p > 0.05$
poorly (grade 3)	–	1	1	NS
Histologic category				
adenocarcinoma	4	7	11	p: 0.3296
adenoacanthoma	–	3	3	$p > 0.05$
				NS
Clinical stage				
I	4	6	10	p: 0.2098
II	–	4	4	$p > 0.05$
				NS
Lymphatic involvement				
none	3	9	12	p: 0.505
present	1	1	2	$p > 0.05$
				NS
Myometrial invasion				
none	–	3	3	x^2: 2.73
inner third	3	3	6	$p > 0.05$
middle-outer third	1	4	5	NS

The relationship among cytoplasmic estrogen receptor, stage, grade, lymph node involvement and myometrial invasion of tumor was investigated and analyzed statistically (Fisher test).

Results

Of these 14 patients, 4 had ER and 10 did not have any ER.All of the 4 patients were Stage I disease. Of these 4 patients with positive ER, 2 had grade 1 disease and 2 had grade 2. There was no ER in patients with adenoacanthoma; but 4 patients with adenocarcinomas had positive ER. Of these 4 patients, 3 had superficial myometrial invasion and one had deep myometrial invasion (Table I). One patient with positive ER had lymph node metastasis, but 3 patients with ER did not have any lymph node metastasis.

No statistical correlation was found between the grade, stage, histopathology, myometrial invasion, lymph node involvement of the tumor and presence of estrogen receptor.

Discussion

Documenter associations of receptor content with histologic differentiation, response to therapy and possible prognosis of endometrial carcinoma have been reported.[2, 4, 5]

Recently, Martin *et al*[14] reported that the presence of cytoplasmic estrogen receptors was a significant prognostic factor in determining survival following primary therapy of patients with endometrial carcinoma of all stages.[1, 9]

Presence of estrogen receptors was significantly associated with histologic differentiation, nuclear differentiation, and histologic documentation of extrauterine metastases.[9] Data pooled from many cases in several reports in the literature showed that assays for cytoplasmic estrogen receptor with positive results were frequent in primary cancers of the endometrium (78 %), the frequency was proportional to the degree of differentiation (undifferentiated 35-46 %, moderately differentiated 72-79 %, well-differentiated 82-96 %).[1, 5, 9, 10, 11]

In this study, of these 4 patients with positive ER, two had grade 1 (50 %) and two had grade 2 disease (50 %).

The stage of the disease and the presence or extent of cervical or myometrial involvement, peritoneal cytology were not related significantly to the presence or absence of receptor.But in other studies, myometrial involvement was found to be related negatively to the concentration

of cytoplasmic estrogen or progesteron receptor.[4, 9, 10, 11] In this series, four patients with positive ER had Stage I and all of them had some degree of myometrial invasion.

In a comparison between endometrial adenocarcinomas and normal proliferative endometrium, the cytosolic receptor levels as measured by dextran-coated charcoal (DCC) assay were lower in the cancers.[12, 13] In a few reports, an effort has been made to correlate the presence of receptor concentrations with tumor type and with specific histologic features. Adenosquamous and clear cell carcinomas did not differ from adenocarcinomas with respect to levels of estrogen and progesterone receptors. In one study clear cell and papillary tumors had distinctly low levels.[9, 10, 11] In this study, all patients with positive ER had endometrial adenocarcinomas.

Little data are available on cytoplasmic ER in relation to response of recurrent of advanced endometrial adenocarcinomas to progesterone therapy. Approximately one-third of unselected patients with advanced or recurrent endometrial carcinoma will respond to progestin therapy.[7]

Although most studies confirm that there is a good correlation between estrogen receptor content and most prognostic factors, even prognosis, in endometrial carcinoma,[7, 12, 14] in the present study we did not find this result. There was no correlation between estrogen receptor content and histologic differentiation, histologic type, clinic stage, lymphatic involvement and myometrial invasion.

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Reevaluation of Endometrial Morphologic Criteria in 340 Infertile Women

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Summary

Female infertility and its' diagnostic laboratory test methods are still controversial topics in gynecology and gynecologic pathology. Endometrial sampling, and dating according to their morphological aspects and serum progesterone measurements are the most reliable methods. In this study, endometrial findings of 340 infertile cases were discussed. Primary infertile cases were 289 (85 %), secondary infertile cases were 51 (15 %) in number. The endometrial biopsy method timing of the biopsy and its effects on the diagnosis were pointed out. The major morphologic probability of diagnostic types were discussed.

Key Words : Infertility, luteal phase defect, anovulatuar cyclus, endometrial dating.

Introduction

Infertility is one of the most frequent clinic problems of gynecology. Various laboratory test methods including serum progesterone level, pelvic ultrasonography, basal body temperature, vaginal cytology,

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and endometrial samplings are used. Morphology of the endometrium is one of the most reliable test methods for ovulation.[1-4] Another difficulty in this particular area comes from the luteal phase defect (LPD).[1-4]

LPD is seen in a many cases. Recently, LPD morphologic types and their importance in female infertility were discribed.[1-4] These controversial ideas stimulated study.

Materials and Methods

Retrospectively, endometrial sampling materials of 340 infertile women were reexamined by the light microscope. The women were selected from our gynecology clinic during a 3-year study period. 289 of them had primary and 51 of them had secondary infertility. Biopsy specimens were obtained from the lateral wall of the uterine fundus with a Novak curette in the first 6th hour of menstruation.

Results

340 endometrial biopsy materials which were taken for diagnose of primary and secondary infertility were studied. The age range of patients was 17 - 41 (average 27.21). 85 % of the cases were primary; 15 % of the cases were secondary infertility. We divided our cases into two group: ovulation positive and negative. Beside these two major diagnostic groups, endometritis, desidual reaction and placental tissue were also diagnose in ovulation positive cases. Endometritis, endometrial hyperplasia, endometrial atrophy, and iatrogenic changes were also seen in ovulation negative cases. There was not significant numeric differentiation in the pathologic diagnosis between the primary and secondary infertile cases. The main pathologic diagnosis in the primary and secondary infertile cases are seen in the Table I.

TABLE I

OVULATION POSITIVE AND NEGATIVE CASES, LUTEAL PHASE DEFECT (LPD) INCIDENCES IN PRIMARY AND SECONDARY INFERTILE CASES

Infertility Types			Ovulation (+)		Ovulation (–)		LPD	
	Cases	(%)	Cases	(%)	Cases	(%)	Cases	(%)
Primary	289	(85)	112	(39)	177	(61)	38	(13)
Secondary	51	(15)	24	(47)	24	(47)	5 (	9,8)

LPD was significant in the ovulation positive cases. LPD age related distribution was summarized in the Figure 1.

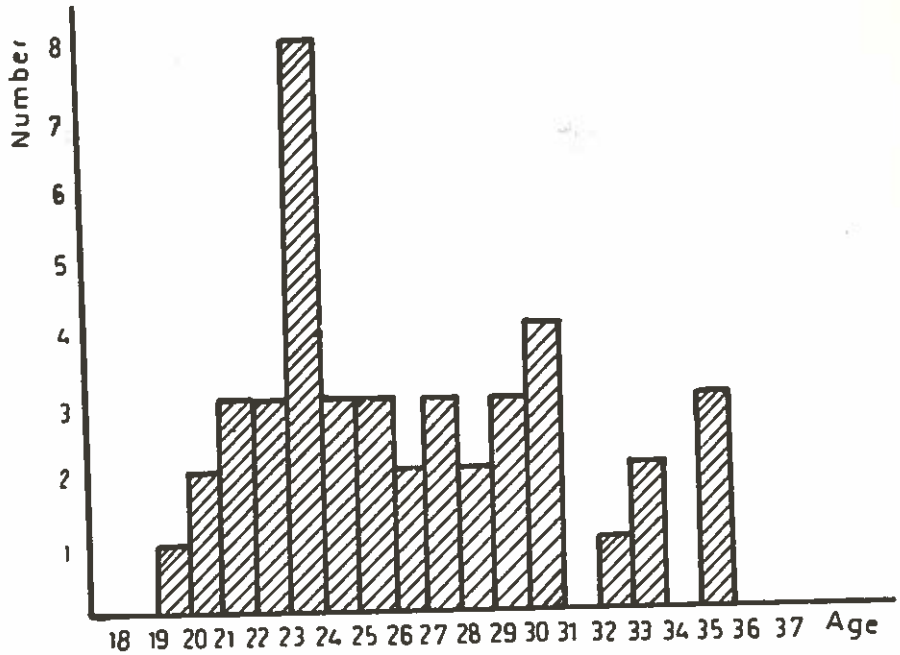

Figure 1
Distribution of lutealphase defects according to ages.

Discussion

The endometrial biopsy serves as a useful and valuable tool in the diagnosis of ovulation. Sampling time is important. 2 to 3 days before the anticipated onset of menses is reccommended.[3] Biopsy specimens should be obtained from the lateral wall of the uterine fundus with a Novak curette.[1] In some articles, two of three endometrial biopsies in separate cycles are proposed.[4] It was also mentioned that the period between the sampling times should be less than 12 months.[4] Anovulation and associated pathologic lesions such as endometritis or endometrial hyperplasia were observed in our cases, just as in the literature.[5, 6, 7] Serum progesterone levels and late luteal endometrial histology should be assessed by evaluation of the luteal phase.[2, 6] Using these two criteria, LPD may accurately diagnosed. LPD was seen in 12 % of our study. In other studies, the percentages ranged from 4 % up to 48 %.[2] LPD includes abnormalities of both progesterone synthesis by the corpus luteum and progesterone alleged rarity of the endometrium.[2] Traditionally, the diagnosis of LPD is obtained by the performance of a well-timed endometrial biopsy.[2, 8] Most investigators agree that an LPD is present when the endometrium is more than two days out of phase in relation to the onset of the next menstrual cycylus.[1]

Presently, two histologic patterns are seen on the endometrial biopsy in LPD.[1,9] 1- Glandular - stromal synchrony type of LPD. 2- Glandular-stromal asynchrony type of LPD.

Progestational therapy, supports for synchron LPD, and clomiphene citrate therapy for asynhcron LPD is advisable.[1] Synchron LPD cases were 3/4 of the LPD cases in our study; this supports the literature.[1]

We conclude: more than one endometrial sampling by a Novak curette, at 2 days before the onset of menses, from the front lateral wall of the uterine corpus, differentiation of asynchron and synchron LPD and evaluation of the serum progesterone levels are all necessary in the pathological evaluation of infertility in women.[1-8]

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Brucellosis

Experience in 86 Patients

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Summary

Eighty-six patients diagnosed as having brucellosis at Hacettepe University Hospitals, between 1978-1986, were reviewed for major causes of admission, important clinical and laboratory features and importance of various diagnostic methods. Fever was the most common complaint on admission. The vast majority of the patients had malaise, weight loss, sweats and chills. Most patients had fever during their acute illness. Hepatomegaly occured in 60 % and splenomegaly in 54 %. On admission, 89 % of the patients had a diagnostic serological response (Standard tube agglutination titers $\geq$ 1: 160). Cultures of bone marrow and blood were positive in 70 % and 62 % of the patients, respectively. Of the last 28 patients from whom both bone marrow and blood cultures were taken, 14 (50 %) had positive blood culture, and 21 (75 %) had positive bone marrow culture ($p < 0.05$). We recommend bone marrow culture for patients in whom brucellosis is suspected.

Key Words: Brucellosis; Bone marrow culture; Blood culture.

Introduction

Brucellosis is a zoonosis endemic to certain areas of the world, caused by microorganism of the genus Brucella. Human infection generally occurs in individuals who are exposed to infected tissues and milk or milk products. There are many more common illnesses that mimic it and sometimes diagnosis is difficult. The definitive evidence of brucella

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infection consists of isolating the etiologic agent. In the absence of a positive culture, the value of specific agglutinin titers has long been recognised.[1] Brucellosis in Turkey is a serious health care problem like in many other developing countries, but there are a few reports dealing with it extensively.[2,3] In this review, 86 patients with brucellosis followed at Hacettepe University Hospitals between 1978-1986 were reviewed. The major causes of admission and importance of various diagnostic methods are emphasized.

Materials and Methods

Patients were selected on the basis of two criteria; a) Brucella agglutination titer $\geq$ 1 : 160 or, b) Positive culture for Brucella melitensis obtained from blood, bone marrow or cerebrospinal fluid. The standard tube test was used for agglutination. Tryptose agar and broth biphasic culture media was used. Forty-four (51.2 %) patients were male and 42 (48.8 %) female, ages ranged from 17 to 69 years (average, 39). Most of the patients were farmers and housewives from rural areas who had a history of direct contact with animals and/or ingesting unpastorized milk products. One patient was a veterinary surgeon and two others were microbiologists working in the microbiology laboratory of the hospital. The duration of symptoms prior to diagnosis varied from one week to one year (mean, 9.7 weeks), less than eight weeks in 34 patients, from eight to fifty-two weeks in 43 patients, more than one year in 2 patients. The difference between two percentages for the dependent and the independent samples were used for statistical analysis.[4]

Results

Fever was the most common complaint at the onset of the disease. The vast majority of the patients had constitutional symptoms. Weight loss was also a major symptom with an average of eight kilograms. In the patients who had arthralgia, the most frequent localisation was knee, and followed by hip, elbow, and wrist. Table I shows the symptoms in the patients.

Fever was intermittent pattern in 68 %, relapsing pattern in 15 % and sustained fever in 17 % of the patients. Sacroiliitis with or without spondylitis was found in 9 patients. There were four patients with neurobrucellosis (two with meningoencephalitis, one with paraparesis, one with spinal epidural abscess). Four patients had cirrhosis of the liver that was diagnosed previously. Nine patients had valvular heart disease along with brucellosis. Seven of them had rheumatic heart disease. In two patients, valvular heart disease (aortic incompetence) was caused by brucellosis. Table II shows the signs in the patients.

TABLE I
SYMPTOMS IN 86 PATIENTS WITH BRUCELLOSIS

Symptom	%
Fever	95
Malaise	90
Weight loss	80
Sweats	75
Chills	72
Anorexia	71
Arthralgia	47
Headache	39
Back pain	38
Musculoskelatal pain	36
Nausea/Vomiting	28
Cough	24
Abdominal pain	20
Jaundice	6
Neurological symptoms (vertigo, tinnitus, disorder of balance)	5

TABLE II
SIGNS IN 86 PATIENTS WITH BRUCELLOSIS

Sign	%
Fever	95
Hepatomegaly	60
Splenomegaly	54
Lymphadenopathy	22
Arthritis	10
Jaundice	6
Orchoepididimitis	2

A sedimentation rate of more than 20 mm in one hour by the Westergren method was present in 51 of 69 patients (74 %). C-reactive protein was positive in 19 of 43 patients (44 %) and latex agglutination was positive in 9 of 19 patients. Table III summarizes the laboratory findings in the patients.

Only 6 of 44 anemic patients had iron deficiency anemia, the reminders assumed to have anemia due to infection. Leukocyte counts in the majority of the patients ranged between 4000 and 10000 per cubic millimeter of blood. Of the 85 patients with symptomatic brucella infection, 76 had serum specimens which produced titers of 160 or higher in the standard tube agglutination test (89 %). Forty of 65 (62 %) patients had positive blood cultures for B. melitensis. Marrow cultures were taken form 37 patients, 26 (70 %) of them revealed B. melitensis. Of the last

28 patients from whom both bone marrow and blood culture were taken, 14 (50 %) had positive blood culture, and 21 (75 %) had positive bone marrow culture ($p < 0.05$). Twenty-eight patients had given various antibiotic combinations including penicillin, streptomycin, lincomycin, chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, clindamycin and antituberculous drugs prior to specific treatment. Twenty of 28 patients had used one or more antibiotics which are known to be effective against Brucella. In the analysis of these patients, we found that prior antibiotic use did not affect the recovery of brucella from blood and bone marrow.

TABLE III
SELECTED LABORATORY FINDINGS IN PATIENTS WITH BRUCELLOSIS

Laboratory Finding	Number	%
Positive agglutinin titers ⩾ 1: 160	* 76/85	89
Positive venous blood culture	40/65	62
Positive bone marrow culture	26/37	70
Anemia +	44/85	52
Leukopenia	15/85	18
Leukocytosis	2/85	2
Lymphopenia	5/85	6
Lymphocytosis	4/85	5
Thrombocytopenia	3/85	4
Elevated SGOT	27/74	36
Elevated SGPT	17/51	33
Elevated alkaline phosphatase	45/72	63
Hyperbilirubinemia	7/49	14
Hyperglobulinemia	30/71	42
Hypoalbuminemia	18/72	25

* The number of patients who have abnormalities/total number of patients.
\+ This means a concentrations of hemoglobin of less than 13 gram per 100 ml of blood in men or less than 12 gram in women.

In general patients received 1 gram of streptomycin / day for three weeks and 2 gram of tetracycline / day for four weeks. In 5 patients trimethoprim-sulfamethoxazole and in 3 patients with endocarditis rifampin were added to this regimen. All patients responded to these combinations. The defervescence period was 5 ± 0.5 days (mean $\pm$ SD).

Discussion

Human brucellosis in Turkey is almost invariably caused by B. melitensis.[2] B. melitensis infection has been described as more invasive, more acute onset and severe course.[5] Fever, chills, malaise, sweats and weight loss were present in the majority of the patients on addmission. The most

common fever pattern was a diurnal intermittent type. The undulating type of fever more frequently seen in infections due to B. melitensis was exist in 15 % of the patients.[6] Sacroiliitis and spondylitis are the most common osteoarticular manifestations in brucellosis.[7] Sacroiliac joint involvement is generally nondestructive, resolves with antibiotic treatment and like two patients in this study radionuclide scans may show areas of increased activity.[8] Since the liver is the largest organ of the reticuloendothelial system, hepatomegaly is more common than splenomegaly as in this study.[9] Impaired liver function tests consisted basically of an increase of alkaline phosphatase and an increased serum transaminase activity are frequently encountered in patients with hepatic involvement, as we found in this study.[10] Anemia and leukopenia are common features in human brucellosis.[11] Although some studies state that relative lymphocytosis is usually present, we found it in a few patients.[9]

The diagnosis of brucellosis is established with certainty by isolating the etiologic agent. In the period of 1960-71 , positive cultures are only found in 17 % of patients in USA and this was largely attributed to the increased use of antibiotics prior to obtaining cultures.[12] Bone marrow cultures are recommended for diagnosing typhoid fever and brucellosis.[13, 14] Gotuzzo *et al*,[14] showed prior antibiotic use affected the recovery of Brucella from blood more than it affected the rate of isolation from bone marrow cultures. We found cultures of bone marrow and blood were positive in 70 % and 62 % of the patients, respectively. Of the last 28 patients from whom both bone marrow and blood cultures were obtained, 21 (75 %) had positive bone marrow and 14 (50 %) had positive blood cultures ($p < 0.05$). In analysis of the patients from whom blood cultures were taken, prior use of antibiotics did not reduce the positivity of blood cultures. Also, prior antibiotic use did not affect the efficacy of bone marrow culture. In one case, blood culture was positive and the bone marrow culture was negative. The reverse was true in 8 patients. Three cases were diagnosed exclusively by bone marrow culture. We recommend bone marrow culture as an adjunct for patients in whom brucellosis is suspected but serological results and blood cultures are negative. In the absence of a positive culture specific agglutinin titers has a great value. On admission, 89 % of our patients had a diagnostic serological response. This could have been higher if the test had been repeated in the fourth week of illness.[12]

Recommended therapeutic approach for brucellosis today is the combination of oral tetracycline plus intramuscular streptomycin[9]. All patients in this study responded to this combination. Two patients had

relapses documented by positive cultures at one week and five months posttherapy. First patient was treated with tetracycline-streptomycin combination and the second patient was treated with ofloxacin 200 mg two times a day for four weeks, successfully. We could not reach a conclusion about a definite relapse rate because of the relatively short period of follow up.

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The Effects of Low Osmolality Contrast Medium (Ultravist R) on Osmotic Diuresis*

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Summary

It is well known that osmotic diuresis resulting from the administration of ionic high osmolality contrast media leads to volume depletion and renal functional impairment. In order to determine the effects of low osmolality nonionic contrast media, renal functions were investigated in 16 normovolemic patients without preexisting hepatic and renal disease before and after angiocardiography with lopromid (Ultravist R), a nonionic, low osmolality contrast medium. We obrserved only a slight decrease in creatinine clearence on the day of angiocardiograpy as compared to the control value (98.62±4.17 ml/min vs 111.06±7.74 ml/min during initial 6-hour period, and 98.43±3.06 ml/min vs 108.31±6.5 ml/min during terminal 17-hour period, $P > 0.05$). There was no significant difference in sodium, potassium and chloride excretion rates on control and angiocardiography days, ($P > 0.05$). Total osmolar excretion rate and residual osmolar excretion rate, which was calculated by subtraction of the molar contribution of electrolytes, urea, and creatinine from the total osmolar excretion rate, were also not different on the control and

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angiography days, (1.04 ± 0.11 mOsm/min vs 0.84 ± 0.08 mOsm/min and 0.64 ± 0.08 mOsm/min vs 0.46 ± 0.09 mOsm/min during initial 6-hour period, and 0.58±0.07 mOsm/min vs 0.44±0.04 mOsm/min and 0.17±0.06 mOsm/min vs 0.12-0.04 mOsm/min during terminal 17 hour period respectively, $P > 0.05$). We conclude that low osmolality, nonionic contrast media do not cause osmotic diuresis, marked volume or electrolyte losses; and we recommend their usage especially in high risk patients with preexisting hepatic, renal, vascular disease, diabetes mellitus and dehydration.

Key Words: Low osmolality contrast media, osmotic diuresis, osmolar clearence, angiocardiography.

Introduction

It has been shown that administration of high osmolality radiographic contrast media may cause various renal complications changing from slight renal functional impairment to acute renal failure.[1-9] These complications may occur more frequently in certain disease states, such as preexisting hepatic, renal, or vascular disease, multiple myeloma, diabetes mellitus and dehydration.[3-5, 10-13] High osmolality contrast agents cause an osmotic diuresis that may precipitate volume losses and hypovolemia, and the decrease in effective plasma volume potentiates nephrotoxicity.[7, 8, 11-19] For this reason prophylactic hydration of patients has been suggested before angiographic procedures.[16-18] However, in patients with diminished cardiac reserve volume load may be quite hazardous. On the other hand the new low osmolality nonionic contrast agents have been proposed to cause less disturbance on coronary blood flow, myocardial contractility and renal functions.[20]

In this study we prospectively assesed the renal functions of 16 patients undergoing angiocardiography with a new low osmolality contrast medium lopromid (Ultravist R).

Materials and Methods

The study group comprised of 16 patients (10 males, 6 females) who underwent angiocardiography at the Department of Cardiology, Hacettepe University, School of Medicine. The mean age was 46 with a range of 32 to 66. The indication for angiocardiography was coronary artery disease in 10 patients, rheumatic valvular hearth disease in 5 patients and mitral valve prolapsus in 1 patient. None of the patients had preexisting hepatic or renal disease and all were normovolemic on the angiography day.

Each patient served as his own control, providing paired comparisons on control and angiography days. On both control and angiography days following an overnight fasting, urine was collected during the first 6-hour period (8.00 a. m. to 2.00 p. m.) and final 17-hour period (2.00 p. m. to 7.00 a. m.) by spontaneous voiding, with venous blood sampling at the beginning and end of each urinary collection period. To minimize inaccuracies of urine colection owing to the lower urinary dead space, and to ensure comparable hydration, 5 % dextrose in 500 cc water was given to all patients at 7.00 a. m. on both control and angiography days. A total of 134 ± 14 cc of contrast was administered. Flushing of cateters resulted in an additional fluid load of 178 ± 34 cc saline for each patient.

Creatinine clearence, sodium, potassium, chloride excretion rates, total osmolar excretion rate, osmolar clearence and free water clearence were calculated from standard formulae on both control and angiography days. The molar contributions of sodium, potassium, chloride urea, and creatinine, measured in the urine by routine analytical methods, were subtracted from total osmolar excretion rate to yield the residual osmolar excretion rate. This was used as an index of contribution of contrast agent to the total osmolar excretion.

Measurements during comparable time periods on control and catheterization days for a given patient were analysed with a two-tailed paired t test.

Results

The investigated renal functional parameters before and after angiocardiography are presented in Table I.

During both initial 6-hour and terminal 17-hour period of the angiography day, creatinine clearence values were slightly depressed compared to the same periods of control day (98.62 ± 4.17 ml/min vs 111.06 ± 7.74 ml/min, and 98.43 ± 3.06 ml/min vs 108.31 ± 6.5 ml/min, $P > 0.05$).

As indicated in Table I, there was no difference between control and angiography days during both initial 6-hour and terminal 17-hour periods, in sodium, potassium, and chloride excretion rates, ($P > 0.05$).

Total osmolar excretion rate and residual osmolar excretion rate values did not show statistically significant difference between control and angiography days, ($P > 0.05$).

Similarly there was no difference in osmolar clearence, urinary flow rate and free water clearence values of control and angiography days, during both initial 6-hour and terminal 17-hour periods, ($P > 0.05$).

TABLE I

THE INVESTIGATED RENAL FUNCTIONAL PARAMETERS BEFORE AND AFTER ANGIOCARDIOGRAPHY

Renal Functions	Initial 6 Hour*		Terminal 17 Hour*	
	Control	Angiography	Control	Angiography
Creatinine Clearance ml/min	111.06± 7.74	98.62± 4.17	108.31±6.5	98.43±3.06
Sodium Excretion Rate µEq/min	80.94±14.58	77.75± 8.12	65.75±5.54	58.13±8.15
Potassium Excretion Rate µEq/min	43.44± 4.68	36.31± 4.95	47.06±5.43	44.06±5.71
Chloride Excretion Rate µEq/min	92.94±15.41	99.38±10.35	74.19±6.0	68.38±8.63
Total Osmolar Excretion Rate mOsm/min	1.04± 0.11	0.84± 0.08	0.58±0.07	0.44±0.04
Residual Osmolar Excretion Rate mOsm/min	0.64± 0.08	0.46± 0.09	0.17±0.06	0.12±0.04
Urinary Flow Rate ml/min	1.02± 0.13	1.12± 0.11	0.84±0.05	0.85±0.09
Osmolar Clearance ml/min	1.36± 0.2	1.18± 0.13	1.35±0.1	1.37±0.25
Free Water Clearance ml/min	-0.28± 0.18	-0.05± 0.11	-0.39±0.16	-0.47±0.18

* $P > 0.05$

Discussion

The nephrotoxicity of ionic high osmolality contrast agents has been studied extensively, and impaired renal perfusion, glomerulotubular injury, obstructive nephropathy have been proposed as probable mechanisms.[20] On the other hand, it has been suggested that, high osmolality contrast media exert a negative inotropic effect on the myocardium and a vasodilatory effect on the periferal vasculature.[22, 23] These may lead a significant fall in systolic blood pressure and renal perfusion. Experimentally it has been shown that high osmolality contrast media infused into the normal kidney produce initially a vasodilatory response due to hyperosmolarity, then a vasoconstriction due to the activation of renin

angiotensine system. This renal vasoconstriction and contrast induced high blood viscosity may also contribute to the decreased flow in the renal microcirculation.[14, 24, 25] Consequently all of these changes may cause a significant decrease in the glomerular filtration rate and associated renal damage.

Low osmolality contrast media have less negative inotropic effect on the myocardium and produce fewer effects on the systemic blood pressure, renal blood flow and blood viscosity.[22, 23] Therefore, the low osmolality contrast media would not be expected to cause a depression in glomerular filtration rate. Although we detected a slight decrease in glomerular filtration rate on angiography day compared to the control value, this was statistically insignificant ($P > 0.05$).

It has been demonstrated by many authors that there is a rise in serum osmolality after the use of high osmolar contrast media and that large numbers of contrast molecules which exsist in the glomerular filtrate cause an osmotic diuresis by decreasing the net reabsorption of water and electrolytes.[15, 16] On the other hand, low osmolality contrast administration would be expected to cause less osmotic diuresis and produce leschanges in water and electrolyte excretion rates. Accordingly, significant changes were not observed in sodium, potassium, and chloride excretion rates between angiography and control days in our study. Also, there was no significant difference in total osmolar excretion rate, residual osmolar excretion rate, osmolar clearence, urine flow and free water clearence values between control angiography days.

In conclusion, our findings indicate that low osmolality contrast media, when used in angiocardiography, do not cause osmatic diuresis, marked volume and electrolyte losses. Therefore prophylactic hydration before angiocardiography is unnecessary. This is particularly important in patients with diminished cardiac reserve, in whom volume load may be dangerous.

Also we may conclude that the new low osmolality contrast media are less likely to be associated with nephrotoxicity and should be considered especially in high risk patients. Their relatively high cost seems to be the only disadvantage.

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Hyporeninemic Hypoaldosteronism Presenting with Hypokalemia

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Summary

A nondiabetic woman with hyporeninemic hypoaldosteronism was presented. She had reduced serum levels of aldosterone in addition to very low plasma renin activity. Contrary to the classic type, our case exhibited hypokalemia, not hyperkalemia.

Key Words : Hyporeninemic hypoaldosteronism, Hypokalemia.

Introduction

Acquired selective aldosterone deficiency in adult patients is a rare clinical entity, in which adrenal production of aldosterone is reduced without associated reduction in adrenal production of cortisol. Aldosterone stimulates H^+ and K^+ secretion in the distal nephron.[1,2] As would be expected from these actions, hyporeninemic hypoaldosteronism is associated with hyperkalemia and a mild hyperchloremic metabolic acidosis.[2-4]

The diagnosis can be made by the demonstration of a low plasma aldosterone concentration as well as low plasma renin activity, neither of which increases after stimulatory maneuvers, such as volume depletion or upright posture.[5] Since the original case reported by Hudson *et al* in 1957, approximately 50 additional cases have been described, but there is no case coursing with hypokalemia (instead of hyperkalemia) except that in an Italian journal and ours.[6,7]

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Case Report

F. Ş., a 55 year-old housewife was admitted to the hospital because of dysuria, nocturia, frequency, fever, nause, and vomiting for 3 months.

Upon physical examination, the blood pressure was 150/110 mmHg, the pulse rate 90 per minute; the thyroid gland was minimally palpable, and the costovertebral angle pain was bilaterally positive. There was no other abnormal finding.

The BUN (blood urea nitrogen) was 16 mg/dl, sodium= 132 mEq, potassium= 3.6 mEq, chloride= 99 mEq per liter of serum. The creatinine was 0.6 mg/dl, SGOT= 44 IU/l, SGPT= 36 IU/l. A urinary culture yielded E. Coli, then tobramycine 5 mg/kg/day was given. On the third day of therapy, the patient felt better and was discharged from the hospital.

She was rehospitalized 3 weeks later, because of fever, chills, nausea, vomiting, and dysuria. The laboratory examinations were: BUN was 5 mg/dl, serum creatinine= 0.8 mg/dl, blood sugar= 98 mg/dl, sodium= 135 mEq, potassium= 2.9 mEq, chloride= 105 mEq per liter of serum. The creatinine clearance was 118 ml/min/1.73 m^2, 24 hour-urinary potassium= 126 mEq, arterial pH= 7.36, HCO^-_3= 18 mEq/l, Pa O_2= 60.1 mmHg, Pa CO_2= 24.1 mmHg.

Table I,II show the results of various renal function tests and biochemical values.

During hospitalization the patient developed an enterobacter septicaemia which originated from the urinary system. She was given amikacin at 15 mg/kg/day.

TABLO I

SOME BIOCHEMICAL VALUES OF SERUM OR BLOOD

Sodium*	130 – 135 mEq/l
Potassium*	2.8 – 2.9 mEq/l
Chloride*	102 – 105 mEq/l
Bicarbonate*	18 – 22 mEq/l
pH of arterial blood	7.36 – 7.56
BUN (Blood urea nitrogen)	5 – 10 mg/dl
Creatinine*	0.6 – 0.8 mg/dl
Magnesium*	1.2 – 1.45 mg/dl
Calcium*	7.5 – 9.5 mg/dl
Phosphorus*	3.3 – 4.4 mg/dl
Blood sugar	98 – 108 mg/dl

* These values were obtained in serum.

TABLO II
RENAL FUNCTION TESTS

Clearance of endogen creatinine	118 ml/min/1.73 m^2
Esbach test (24 hour-proteinuria)	negative
Urine density	1018
Urine osmolality	650 mOsm /kg
Plasma osmolality	296 mOsm /kg
Urine pH	6.7
Tubular reabsorption of phosphorus (TRP)	% 70

	Urinary*	Serum*
Sodium	138 mEq/24 hours	138 mEq/l
Potassium	126 mEq/24 hours	2.9 mEq/l

* These values were obtained simultaneously

After she had recovered from the septicaemia, serum potassium levels were found between 2.4 - 3.1 mEq/l. She was given 1200 mg/day (30.76 mEq/day) K^+ - enteric tablets, but hypokalemia persisted. Having completed hormonal studies at this time, a diagnosis of hyporeninemic hypoaldosteronism with hypokalemia was confirmed (Table III).

TABLO III
SOME OF THE HORMONAL STUDIES

		Normal Range
Plasma renin activity		
supine position	0.16 ng/ml/h	(0.51 – 2.64)
upright position	0.42 ng/ml/h	(0.98 – 4.18)
Plasma aldosterone	28.5 pg/ml	(40 – 310)
Cortisol (at 07 a.m.)	14.9 µg/dl	(5 – 23)
Dehydroepiandrosterone sulphate	890 ng/ml	(80 – 1000)
17 – Hydroxyprogesterone	0.95 nmol/l	(0.12 – 1.5)
17 – Ketosteroids (urinary)	3.73 mg/dl/day	(3 – 10)
Thyroxine	11.20 µg/dl	(4.5 – 12)
Triiodothyronine	1.60 ng/ml	(0.8 – 2)

The patient has been monitorized at regular intervals, but hypokalemia and low levels of both aldosterone and plasma renin activity have continued.

Discussion

In adult patients, the isolated deficiency in aldosterone secretion is a consequence of impaired or absent renal secretion of renin. This syndrome is known as hyporeninemic hypoaldosteronism. Adrenal

glucocorticoid production is normal in this entity.[8] Since aldosterone is required to augment renal excretion of potassium and hydrogen ions, this disorders result in hyperkalemia, sodium wasting, and metabolic acidosis, to which impaired renal ammonia production, in the presence of hyperkalemia, also contributes.[1-4, 9]

Numerous factors are considered for the pathogenesis of hypoaldosteronism (Table IV) :

a) Hyporeninemic hypoaldosteronism (as in our case)

b) Adrenal insufficiency : i)primary hypoaldosteronism (Addison's disease), ii)secondary hypoaldosteronism (hypothalamic-hypophiseal disfunction).

c) Enzyme deficiencies (involving adrenal cortex)

d) Aldosterone resistance (synonims: pseudohypoaldosteronism, aldosterone receptor deficiency) : (i) with normal functions, (ii) with chronic renal failure.

e) K^+ sparing diuretics (triamterene, spiranolactone) : These diuretics inhibit Na^+ reabsorption and K^+ and H^+ secretion at the aldosterone sensitive site in the distal tubule.

TABLO IV
DIFFERANTIAL DIAGNOSIS OF HYPOALDOSTERONISM

	Renin secretion	Aldosterone secretion	Cortisol secretion	Responsiveness to Aldosterone
Hyporeninemic hypoaldosteronism	↓	↓	N	+
Adrenal insufficiency	N ↑	↓	↓	+
Adrenal cortex enzyme deficiency	N ↑	↓	N ↓	+
Aldosterone resistance	N ↑	↑	N	−
K+ sparing duretics	↑	↑	N	∓

N : normal ↑ : increased ↓ : decreased + : positive − : negative

With the only exception of hyporeninemic hypoaldosteronism, as can be seen in the Table IV, renin secretion tends to be normal or increased, because of Na^+ loss in the urine.[10]

Hyperkalemia usually does not occur in patients with renal failure until the late onset of oliguria. However, some patients with mild to moderate renal failure and a normal urine output develop hyperkalemia plus metabolic acidosis; this is probably due to the associated hyporeninemic hypoaldosteronism.[11, 12]

The factors leading to the defect in renin release has been attributed to a deficiency of renin secretion by the kidney.[9] This syndrome may also occur in patients, with normal renal function, as in our patient.

The findings in our patient fulfill the criteria for hyporeninemic hypoaldosteronism; the plasma renin activity and aldosterone levels were decreased and adrenal-thyroid functions were normal. But our patient had hypokalemia instead of hyperkalemia, which is the expected classical finding. To our knowledge, our subject is only the second case, presenting with hypokalemia, reported in the literature. One similar case has been described elsewhere.[7]

The possible causes of hypokalemia in our patient may be : (a) decreased dietary K^+ intake (because of vomiting with septicaemia), (b) vomiting (this may cause hypokalemia-despite the low levels of aldosterone-by two mechanism : first, by external K^+ loss and the second, by increasing urinary K^+ excretion), (c) hypomagnesemia (due to vomiting).[13]

We observed the blood pH between 7,36 - 7.56. This finding probably reflected the sum of the effects of hypoaldosteronism, and vomiting, although one would expect also metabolic acidosis in this syndrome.

In summary, this syndrome is understandable as a hyporeninemic hypoaldosteronism, different from most of the cases in the literature, in that the patient presented with hypokalemia which probably originated from a mild nephropathy, involving the juxtaglomerular apparatus.

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Pleural Melanoma with Subsequent Appearance of the Primary in the Skin

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Summary

A patient with malignant melanoma, who presented to us with pleural effusion, darkening of pleural fluid and urine, and late occurrence of the primary site in the skin, is introduced. His diagnosis was established by needle biopsy of the pleura. It is suggested that metastatic malignant melanoma should always be taken into account in case of dark-colored pleural fluid obtained via thoracentesis.

Key Words: Malignant melanoma, pleural metastasis, tumors with unknown primary.

Introduction

Malignant melanomas are relatively uncommon tumors constituting about 1.5 percent of all cancers.[1, 2] They are well known for their unpredictable and capricious behaviours; spontaneous regressions, though rare, have been reported in the literature.[3] In addition, metastases from melanoma may appear without any trace of its primary lesion; an incidence of 4 % of all malignant melanoma may occur in this way.[4-9]

The current report is rather unique because there are some unusual and interesting events regarding this patient's melanoma:

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a) A primary cutaneous tumor or a precancerous skin lesion was absent in the initial stage.

b) A primary site in the skin occurred several days after the pleural metastasis with effusion was observed.

c) The diagnosis of melanoma was established simply by a percutaneous needle biopsy of the pleura.

d) Both the urine and pleural fluid darkened on standing; this prompted us to search for the possibility of a melanoma.

Case Report

A 51-year old man was admitted with dyspnea and unilateral pleural effusion. He was healthy until 6 months previously, when a nonproductive cough developed. Two months later he became dyspneic, even upon mild exertion, and complained of stabbing pain in the left side of the chest. Fatigue and anorexia were present; and he had considerable weight loss over the last 20 days. There was no history of occupational exposure to chemicals, fever, hemoptysis, diabetes mellitus, coronary insufficiency, tuberculosis or surgery regarding skin or eyes, etc.

On physical examination, the body temparature was 36.7° C, the pulse 110/min., and the respirations 28/min. The blood pressure was 110/60 mmHg. He was in moderate pain and tachypneic with peroral cyanosis. The skin was entirely normal, i.e., there was no pigmentation, nevus, nodule in the skin. No lymph node enlargement was detected except bilateral supraclavicular microlymphadenopathy. The examination of the chest displayed signs of a large left-sided pleural effusion. Pleural friction rub was not heard. The liver was palpable 3 cm below the right costal margin. There was no splenomegaly, clubbing of the fingers, ascites or peripheral edema.

The routine urinalysis was normal. The hemoglobin was 14 g/dl, and the prothrombin time 16 seconds (with a control of 12 seconds). The results of biochemical tests were normal except for a serum level of the albumin of 3.2 g/dl, the globulin 3.6 g/dl, the SGOT (aspartate aminotransferase) 61 IU/l, and the SGPT (alanine aminotransferase) 66 IU/l. The serum LDH (lactic dehydrogenase) was 110 U/l (normal: 88-190 U/l). The PaO_2 was 64 mmHg, the $PaCO_2$ 33 mmHg, and the blood pH 7.53 (These were compatible with hypoxia plus respiratory alkalosis). A tuberculin skin test was negative. The liver scanning showed nonspecific hepatomegaly.

X-ray films of his chest disclosed the left pleural effusion (Figure 1); and the heart was pushed slightly to the right. A left-sided thoracentesis

yielded 950 ml of pleural fluid that soon became darkened when it was left standing in air (Figure 2). The fluid contained 3200 white cells per mm^3 (most of them were lymphocytes, and no erythrocyte was detected); the glucose content was 74 mg/dl, the protein 6.6 g/dl and the LDH 84 U/1. The cytologic examination of the pleural fluid for tumoral cells and bacteria was negative. The bronchoscopy revealed no abnormality. Meanwhile it was incidentally noted that freshly-voided urine, left standing in light and air or mixed with a few drops of $FeCl_3$ turned dark (no hematuria was present). Then, on the 10th day of admission, a repeated pleural aspiration and percutaneous pleural biopsy was carried out simultaneously by use of Abrams' pleural biopsy punch. This revealed malignant melanoma in the pleura (Figure 3). Macroscopically, the color of this specimen was dark brown.

Figure 1
Chest X-ray: Left pleural effusion.

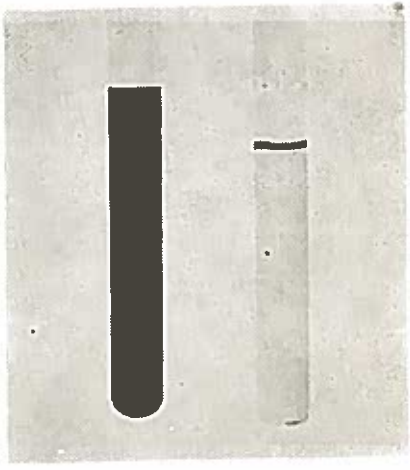

Figure 2
Pleural fluid darkened on standing.

After the diagnosis of melanoma was established, we repeatedly attempted to find the primary focus; but examinations of the skin, nail beds, genital and anal regions, the upper gastrointestinal series, ano-rectoscopy, the "blind" biopsy of the left scalene lymph node were all negative. The eyes and otolaryngologic examination were also normal. On the 22nd day of hospitalization, i.e., 12 days after the diagnosis of pleural melanoma, a minute bluish-black pigmented nodule with a clear-cut margin was detected on the left side of the neck skin (Figure 4), which grew quite rapidly. It was excised and the histology was diagnostic of malignant melanoma (Figure 5). Although a chest tube drainage, intrapleural talc and DTIC (dimethyl triazenoaminoimidazocarboxamide)

200 mg/m²/day for 5 days were applied, the patient died of respiratory failure on the 25th day of admission.

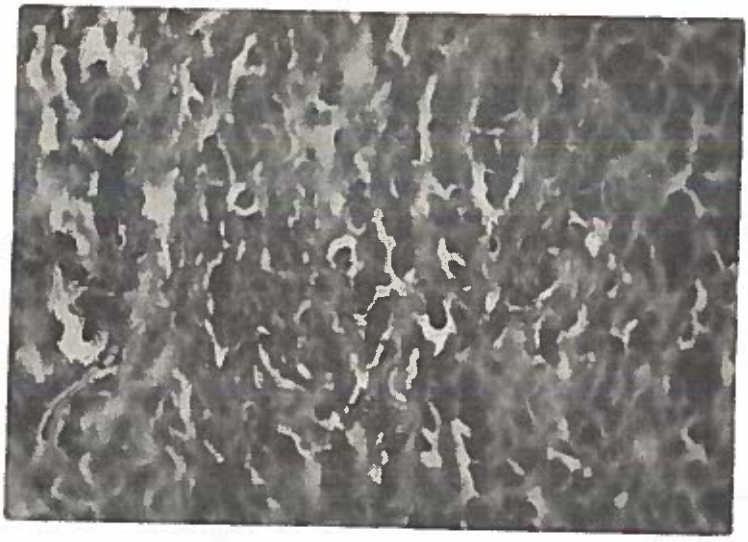

Figure 3
Atypical melanocytes infiltrating the pleura H., E. X 450.

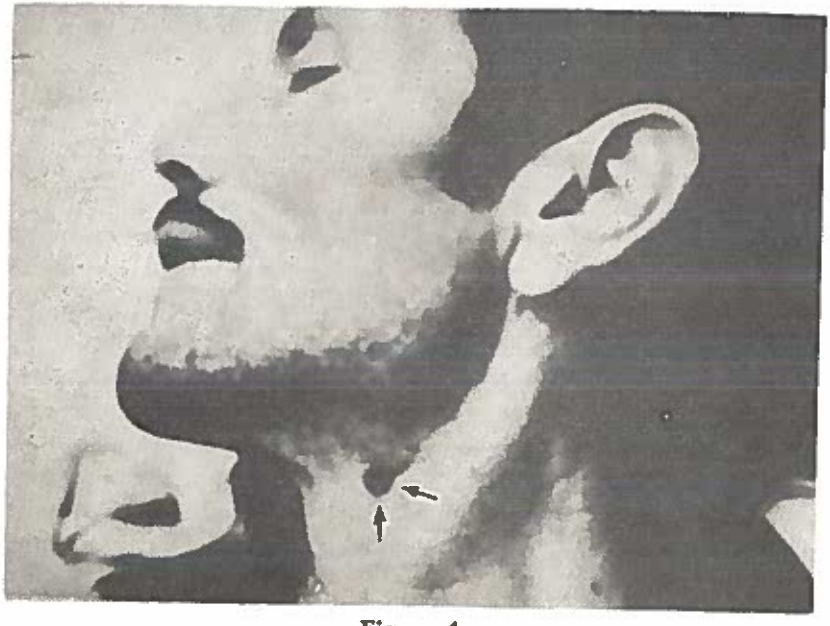

Figure 4
A bluish-black melanoma nodule on the neck skin.

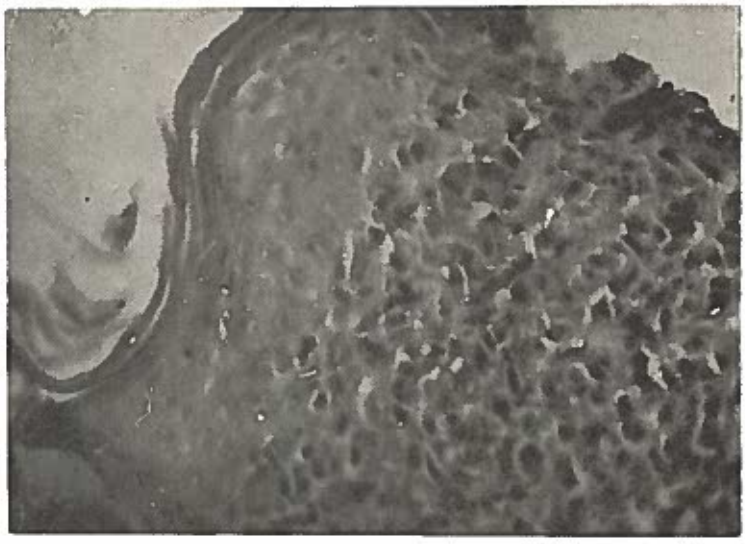

Figure 5
Atypical melanocytes in the skin H., E. X 450.

Discussion

Metastatic melanoma with unknown primary origin has been reported to occur in a small percentage of patients ranging from 2 % to 9 %.[4, 5, 6, 10] Spontaneous resolution of the primary tumor due to immunologic causes is presumably the most aggreable explanation for metastatic melanoma of unknown primary site.[3, 11] But in the current case, although in the initial stages it was accepted as a malignant melanoma with unknown primary, a melanoma nodule subsequently appeared in the skin. Such a situation has never been described in the medical literature. We supposed that in the present case, primary cutaneous melanoma may have existed in a silent intraepidermal, preinvasive form for an indefinite period of time and manifested itself only after pleural metastasis had occurred.

In one report involving patients with metastatic melanomas of the lungs, over a 25-year period, 98 % of cases showed the characteristic pattern of multiple discrete nodules.[12] In another review, the occurrence of a lymphangitic pattern in 7.6 percent and a miliary pattern in 7.6 percent was noted.[13] Pleural effusion, the most prominent sign in our case, is probably a quite unusual form of intrathoracic metastatic malignant melanoma and has not been encountered previously.

Although Poellein *et al*[14], reported 2 cases with metastatic melanoma diagnosed by means of cytologic examination of pleural fluid;

in our case, the definite diagnosis was simply established by a percutaneous needle biopsy of the pleura. Other cases with metastatic malignant melanoma in the thorax, diagnosed by "closed" pleural biopsy (without need of thoracotomy), was not found in a search of the literature from 1974 to 1986.

The urine as well as the pleural fluid of our subject spontaneously darkened upon standing or by adding $FeCl_3$. In reality, in about 10 % of the melanoma patients with widespread metastases, moderate amounts of thyrosine oxidation products formed in the tumor are carried via blood to the kidney and excreted in the urine. In air they become oxidized to melanin, giving the urine a dark color (melanuria).[1, 15] But, to the best of our knowledge, the additional sign of darkening of the *pleural fluid* has not been reported so far. We presume that pigments released by melanoma cells entered into the pleural space directly or via bloodstream. Therefore, in any case where a dark-colored pleural fluid is obtained by thoracentesis, the possibility of a metastatic melanoma should be seriously considered.

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Intestinal Bezoars Causing Obstruction After Gastric Surgery

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Summary

The incidence of phytobezoars is increased after gastric surgery. Due to the disruption of pyloric function, these bezoars can pass into the small intestine and cause obstruction. If unresponsive to conservative measures, the best method of treatment is crushing the bezoar and milking it into the caecum operatively.

Key Words: Gastric surgery, intestinal bezoar, intestinal obstruction.

Introduction

In recent years many entities have been described as new post-gastrectomy syndromes, and one of them is the gastric bezoar observed after gastric surgery.[1] A bezoar is, by definition, a mass of foreign and intrinsic material in the stomach or intestines. Phytobezoars, composed chiefly of plant or vegetable fibers, are the most commonly seen. The incidence of this type of gastric bezoar is increased after gastric surgery. The bezoar formed in the stomach can easily proceed into the small intestine, and cause obstruction there.

In this paper two patients with intestinal bezoars, after gastric surgery, which caused intestinal obstruction are reported. This uncommon clinical entity is rarely diagnosed preoperatively. For the treatment of this type of intestinal obstruction, medical and surgical measures are used. Different forms of surgical treatment are discussed in the article.

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Case Reports

Case 1: A 42-year-old female patient was admitted with complaints of abdominal pain, nausea, and vomiting for three days. She was abstinated for three days. Her past history revealed a truncal vagotomy and Heinecke-Mikulicz pyloroplasty for chronic duodenal ulcer. At physical examination an upper midline laparotomy scar, mild distention, and hyperkinetic bowel sounds were found. Erect plain abdominal X-rays showed air-fluid levels in the small intestine (Figure 1). Intestinal obstruction was thought to be due to postoperative adhesions and a conservative treatment was planned. Nasogastric decompression and i.v. fluids did not help to relieve the obstruction, and at the end of 48 hours laparotomy was performed. An intestinal bezoar obstructing the lumen at 160 cm. distal to the ligament of Treitz was found, and it was crushed with fingers and milked into the caecum. No other bezoars in the stomach or intestines were found. The patient had an uneventful postoperative course.

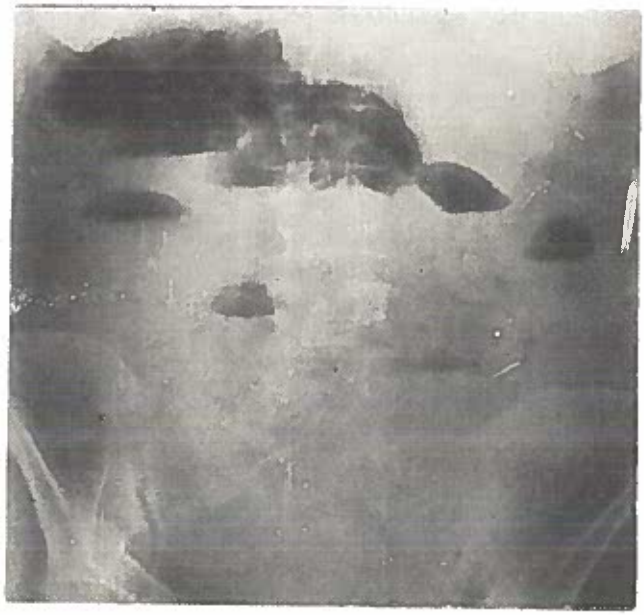

Figure 1
Erect plain abdominal x-Ray of case 1 shownig air fluid levels with distended small bowel loops.

Case 2: A 34-year-old male patient, who had undergone a bilateral truncal vagotomy and Heinecke-Miculicz pyloroplasty for chronic duodenal ulcer three years ago, was admitted with intestinal obstruction

which was thought to be due to postoperative adhesions (Figure 2). Laparotomy was performed after three days when conservative measures failed. An intestinal bezoar obstructing the lumen was found at 80 cm. proximal to the ileocaecal valve. Another bezoar was found in the ileum, and both were crushed with fingers and milked into the caecum. No bezoar was found in the stomach. The postoperative course was uneventful except for a wound infection.

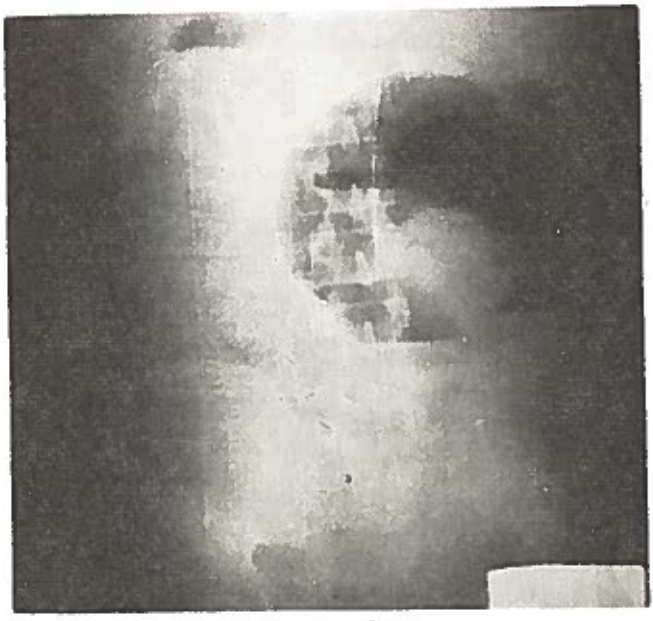

Figure 2
Erect plain abdominal x-Ray of case 2 showing distended loops of small bowel.

Discussion

Gastric bezoars can be formed in normal stomachs but they are more frequently seen after gastric surgery.[1-7] Diminished secretion of hydrochloric acid and pepsin after vagotomy causing decreased peptic and proteolytic activity, and hypoperistalsis have been blamed for the increased incidence of gastric bezoars after gastric surgery.[8] Inadequate mastication of food rich in fiber such as persimmon[4, 5] orange pulp,[7] and many others, further increase the risk.

Gastric bezoars have also been reported in diabetic[2, 9] and hypothyroid[10] patients displaying gastroparesis. Recently, gastric bezoars, after cimetidine therapy, which causes hypoacidity and gastric hypomotility, have been reported.[11]

Gastric bezoars have been reported after all kinds of gastric surgery, including highly selective vagotomy.[3, 4, 12] The loss of normal pyloric function after gastric surgery allows the bezoar formed in the stomach to pass into the small intestine. This may then cause intestinal obstruction. Cases with intestinal obstruction due to bezoars have also been reported in people without any previous history of gastric surgery.[13]

The average interval between gastric surgery and bezoar formation varies between 5 and 7.7 years.[2, 12]

The methods of treatment for bezoars, such as clear liquid diets,[2] nasogastric saline lavage and suction,[7] enzymatic dissolution[14, 15] and endoscopic fragmentation[3] have been successfully employed for gastric bezoars. If intestinal obstruction due to bezoars occurs, the treatment is the same as with other types of intestinal obstruction.

Kraus and Moriel operated on 95 patients with intestinal obstruction due to bezoars who had previously undergone gastric surgery. In 60 of their patients the bezoar was successfully milked into the caecum.[5]

In our patients both of whom had vagotomy and Heinecke-Miculicz pyloroplasty for chronic duodenal ulcer six and three years prior to the last admission consecutively, no specific dietary history was obtained. But in Turkey, a high fiber diet is usually consumed.

In both of our patients, the intestinal obstruction was thought to be due to postoperative adhesions and the correct diagnosis was made at laparotomy. The intestinal obstruction was not relieved with conservative measures and laparotomy was performed in both cases. The bezoars obstructing the intestinal lumen were crushed with fingers and milked into the caecum successfully in both cases. No gastric bezoars were encountered in either of the patients. The postoperative course was uneventful.

The treatment of intestinal bezoars causing obstruction should be the same as for other types of intestinal obstructions. Conservative measures should be tried first. If the obstruction is not relieved conservatively, laparotomy and milking the bezoar into the caecum should be tried. Enterotomy can be performed if this is not possible. Enterotomy may be indicated if the diagnosis is uncertain. Intestinal resection for this benign condition should be reserved only for cases with intestinal ischemia or gangrene. The full length of the intestinal tract should be searched for multiple bezoars at laparotomy.

We are convinced that in patients presenting with intestinal obstruction after gastric surgery, intestinal bezoars should be kept in mind as an

etiological cause; and if unresponsive to conservative measures, laparotomy should be carried out. Crushing the bezoar with the fingers and milking it into the caecum is the best method of treatment . It gives low morbidity and mortality.

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Surgical Treatment of an Extensive Lesion of Lymphangioma Circumscriptum of the Vulva

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Summary

A 52 year-old postmenopausal female patient applied to our clinic complaining of inability to have coitus due to a lesion in her vulva. It was found that, despite various modes of therapy given become to the patient who had had the same complaint for ten years, the lesion had enlarged. The clinical appearance suggested the presence of a case of lymphangioma circumscriptum. This diagnosis was confirmed by biopsy studies. Total vulvectomy was performed with the removal of the deep subcutaneous fat tissue. No recurrence was noted over a follow-up period of a year and a half. The present study aims at demonstrating that extensive surgical treatment might be successful in cases where this lesion which is encountered quite infrequently occurs in the vulva in an extensive fashion.

Key Words : Lymphangioma circumscriptum, vulva, surgical treatment.

Introduction

Lymphangiomas are cases of malformation rather than true neoplasms.[1,2] They are seen quiet rarely. They are mostly of congenital nature occurring immediately after birth or in the first year of life.[1,2] They

may develop very rarely. They are of three types, lymphangioma circumscriptum being the most infrequent one. [1,2] The term lymphangioma circumscriptum was first used by Morris in 1889.[3] The lesion manifests itself in the subepidermal vesicles.[1,2] There are lymphatic cisterns deep set in the subcutaneous fat tissue. These cisterns are developmental anomalies and are not connected to the deep lymphatic system.[1] However, they are connected to the dermal lymphatics along dilated lymphatic vessels in cases of lymphangioma circumscriptum.[1] The vesicles forming under the skin are saccular dilatation of superficial lymphatics.[1] They are translucent, pale and 3-4mm in diameter.[1,2] When they bust open a mucoid fluid comes out. This fluid contains proteinaceous material and lymphatic fluid.[1,2] Infection and increment in the size of the lesion are frequently seen.[1,2] Lymphangioma circumscriptum has been reported to occur most frequently in the upper trunk, the neck, the axilla and the tongue.[1,2] Varying modes of therapy have been performed in cases of lymphangioma. In local cases, cryotherapy and cauterization have been reported to prove effective.[2] In local and diffuse cases radiotherapy has not only proven ineffective, but irradiation has given rise to some side effects.[1,2] Surgical inter vention has been attempted, yet recurrences were frequent.[1,2] The process has been found to recur at sites of primary suture and in the graft in cases where the defect was grafted.[3] It was Whimster[4] who first suggested that recurrence would be the rule unless the deep cisterns are totaly removed.

In 1977, Jordan *et al*[5] observed, in a case of lymphangioma circumscriptum of the hip, that the lesion did not recur after the total removal of the skin and the subadjacent subcutaneous fat tissue.

A case of vulvar lymphangioma circumscriptum with such extensive spread as to prevent coitus is unique in the literature. Thus, in the outset, the authors were unable to predict the extent to which they might be successful. Furthermore, occurence of the lesion at such an advanced age was an additional feature of the case.

Case Report

A postmenopausal female patient 52 years old, applied to our clinic complaining of a lesion in the vulva. She stated that, due to this lesion, she was so uncomfortable as to be unable to wear underclothes and have coitus. It was found that these complaints had been present for ten years. Previously, when the lesion was in a local state, it was cauterized and various therapies were given in an effort to prevent superinfection. Yet, it was found that the lesion had progressed to spread to the entire vulva. On clinical examination, large groups of flat papillomas and infected

pseudovesicles (0.3-0.5 mm in diameter) were observed in the vulvar skin. The skin was thickened and deformed (Figure 1). Biopsy was obtained following antibiotic therapy for superinfection. Pathological examination of the biopsy revealed that the epidermis had become thinner at some places with the papillae having become elongated and acanthosis in some areas. There were sections of enlarged lymphatic vessels immediately underneath the epithelium. These were surrounded by the endothelium and contained proteinaceous fluid and lymphocytes in their lumina. A diagnosis of lymphangioma circumscriptum was established. Total vulvectomy was carried out on August 12, 1985, consisting of the removal of the deep subadjacent subcutaneous tissue (Figure 2). No postoperative complications occurred. Pathological examination confirmed a possible clinical diagnosis of lymphangioma circumscriptum elephantiasis. The microscopic appearance of the lesion is shown in Figures 3, 4.

The patient was followed up for more than a year and a half. No recurrence was recorded. The patient felt extremely comfortable upon the elimination of the lesions. The patient and her husband stated that they had regained the ability to have coitus (Figure 5).

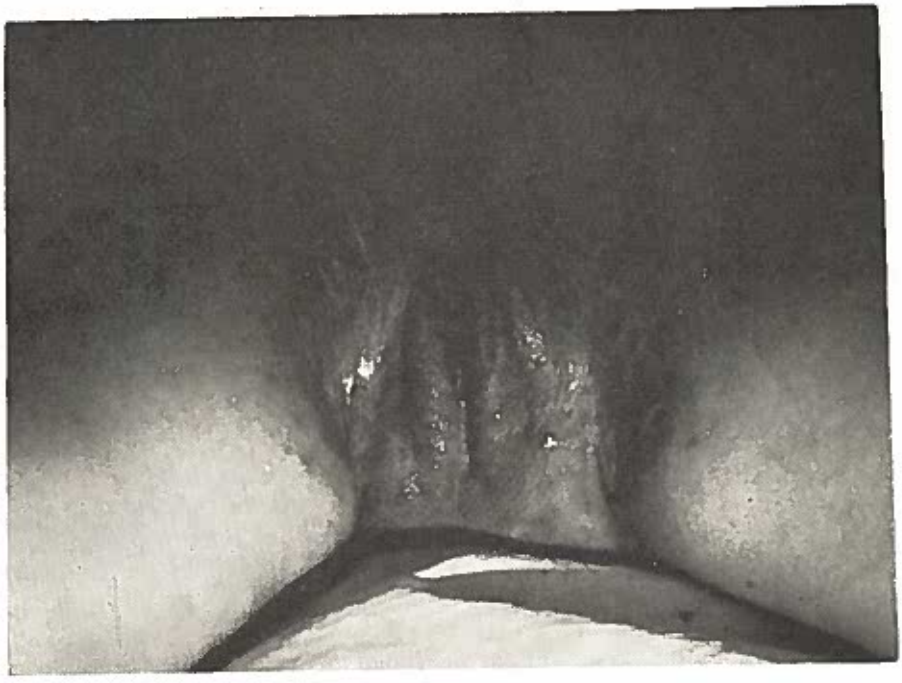

Figure 1
Preoperative appearance of the case of vulvar lymphangioma circumscriptum.

Figure 2
Intraoperative appearance. Total vulvectomy was carried out, consisting of the removel of the deep subadjacent subcutaneous tissue.

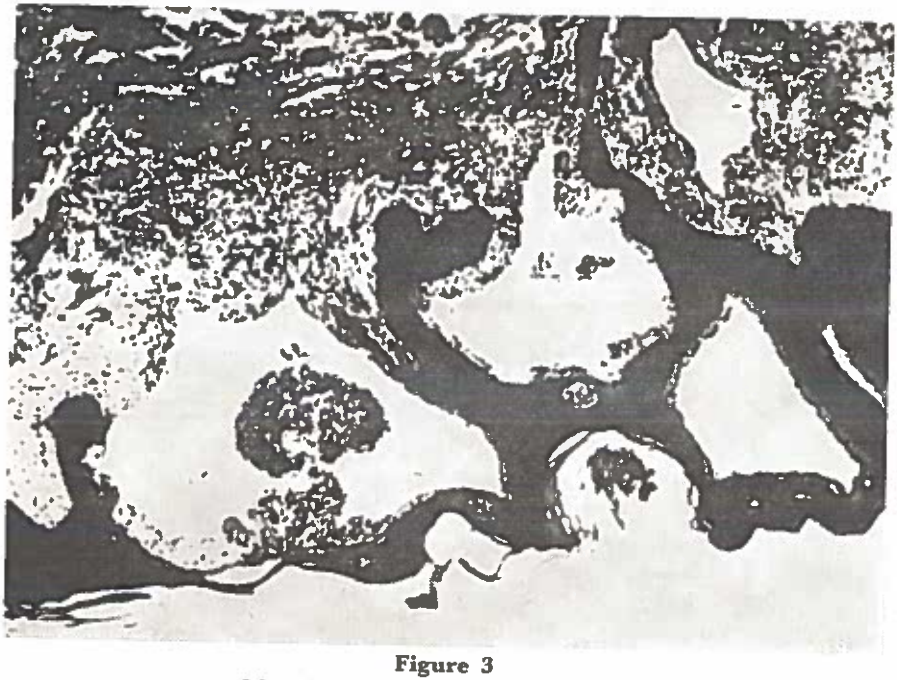

Figure 3
Microscopic appearance of the lesion.

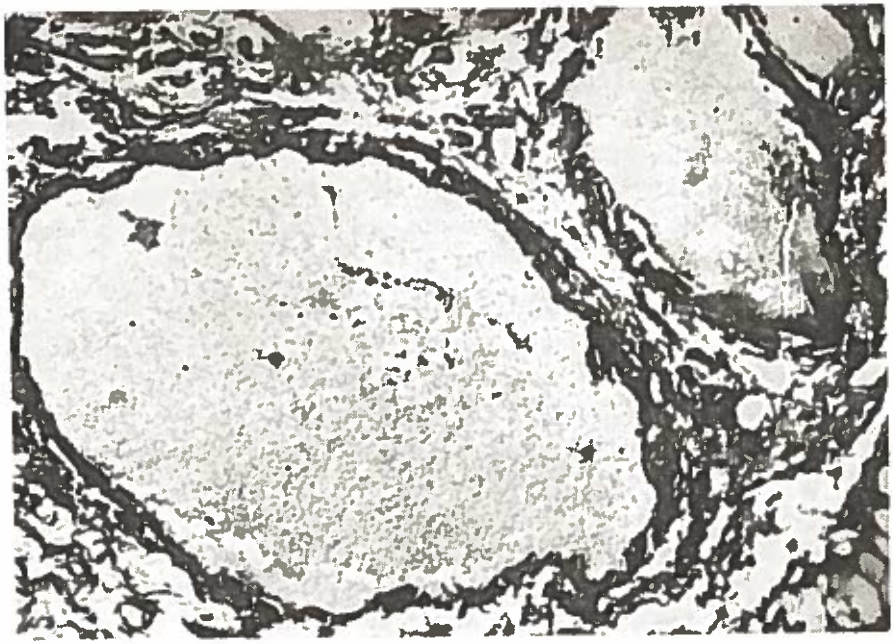

Figure 4
Microscopic appearance of the lesion.

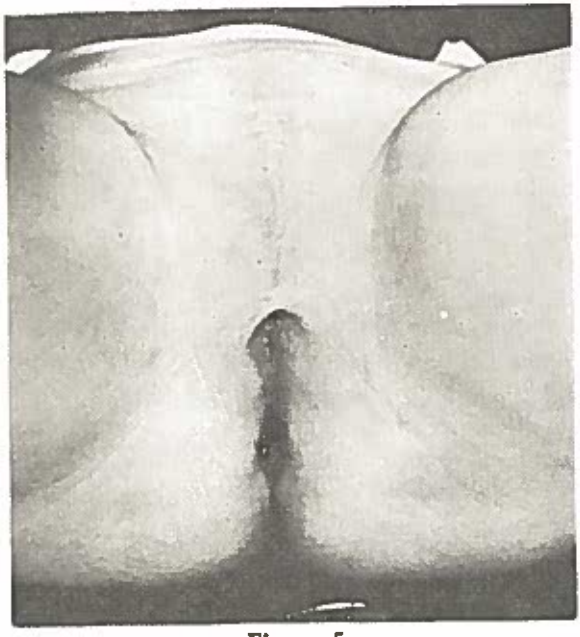

Figure 5
Appearance 18 months postoperatively.

Discussion

Lymphangioma circumscriptum is a rarely encountered disease and is usually considered to be of congenital origin.[1,2] It is seen at birth and within the first postnatal year.[1,2] A very small number of cases have been reported in the adult age group.[1,2] In the present case, the quite advanced age of the patient and hindrance of coitus due to pain are striking features. Inability to have coitus constituted a great problem for the family.

Although two cases of vulvar lymphangioma circumscriptum with small lesion have been reported in the literature, the authors have been found no cases of vulvar lymphangioma circumscriptum with such wide spread.[5,6] Therefore, the authors were not able to initially predict to what extent therapy would prove effective. These lesions are insensitive to irradiation, and surgery was the only treatment of choice. Our treatment was based on the previous studies by Whimster[4] and Jordan[3] which described cases of lymphangioma circumscriptum occuring in other parts of the body. We performed total vulvectomy with the removal of deep subadjacent subcutaneous fat tissue containing the cisterns.

Recurrence was not observed over a follow up period of 18 months. There was no keloid formation. Restoration of the patient's comfort and regaining of the possibility of comfortable coitus seem to be favorable results of the present study.

In conclusion, the authors are of the opinion that, if total vulvectomy is indicated in cases of vulvar lymphangioma circumscriptum, the total removal of the subadjacent subcutaneous fat tissue as far as the fascia is recommendable in an attempt to prevent recurrence.

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In-Vitro Fertilization and Embryo Transfer

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The birth of "test-tube babies" makes headline news and has brought new hope to many infertile couples. In this article, the authors briefly relate the history of *in vitro* fertilization and embryo transfer, than describe its current potential use for future research.

To date, over 4000 births have been reported in several countries following the successful results of *in vitro* fertilization of human eggs and subsequent embryo transfer. Although this technique was originally developed to treat infertility caused by tubal pathology, it is now more widely used in the treatment of other forms of infertility, such as poor male sperm quality, cervical hostility, endometriosis, unresponsiveness to conventional therapy, and unexplained infertility.

In addition to the clinical application of *in vitro* fertilization and embryo transfer (IVF and ET), the technique may be used for research into the causes of certain types of infertility. This fundamental research will increase our knowledge of factors involved in oocyte maturation, fertilization and early embryonic development. With this technique we can also evaluate the effectiveness of some contraceptive agents before clinical trials are undertaken.

In the past decade, there have been remarkable achievements in human IVF and ET. However, the fundemental research began nearly two centuries ago. It is thus appropriate to acknowledge some of these novel discoveries.

Development of IVF

Cruickhank (1797) was probably the first to recover the mammalian embryo at the pre-implantation stage from the rabbit oviduct.[1] In 1827,

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Baer recovered oocytes from graafian follicles of the dog; and in 1897, Heape, in Cambridge, successfully recovered a pre-implantation stage embryo of a rabbit by flushing the oviduct, and then transferred it to a foster mother.[2,3] Later, in 1924, Estes described ovarian implantation into the uterine cavity.[4] As a result of this procedure ovarian function have been preserved and human pregnancies have been reported.

In 1939, Pincus and Saunders recovered unfertilized oocytes from fallopian tubes.[5] They reported that human oocytes should achieve meiotic maturation *in vitro*, and in 1944 Rock and Menkin reported fertilization and cleavage of the human oocyte *in vitro*.[6] Hammond, in 1949, found a complex medium that could facilitate mouse blastocyte development *in vitro*.[7] In 1958 McLaren and Biggers showed that mouse embryos could be cultured from eigth cells to blastocyte, and transferred to the uterus of a foster mother, producing normal offspring.[8]

Proof of successful fertilization

Although there had been several attemps at *in vitro* fertilization, the criteria used to prove fertilazation were unconvicing. By now it had become evident that the development of an individual may occur in the absence of fertilization by a spermatozoon-a condition known as parthenogenesis. Therefore, cell division *in-vitro* did not necessarily mean successful fertilization. A novel experiment by Chang in 1959 was the first convincing proof that fertilization *in-vitro* can occur.[9] He incubated the oocyte with sperm from males with specific genetic traits which were not present in the female donating the ova. The presence of the male traits in the offspring confirmed that geneticinformation was conveyed to the ova.

Human fertilization

In 1969, Edwards, Bavister and Steptoe successfully matured human oocytes *in vitro* with subsequent fertilization.[10] Fertilization and cleavage of human oocytes was reported by Edwards, Steptoe and Purdy in 1970.[11] In 1976, a group in Australia reported successful *in vitro* fertilization of human oocytes.[12] Also in 1976, a remarkable finding was made by Yanagimachi and Rogers in Hawai.[13] They reported the use of a zona-free animal ova as a test system for the assessment of fertilizing capacity of human spermatozoa. In the same year, Steptoe and Edwards reported re-implantation of a human embryo with subsequent tubal pregnancy;[14] and in 1978 they announced the first live birth, Louise Brown, after re-implantation of a human embryo.[15] In October 1978, the Washington Post reported the birth of a second "test tube" baby in India.[16]

In 1978, Rudak, Jacobs and Yanagimachi reported direct analysis of the chromosome constitution of human spermatozoa following successful fertilization with zona-free hamster oocytes.[17] This technique allowed us, for the first time, to study the genetic make-up of human spermatozoa.

At this stage, it was believed that *in-vitro* fertilization and subsequent birth could be achieved only in natural cycles; however in 1981, Trounson and colleagues reported human pregnancies by fertilization *in vitro* and embryo transfer in controlled ovulatory cycles.[18]

Human embryo transfer has been performed at different stages of cleavage, and pregnancy has been achieved by transferring embryos as early as in the pro-nuclei developmental stage. However, in 1982, Craft *et al.* reported human pregnancies following oocyte and sperm transfer into the uterus.[19] Cryopreservation of human embryos was successfully achieved and nearly 50 % of the embryos survived following this procedure.[20] Most recently, a pregnancy was reported in a patient with primary ovarian failure, after IVF and embryo donation.[21]

Monitoring of follicular development and timing of ovulation

The success of IVF depends on the quality of pre-ovulatory oocytes. The oocytes recovered just before ovulation have potential for fertilization and normal cleavage. Oocyte maturation and changes in the pre-ovulatory follicle are completed following the surge of luteinizing hormone (LH) just before ovulation. In order to recover mature pre-ovulatory oocytes, it is essential to monitor follicular development and determine the onset of LH surge.

Follicular development

Follicular development can be monitored by noting changes in the cervix and cervical mucus, estimation of estrogens in plasma and urine and by ultrasonic visualization of the pre-ovulatory follicle.[22, 26] However, there are individual variations in response of the cervical mucus to estrogen. The measurement of either plasma or urinary estrogen can accurately reflect the development of pre-ovulatory follicles. Similarly, in both spontaneous and clomiphene citrate induced cycles there is a wide variation in pre-ovulatory estrogen peak.

Using ultrasonic visualization of the ovulatory follicle, it is possible to assess the site, number and size of pre-ovulatory follicles.[22-26] The mean diameter of pre-ovulatory follicle in 18 sponteneously ovulating women was 21.3 mm (range 17 to 23.5 mm). Because of this wide range in the size of follicles at the time of ovulation, it is not possible to determine

accurately the time of ovulation by scan alone.[25, 26] The potential usefulness of the scan lies in the determination of the number and site of developing follicles. Thus, the combination of urinary or plasma estrogen determination and ultrasound will provide adequate information with respect to the timing of human chorionic gonadotropin(HCG)administration in induced cycles, so preventing both over-stimulation and multiple ovulation. In patients with only one accessible ovary for oocyte recovery, the scan will provide information regarding the site of developing follicle. The scan can also be used before oocyte recovery in order to exclude recent ovulation, thus preventing unnecessary operations.

Timing the ovulatory surge of LH

The most critical event for the pre-ovulatory follicle is the onset of the ovulatory surge of LH. Ovulation occurs between 32 and 40 hours after the start of the LH surge in most women.[27] The mature pre-ovulatory oocytes can be recovered just before ovulation. Oocytes recovered in the earlier stages (immature eggs) have little potential for fertilization and normal cleavage.

As a result of LH surge, a progressive rise in plasma progesterone can be seen until the transitional fall progesterone level, between 32 and 40 hours after the start of the LH surge, at the time of ovulation.[28] There is also a substantial increase in the levels of prolactin beginning at the start of and lasting for the duration of the pre-ovulatory LH surge.[29,30]

In most women the LH surge starts in the early hours of the morning.[29] As ovulation occurs between 32 and 40 hours after the start of the LH surge, it seem that most women ovulate in afternoon and early hours of the evening. Thus, the mature pre-ovulatory oocytes can be recovered during the working day in most women.

It is not clear why the LH surge should begin at night, but it is probably caused by sleep-related alteration in hypothalamic function. It is known that during puberty there is an increase in the LH secretion during the night, associated by sleep.[29, 30]

Artificially induced cycles

Several problems arise from using the natural cycles. First, a single follicular development occurs in most cases and therefore only one oocyte can be recovered. Second, there is little warning in relation to the timing of oocyte recovery and so the operation may be performed at an inconvenient time. To overcome these problems the cycle can be induced.

Follicular development can be induced either by clomiphene citrate,

human menopausal gonadotropin, or a combination of both. These regimes facilitate multiple follicular development. The follicular development is monitored by serial measurements of plasma or urinary estrogens and ultrasonic visualization of pre-ovulatory follicles. When follicular development is complated, HCG is administered and oocytes can be recovered 35 to 37 hours later.

A major problem with induced cycles is judging the timing of HCG administration. The optimum time for this is when follicular development is completed and just before the endogenous LH surge. If HCG is given in the early stage of follicular development, it is likely that immature oocytes will be recovered. On the other hand, if HCG is given after the endogenous LH surge, ovulation may occur earlier than expected. Therefore, in induced cycles, maximum success can be obtained by monitoring follicular development by using a combination of estrogen measurements and ultrasonic visualizations. At the same time, it is essential to determine the LH level before HCG administration.

Oocyte aspiration

In 1970, Steptoe and Edwards reported laporoscopic recovery of pre-ovulatory oocytes after priming the ovaries with gonadotropins.[31] It is essential that an ovary containing a pre-ovulatory oocyte is accessible, and therefore it is customary to perform a laparoscopy first in order to determine the accessibility of the ovary for the laparoscopic aspiration. In patients with one accessible ovary, ultrasonic visualization is used before surgery to determine the site of the developing follicle, thus avoiding unnecessary surgery.

The more recently described method of ultrasonographically guided follicle aspiration is increasing in popularity and is currently being used in a growing number of centres. Lenz and Lauristen first advocated the use of an ultrasonically guided needle for aspiration of the pre-ovulatory follicle instead of laparoscopy.[32] This procedure is performed under local anaesthesia and is easy to perfom. It also allows for oocyte retrieval in patients with dense pelvic adhesions, rendering laparoscopy impossible. The ultrasonographically guided approach can be employed either transabdominally or transvaginally.[33]

In the future, ultrasonically guided aspiration may replace laparoscopic oocyte recovery, providing new hope for those patients whose ovaries are inaccessible laparoscopically. However, further evaluation of this technique is required before it can be performed routinely.

Fertilization in-vitro

The pre-ovulatory oocyte is surrounded by a mass of cumulus cells. This can be identified even by the naked eye. At present, the only criterion for the assessement of the stages of maturity of the oocyte is by the presence of cumulus cells around the pre-ovulatory egg.

The recovered oocyte is incubated in culture media at 37°C for 4 to 6 hours before insemination. This pre-incubation stage assists the final maturation of oocyte.[34] A fertilization rate of 80 to 90 % is a generally accepted figure with mature pre-ovulatory oocytes, providing there is no pathological disorder in the spermatozoa.

Several combinatione of culture media can be used and each is supplemented with serum albumin or homologous human serum. The oocytes are fertilized within a few hours, and normally within 28 to 35 hour of insemination the embryos reach a two-to four-cell stage of development and the eight-cell stage by 54 to 70 hour.[35]

Although it is important to know that the embryo contains the normal chromosome complement, it is not possible to ascertain this in the embryo intended for transfer to the uterus. Thus, the normality of the embryo can be judged only by the following parameters: (a) the dividing cells should be equal in size, they must occupy most of the space within the zona pellucida, and they also should be uniform in shape; and (b) cell division should be progressive and the cleavage rate must be within the normal range, as described by Edwards *et al.*[36]

Embryo transfer[37]

The embryo is transferred transcervically in almost all cases. Implatation can occur after replacing the embryo at almost any stage from pro-nuclei development to blastocyst. Recently, Craft *et al* showed that human pregnancy can occur even after oocyte and sperm transfer into the uterus.[19] The oocyte was incubated with sperm for only one hour before intrauterine transfer.

Success of in vitro fertilization

Even with improved tehniques of *in-vitro* fertilization, one cannot ignore the process of embryonic loss that normally occurs in the genital tract. Robinson, in 1921, suggested that embryonic loss is so widespread in mammals that it should be accepted as a normal process.[38] Leridon constructed a life table for intrauterine mortality throughout pregnancy, in which 69 % of ova exposed to spermatozoa were lost by the time of expected birth.[38] The major factor in embryonic death is chromosomal inbalance arising during maturation of oocytes, spermatozoa and fertilization.

Besides natural embryonic deaths, one would expect an increased incidence of embryonic loss in IVF and ET. First, the quality of sperm reaching and fertilizing the ovum *in-vitro* may be different from those reaching the ovum in the fallopian tube. Thus, fertilization with abnormal sperm may occur. Second, in normal conditions the fertilization of the oocyte by more than one sperm is prevented. This mechanism may break down in IVF system, thus resulting in a higher incidence of polypoid embryos.[39, 40] Third, super-ovulation itself may be associated with an increased incidence of chromosomal abnormality. Finally, there can be asynchorny between embryonic development and the uterine environment, thus the embryo may be transferred into the uterus at inappropriate time.[41]

The prelimanary report from the National Australian Register of IVF pregnancies shows the following outcome: single live births 34.6 %, multiple live births 12.7 %, biochemical pregnancies 21.8 %, ectopic pregnancies 5.4 %, abortions 23.6 %.[42]

The success of IVF and ET is closely related to the number of embryos transferred. Craft *et al* reported 6.5 % clinical pregnancy when one embryo was transferred. This figure increased to 26 % when two or more embryos were transferred.[19]

Currently the success of IVF ranges from 10-25 % per laparoscopy in well established units.[43] The average number of treatments persued by couples are at least two, but possibly 3-4. The average number of laparoscopies for 202 couples followed over a range of 18 to 30 months from 1982 to 1984 was 2.2.[44]

The chance of a multiple pregnancy also increased with the number of embryos transferred, but the risk of twins (20 %) was far out weighed by the relatively poor result transferring a single embryo. The early experience of freeze-thawing of embryos and donor oocyte ET is encouraging.[45]

Research aspects of in-vitro fertilization

IVF and ET techniques have potential application for many areas of research.[46] Some of these fundemental research applications are: expansion of knowledge about contraception, the diagnosis and treatment of male and female infertilty, factors regulating oocyte maturation, a better understanding of cancer, and even a knowledge of evolutionary origins.

Conclusion

IVF and ET have proved to be relatively safe and reproducible in the hands of experts. The technique has become simpler and is accepted by most communities. Recently, in August 1987, IVF and ET procedure was accepted by Turkish *law*.

One may expect an increase in abnormality following IVF. However, because of the efficiency of the female reproductive tract in rejecting abnormal embryos, the chances of delivering an abnormal child are probably close to those of natural conception.[46] This comment may be rather premature, as most of the babies born following IVF were treated in highly specialized centers. It is rather bothersome to see that the practice of IVF is now being widely engaged in by specialized groups, even as an office procedure in some countries.

Simplification of the technique make IVF and ET attractive. Therefore a few pregnancies may be achieved in the hands of inexperienced personal with minimal facilities. However, a high success rate is dependent upon the quality of oocytes recovered, and this can be achieved only when follicular development is precisely monitored. Patients which are referred for IVF have almost all suffered years of disappointment and have undergone several surgical procedures. These couples will go to any length to achieve pregnancy, and therefore, should be treated in centers which have the greatest expertise and experience in the field of endocrinology and cytogenetics.

There is serious concern about IVF and ET. The initial fear was to produce abnormal babies following this technique. Now, there is concern about the freezing of human embryos, the donation of human eggs and, especially research on the early embryo. This research will advance our knowledge of human reproduction and the treatment of genetic disorders, but it should be performed in recognized institutions under the supervision of central bodies. Similarly, IVF and ET should be performed in a few accredited and recognized centers rather than as an office procedure, thus preventing disappointing results and inappropriate experiments.

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Determination of Aminoglycoside Modification Enzymes by "MIC-ratio" Method and its Comparison with Radioactive Assay

Semra Kocabıyık* / N. Gürdal Alaeddinoğlu*

Summary

One hundred and twenty-five gentamicin resistant Gram-negative bacilli, isolated from clinical material were tested for their sensitivities (MICs) to gentamicin, tobramycin, amikacin, netilmicin and two ethyl derivatives of netilmicin (2'-netilmicin and 6'-netilmicin). The susceptibility profiles thus obtained were used to classify the isolates into groups (Aminoglycoside Resistance Patterns) that corresponded to known enzymatic inactivation mechanisms. To set up a biochemical basis for the MIC ratio method, enzymatic activities conferred by the respective resistance patterns were assayed by Phosphocellulose Paper Binding Assay in cell-free sonicates of ten representative strains exhibiting various aminoglycoside modification patterns. The justification made upon enzyme measurments was that MIC ratio method could equally be used for epidemiological purposes as actual identification of the enzymes.

Key Words: Aminoglycoside Modifying Enzymes, Adenylyltransferase, Phosphotransferase, Acetyltransferase.

Introduction

In clinical situations the aminoglycoside resistance in bacteria is usually enzyme mediated.[1-4] It has been well documented that many of the structural genes for these enzymes are carried on plasmids[5-7] and

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transposable elements,[8-12] and this in turn, has led to their widespread dissemination.

In an epidemiological study, identification of a particular enzyme may be of great help in choosing an alternative chemotherapy.

Aminoglycoside modifying enzymes may be identified: (i) by their spectrum of activity against various aminoglycoside substrates, as determined by the incorporation of radiolabelled cofactors (Phosphocellulose Paper Binding Assay, PPBA);[13, 14] (ii) by the ratio of the MICs of various aminoglycosides for the organism which produces the enzyme,[15] or (iii) by identification of the modified product involving analytical techniques, such as mass spectrometry and nuclear magnetic resonance[16] or high pressure liquid chromatography.[17] Recently DNA hybridization methods have been described to record specific resistance determinants throughout large populations of clinical microorganisms.[18-20]

MIC ratio method developed by Miller *et al.*[15] is based on observed expression of the enzyme present and has proved itself to be a rapid screening procedure for large numbers of clinical isolates for the resistance determinants. Classification of the clinical strains in that way may be equally useful for epidemiological purposes as actual identification of the enzyme. The method however, does not utilize direct measurment of the enzymes. The present study aims to evaluate the survey method on a biochemical basis by use of PPBA. This would also allow us to compare two methods considering the factors such as reliability, practibility and cost.

Materials and Methods

1) Bacterial Strains: Strains were isolated from clinical specimens submitted to Hacettepe University Hospital, Microbiology Laboratory from June 1984 to November 1984. The bacteria were identified to be resistant to gentamicin by conventional disk diffusion test, and were later confirmed by routine tube dilution technique in our laboratory. Resistance to gentamicin was defined by a MIC of $\geqslant$ 8 mg/l.

In control procedures to ensure precision and accuracy of the susceptibility test results ATCC reference strains *E.coli* 25922 and *Pseudomonas* 27853 were used.

2) Maintenance of Bacteria: Cultures for routine uses were prepared on Nutrient Agar (Oxoid) plates which were stored at 4°C and subcultured monthly. Whenever the clinical isolates were grown, the growth medium was supplemented with 5-10 mg/l gentamicin in order to prevent segregation of plasmids.

3) Stock Cultures: Stock cultures of the bacteria were prepared as 'stab' cultures in Soft Nutrient Agar (10g Tryptone, 5 g Yeast Extract, 1 g D-Glucose, 5 g NaCl, 6 g Agar in 1 l of distilled water) which was supplemented with 5-10 mg/l gentamicin for clinical isolates. Renewal of stock cultures were made every six months.

4) Antibiotic Sensitivity Test: Susceptibilities of strains to six aminoglycosides were determined by the conventional tube dilution method.[21] Two-fold dilutions of the antibiotics tested were made in unsupplemented Mueller-Hinton Broth (Oxoid) with a concentration range between 0.125 and 64 mg/l. Inoculum size was adjusted so that the tubes would contain about 10^5 CFU/ml. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic completely inhibiting the growth of bacteria after incubation at 37°C for 18 h.

5) Survey of Aminoglycoside Resistance Patterns (AGRPs): The MIC ratio procedure originally suggested by Miller *et al.* was followed to classify clinical isolates into resistance patterns which correspond to known enzymatic inactivation mechanisms.[15]

The classification of the strains was based on the changes in relative susceptibilities (MICs) to six aminoglycosides, gentamicin (Gm), amikacin (Ak), netilmicin (Nm), 2′-netilmicin (2′-Nm) and 6′-netilmicin (6′-Nm) from the MIC break-points. Breakpoints which separates sensitive organisms from the resistant ones were 8 mg/l for gentamicin, tobramycin and netilmicin, and 32 mg/l for amikacin and two ethyl derivatives of netilmicin. Out of the 8 AGRPs which occur in gram-negative bacteria 5 can be distinguished on the basis of their susceptibility to Gm, Tm, Ak and Nm as follows.

AGRPs	Increased ($\geq$ break point) MICs	Normal ($<$ break point) MICs
ANT (2″)	Gm, Tm	Nm, Ak
AAC (6′)-I	Tm, Nm, Ak	Gm
ANT (2″) + AAC (6′)	Gm, Tm, Nm, Ak	
AAC (3)-I	Gm	Tm, Nm, Ak
AAC (3)-Ia	Gm, Nm	Tm, Ak

The final 3 AGRPs all had increased MICs to Gm, Tm, and Nm. They could, however, be distinguished on the basis of their susceptibility to 2′-Nm and 6′Nm. Such strains were subclassified as follows:

AGRP	Increased MICs	Normal MICs
AAC (2′)	6′-Nm	2′-Nm
AAC (6′)-II	2′-Nm	6′-Nm
AAC (3)-II	2′-Nm, 6′-Nm	

6) Preparation of the Cell-Free Extracts: The bacterial cells growing exponentially in 100 ml culture were harvested by centrifugation at 7 500 g using Sorvall SS-34 rotor for 20 minutes and washed with Tris-HCl buffer (0.1 M, pH 7.5 with 1 mM 2-mercaptoethanol). The washed cells were suspended in 5.0 ml of the same buffer solution (20Xconcentration). The suspension was cooled in ice bath and was subjected to five 20 sec burst, with intermittant cooling, of a sonicator (Ultrasonic Membrane Disintegrator, Fisher) at 100 output. The supernatant (S 100) obtained by centrifugation at 40 000 rpm for 30 minutes, using Beckman 50 Ti rotor, was designated as the 'cell free extract'.

7) Phosphocellulose Paper Binding Assay (PPBA): The presence of aminoglycoside-modifying enzymes in crude cell-free preparations was demonstrated by Phosphocellulose Paper Binding Assay (PPBA).[13] The assay mixture consisted of 10 µl of buffer (1:1 mixture of Tris 10 mM, pH 8.1 and $MgCl_2$, 8.6 mM), 10 µl cell-free extract and 5 µl aminoglycoside solution (1 mg/ml). Depending on whether phosphotransferase or adenylyltransferase or acetyltransferase activity was to be determined, the mixture was supplemented with 10 µl [γ - ^{32}P] ATP (10 µCi/ml) or 10 µl [α - ^{32}P] ATP (10 µCi/ml) or 4 µl [^{14}C] Acetyl coenzyme A (7.5 µCi/ml), respectively. The amount of radioactivity specifically associated with each test aminoglycoside was counted by use of a Beckman Liquid Scintillation Spectrometer. The site of modification was deduced through comparing the relative amount of radioactivity bound by a given set of substrates (aminoglycosides). The substrates were selected so that they could allow to infere modification positions, since each could be blocked at one or more possible target site(s).

Results

1) Aminoglycoside Resistance Patterns (AGRPs): The aminoglycoside resistance patterns of the representative clinical strains determined by use of the *in-vitro* susceptibilities of the strains for six aminoglycosides (Gm, Tm, Ak, Nm, 2′-Nm and 6′-Nm) are presented in Table I. These six aminoglycosides were the key antibiotics for analysis of modification patterns brought by the enzymes.

2) Enzymatic Adenylylation of the Crude Enzyme Preparations Measured by PPBA: Gm C, Gm A, Neo and Km A were used as substrates to find out adenylylation positions. Substrate profiles determined in this study are given in Table II. No adenylylation activity could be detected by cell free extracts of the strains HT835, HT839, HT8439 and HT838. The remaining six strains were all found to have ANT (2″) activity. The enzyme activity predicted through interpretation of the counts from the

TABLE I

AMINOGLYCOSIDE RESISTANCE PATTERNS OF TEN CLINICAL ISOLATES WITH RESPECT TO SENSITIVITIES TO SIX AMINOGLYCOSIDES

Bacteria	Strain	AGRP	MIC (mg/l)					
			Gm	Tm	Ak	Nm	2′-Nm	6′-Nm
Proteus spp	HT 835	AAC (3)-II	32	32	16	64	> 64	> 64
E. coli	HT 839	"	> 64	64	16	64	64	> 64
Proteus spp	HT 843	"	> 64	32	2	> 64	64	64
Pseudomonas spp	HT 837	ANT (2″)	> 64	64	4	8		
Citrobacter spp	HT 8410	"	32	64	4	1		
Pseudomonas spp	HT 8469	AAC (3)-I	> 64	2	2	2		
Enterobacter spp	HT 8439	AAC (6′)-II	> 64	64	2	8	32	4
Klebsiella spp	HT 833	"	> 64	> 64	4	> 64	> 64	2
Pseudomonas spp	HT 838	AAC (2′)	> 64	64	4	> 64	4	32
Proteus spp	HT 8313	Permeability	> 64	> 64	> 64	32	> 64	> 64

TABLE II

SUBSTRATE PROFILES OF AMINOGLYCOSIDE ADENYLYLTRANSFERASES OF CELL SONICATES

Bacteria	Strain	AGRPs	Radioactivity bound (c.p.m.)					Enzyme (s) Predicted
			No Antibiotic	GmC_1	GmA	Neo	KmA	
Proteus spp	HT 835	AAC (3)-II	45	101	84	86	113	-
E. coli	HT 839	AAC (3)-II	86	257	294	97	207	-
Proteus spp	HT 843	AAC (3)-II	132	5870	6474	97	7031	ANT (2″)*
Pseudomonas spp	HT 837	ANT (2″)	105	1081	776	238	928	ANT (2″), ANT (4′)
Citrobacter spp	HT 8410	ANT (2″)	206	9388	9810	118	12327	ANT (2″)*
Pseudomonas spp	HT 8469	AAC (3)-I	105	1535	1209	339	2068	ANT (2″), ANT (4′)
Enterobacter spp	HT 8439	AAC (6′)-II	50	135	n.t	104	108	-
Klebsiella spp	HT 833	AAC (6′)-II	101	2150	3258	123	1384	ANT (2″)
Pseudomonas spp	HT 838	AAC (2′)	52	116	n.t	64	76	-
Proteus spp	HT 8313	Permeability	75	1491	472	117	425	ANT (2″)

n.t. = not tested, GmC_1 = Gentamicin C_1, GmA = Gentamicin A, Neo = Neomycin, KmA = Kanamycin A.
* = considerable activity.

TABLE III

SUBSTRATE PROFILES OF AMINOGLYCOSIDE ACETYLTRANSFERASES OF CELL SONICATES

Bacteria	Strain	AGRPs	Radioactivity bound (c.p.m.)					Enzyme(s) Predicted
			No Antibiotic	GmB	GmC_1	Ak	KmA	
Proteus spp	HT 835	AAC (3)-II	450	8904	21331	2210	3	AAC (3), AAC (2′)
E. coli	HT 839	AAC (3)-II	452	11175	13185	474	4032	AAC (3)
Proteus spp	HT 843	AAC (3)-II	490	18025	18930	954	596	AAC (3)*
Pseudomonas spp	HT 837	ANT (2″)	53	190	155	261	205	-
Citrobacter spp	HT 8410	ANT (2″)	64	691	1025	675	683	AAC (2′)
Pseudomonas spp	HT 8469	AAC (3)-I	85	2129	2917	958	1684	AAC (3)
Enterobacter spp	HT 8439	AAC (6′)-II	56	66	106	65	53	-
Klebsiella spp	HT 833	AAC (6′)-II	412	15604	12107	3319	3923	AAC (3)*, AAC (6′)
Pseudomonas spp	HT 838	AAC (2′)	82	2047	1974	1900	1942	AAC (3)
Proteus spp	HT 8313	Permeability	95	12782	22100	8116	6341	AAC (3)*, AAC (2′)

Gen B = Gentamicin B, Gen C_1 = Gentamicin C_1, Ak = Amikacin, Km A = Kanamycin A.
* = considerable activity.

TABLE IV

SUBSTRATE PROFILES OF AMINOGLYCOSIDE PHOSPHOTRANSFERASES OF CELL SONICATES

Bacteria	Strain	AGRPs	Radioactivity bound (c.p.m.)						Enzyme(s)
			No Antibiotic	Neo	Ak	Tm	GmC_1	GmA	
Proteus spp	HT 835	AAC (3)-II	56	126	454	147	206	322	-
E. coli	HT 839	AAC (3)-II	55	151	60	189	62	176	-
Pseudomonas spp	HT 837	ANT (2″)	55	146	71	212	521	76	APH (2″)
Citrobacter spp	HT 8410	ANT (2″)	62	149	82	399	8740	43	APH (2″)*
Proteus spp	HT 8313	Permeability	55	120	51	774	51	58	-

Neo = Neomycin, Ak = Amikacin, Tm = Tobramycin, GmC_1 = Gentamicin C_1, GmA = Gentamicin A
* = considerable activity.

crude enzyme preparations of the strains with ANT (2″) AGRP (strains HT 837 and HT 8410), confirmed enzyme activity which was specified by the profile. The strains HT843, HT8469 and HT833 although classified previously into a variety of acetylation (AAC) profiles, displayed evidence for a considerable ANT (2″) activity as well.

3) Enzymatic Acetylation of the Crude Enzyme Preparations Measured by PPBA: In this assay, Gm B, Gm C, Ak and Km A were used as substrates (Table III). Cell free preparations of all the strains except two (strains HT 8439 and HT 838) had the acetylation activities specified by the respective AGRPs. No acetylation activity could be detected in cell free extract of strain HT 8439, despite its AGRP conferred AAC (6′)-II activity. In an another example of the same profile however, (HT833), AAC (6′) activity was detected together with a considerable AAC (3) activity. Instead of AAC (2′) activity as specified by the respective AGRP, AAC (3) activity was confirmed with cell-free extract of strain HT838. It is interesting to note that significant AAC (3) activity was measured with crude enzyme preparation of the strain HT8313, although classified into permeability type resistance pattern.

4) Enzymatic Phosphorylation of the Crude Enzyme Preparations Measured by PPBA: Strains tested and substrate profiles determined by PPBA are listed in Table IV. These five strains amongst ten were found promising in terms of phosphorylation activity in a preliminary assay, run with a low specific activity γ - ^{32}P ATP, Neo, Ak, Tm, Gm C_1 and GmA were used as selective substrates in this test. Two of the strains which were classified into ANT (2″) AGRP displayed APH (2″) activity as well.

Discussion

Enzymatic activities conferred by the respective AGRPs were assayed by PPBA with cell free sonicates prepared from ten representative strains with different AGRPs. Determination of the enzymes through PPBA largely confirmed the existance of the enzymes specified by the AGRPs (Tables II and III). ANT (2″) and AAC (3) activities determined by radioactive assays were always well correlated with the indicative enzymes of ANT (2″) profile and two AAC (3) profiles AAC (3)-II and AAC (3)-I. But in one of the cases *Pseudomonas* (HT 838), instead of the modifying activity specified by the AGRP, AAC (3) activity was determined in cell free preparation. Such discrepancy was also observed by Van de Klundert *et al.*[22] in their study which combined sensitivity data to identification of aminoglycoside modifying enzymes in clinical isolates. For certain antibiotics (gentamicin, kanamycin) the sensitivity phenotypes obtained were found to be well correlated with the absence

or presence of a particular enzyme, whereas for other antibiotics (e.g. neomycin, netilmicin) this correlation was less obvious.The failure of the method at this point may be attributed to a lesser enzyme activity, or iso-enzymes with unusual substrate profiles.[23]

One point which was evident from the data of PPBA is that more than one enzymatic activity existed at the same time in a given resistant strain. For example, *E. coli* HT 839 strain had only AAC (3) activity, while *Citrobacter* HT 8410 exhibited ANT (2″), AAC (2′) and APH (2″) activities altogether (Tables II-IV). Thus, the fact that some modifying enzymes (e.g., Phosphotransferases) or combinations of enzymes do not occur in the classification scheme does not necessarily imply that these enzymes or a combination never occur in nature. Existence of more than one modification enzyme in a given strain, furthermore, might have important clinical implications. For example, aminoglycoside cross-resistance, and high-level aminoglycoside resistance might reflect a synergistic interaction of multiple aminoglycoside modification activities.

One of the drawbacks of the survey method followed that should be kept in mind is, that the phosphorylating enzymes have not been identified in these experiments, since they do not cause resistance to any of the six aminoglycosides tested. Similarly, none of the enzymes has been studied which cause resistance to streptomycin and spectinomycin.

Another point that became clear with the radioactive assays was that the classification into the "permeability" type resistance group did not exclude the existance of enzyme mediated resistance mechanism. For instance, a strain (*Proteus* HT8313) exhibiting permeability profile also harbored ANT (2″) AAC (3) and AAC (2″) activities (Tables II and III).

In conclusion, apart from the above mentioned shortcomings, the Miller's classification scheme, within the limited scope of its substrate range, predicts modification enzymes quite correctly. The method seems to be convenient in a screening program for recognizing the frequently occurring aminoglycoside modifying enzymes in a large number of clinical isolates from a wide variety of sources. Its simplicity and the use of inexpesive substances as compared to direct measurment techniques are additional advantages.

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Acetazolamide Potentiated Hypercalcemic Effect of Hypervitaminosis D in Mice

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Summary

Forty male albino mice were used in this study. Twenty of them were injected with vitamin D in a single intraperitoneal dose of 75 000 U. Ten mice also received acetazolamide orally (in drinking water) at a daily dose of 75 mg/kg body weight throughout the study in addition to vitamin D. Another ten animals were controls. 60 hours after vitamin D injection, blood ionized calcium levels were found to be significantly higher in acetazolamide + vitamin D treated animals than vitamin D treated or control animals (1.65 ± 0.07 mmol/L, 1.36 ± 0.05 mmol/L and 1.16 ± 0.04 mmol/L, $p < 0.01$).

Key Words: Acetazolamide, Carbonic anhydrase inhibitor, Hypercalcemia, Vitamin D intoxication.

Introduction

There are conflicting views concerning the effects of carbonic anhydrases in calcification.[1-8] For example, acetazolamide, a carbonic anhydrase inhibitor, antagonizes parathyroid hormone-induced hypercalcemia[3, 6, 7] and thyrocalcitonin-induced hypocalcemia.[8] To our knowledge there is no report concerning the effects of acetazolamide in hypercal-

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cemia due to hypervitaminosis D. We planned this study with mice in order to shed some light on this problem.

Materials and Methods

Forty male albino mice weighing 25-35 g were used in this study and 20 of them were injected with vitamin D in a single intraperitoneal dose of 75 000 U. Ten mice also received acetazolamide orally (in drinking water) at a daily dose of 75 mg/kg body weight throughout the study in addition to vitamin D injection. The other ten animals were separated as controls and received no drugs. Blood samples were taken for ionized calcium analysis 60 hours after the injection. Serum vitamin D and its metabolites and acetazolamide levels could not be determined.

Results were expressed as the mean ± SEM. Student's t test was used to compare the means.

Results

At the end of the study blood ionized calcium levels following administration of vitamin D to mice were significantly higher than the controls (1.36 ± 0.05 mmol/L and 1.16 ± 0.04 mmol/L, $p < 0.01$). When vitamin D and acetazolamide were administrated together, the hypercalcemic action of vitamin D was potentiated (1.65 ± 0.07 mmol/L and 1.36 ± 0.05 mmol/L, $p < 0.01$).

Discussion

There is some evidence that carbonic anhydrase plays an important role in the calcification process in various animals, including invertebrates.[1] Carbonic anhydrase is present in human osteoclasts, but not in osteoblasts.[2] Acetazolamide, a carbonic anhydrase inhibitor, can enter bone cells and inhibit the enzyme and osteoclastic activity in rats *in vitro*.[3,4] But, complete carbonic anhydrase inhibition in rats for years, even from birth, does not produce bone abnormalities.[4] These drugs have been in clinical use for many years, with no associated abnormalities in bone or calcium balance reported. There is a correlation, however, between carbonic anhydrase II deficiency and osteoporosis with renal tubular acidosis and cerebral calcification in man.[5] The use of carbonic anhydrase inhibitors has also been suggested in osteoporosis on the basis of the inhibition of the enzyme in the osteoclasts.[6]

Acetazolamide has some parodoxical effects. Parathyroid hormone-induced hypercalcemia is repressed by acetazolamide[3, 6, 7] but acetazolamide also antagonize the hypocalcemic effects of thyrocalcitonin.[8] We have shown that acetazolamide potentiated hypercalcemic effect

of hypervitaminosis D. At present, there is no conclusive evidence that acetazolamide therapy is significantly associated with an increased risk of hypercalcemia in vitamin D intoxication in humans. The results however, are likely to be of clinical importance and new studies are needed.

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The Left Ventricular Hemodynamics and Regional Wall Motion in Various Artificial Pacing Modalities

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Summary

Only a few studies have suggested the deleterious effects of right ventricular pacing on left ventricular function as a result of incoordinated contraction of muscle fibers due to abnormal depolarization sequence. This hypothesis, however, has not been proven. To test this hypothesis we investigated the hemodynamics and left ventricular regional wall motion (RWM) by digital subtraction ventriculography during AOO, DOO, VVI pacing and sinus rhythm in 16 patients with coronary heart disease. We found a 24.9 % increase in cardiac index during DOO and a 43.28 % increase during AOO modes as compared to VVI pacing ($p < 0.01$). Cardiac index was demonstrated to be 14.9 % less during DOO than AOO pacing ($p < 0.01$). Additionally, by computerized percent radial shortening analysis we found a similar RWM pattern in sinus rhythm and AOO pacing ($p > 0.05$) and a significantly different RWM pattern when we compared AOO and DOO and AOO and VVI pacing ($p < 0.05$). Our findings showing the continuation of the impaired left ventricular regional wall motion of right ventricular pacing in dual chamber stimulation, which has the advantage of atrial contribution, suggest that atrial pacing is more physiological than dual chamber pacing in patients with normal A-V conduction.

Key Words: Cardiac pacing, Left ventricular functions, Left ventricular wall motion.

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Introduction

Since the first permanent cardiac pacemaker implantation, tech nology has evolved from single chamber fixed rate pacing to demand systems, dual chamber pacing and eventually to biosensors.

Most of the recent studies have mainly focused on the detection and solution of the sophisticated pacing modalities and on the comparison of the hemodynamic benefits of the different pacing modalities.[1, 2] Previous reports on the hemodynamic sequelae of artificial cardiac pacing have emphasized the role of atrial systole and atrioventricular synchronisation and the term "physiologic pacing" has been accepted widely for dual chamber pacing.[3]

On the other hand, only a few studies have drawn attention to the deleterious effects of right ventricular stimulation.[4, 5] In a recent study Verna, *et al*[6] have shown non-invasively the presence of asynchronous contraction even during dual chamber pacing, contributing to the impairment of ventricular function. To our knowledge, however, the concept emphasizing the importance of the natural atrioventricular conduction in the synchronized contraction of the left ventricular muscle fibers has not been proven by direct contrast angiography.

Accordingly, we studied the left ventricular hemodynamics and regional wall motion in atrial, atrioventricular and ventricular pacing modes by digital subtraction angiography to point out the functional consequences of right ventricular stimulation.

Materials and Methods

Patients: The study group comprised 16 patients referred for coronary angiography with a diagnosis of coronary heart disease. Five patients were excluded because of the technical inadequacy of the left ventriculograms. Thus 11 patients (7 men and 4 women) were studied and their ages ranged from 31 to 64 (mean age was 43). The procedure was explained and a signed consent was obtained from all patients. According to the coronary angiographic anatomy 4 patients were categorized as 3 vessel disease, 2 patients as 2 vessel disease and 3 patients as one vessel disease and coronary angiograms were normal in 2. Thallium 201 studies showed reversible ischemic defect in the latter two cases.

Digital Subtraction Ventriculography (DSV): DSV studies were performed by a Philips DVI-CV system equipped with a Philips Optimus M 200 X-ray generator. The system uses TID (time interval diffirence) mode for subtraction. The resulting digitized and subtracted images are dis-

played in real time during acquisition and stored in analog form on videotape. After an overnight fasting without any premedication, left ventriculograms were performed by means of a catheter inserted percutanously through femoral artery and advanced to the left ventricle.

All ventriculographic images were obtained with the patient positioned in the 30° RAO projection while 25 ml of contrast material (sodium meglumine ioxithalamate-Urographine^{R}, 50 % diluted with saline) were administered in a rate of 8 ml/sec. Patients were requested to suspend respiration at end-inspiration.

All of the ventriculographic images were subjected to postprocessing program of the DVI unit which was also equipped with a digital angiography computer. End diastolic volume, end systolic volume, ejection fraction and cardiac index were measured according to the single plane area-length method of Sandler and Dodge[7] by this microprocessor controlled automated system. End diastolic and end systolic volumes were selected as the largest and smallest volumes respectively with exclusion of any post-extrasystolic beat. Regional wall motion was also analyzed by the same computer as percent radial shortening in six segments.[8]

Stimulation Protocol: While the patient remained in supine position two bipolar electrodes were advanced to the high right atrium (at the superior vena cava and right atrial junction) and the right ventricular apex via femoral vein. After having had the stabilized electrode positions, a digital subtraction left ventriculogram was performed and subsequently were repeated in VVI, DOO and AOO pacing modes which were selected randomly. The pacing rates were slightly over the patient's own sinus rate and the A-V delay in DOO mode was adjusted slightly shorter than the patient's PR interval. The output and pulse duration were constant during all pacing modalities. We used Telectronics^{R} 2401 dual chamber analyser as external pulse generator.

Statistical Analysis: The data were analysed by Student's t test for paired comparison.

Results

All studies were performed without any complication. The hemodynamic data obtained in 11 patients with coronary heart disease in atrial (AOO), ventricular (VVI) and atrioventricular pacing (DOO) modes are presented in Table I. While there were no significant changes in the ejection fraction between AOO and DOO pacing modes ($p > 0.05$), conversion from AOO to VVI pacing ($p < 0.01$) and from DOO to VVI pacing ($p < 0.05$) caused significant changes in the ejection fraction. On

TABLE I

HEMODYNAMIC DATA IN VARIOUS PACING MODALITIES

Hemodynamic Data	Stimulation Modes A O O ‡	V V I ‡	D O O ‡	Statistical Evaluation	
Ejection Fraction (%)	60.30 ± 5.10	51.30 ± 4.90	60.10 ± 5.60	AOO-VVI	$p < 0.01$
				DOO-VVI	$p < 0.05$
				AOO-DOO	$p > 0.05$
Stroke Volume Index (ml/m^2)	45.63 ± 4.68	31.18 ± 3.89	40.09 ± 4.71	AOO-VVI	$p < 0.01$
				DOO-VVI	$p < 0.01$
				VVI-DOO	$p < 0.01$
Cardiac Index ($L/min/m^2$)	4.61 ± 0.46	3.22 ± 0.36	4.02 ± 0.44	AOO-VVI	$p < 0.01$
				AOO-DOO	$p < 0.01$
				DOO-VVI	$p < 0.01$

‡ mean ± SEM

TABLE II-A

LEFT VENTRICULAR REGIONAL WALL MOTION IN NORMAL SINUS RHYTHM AND ATRIAL PACING

Case	Left Ventricular Wall Motion Normal Sinus Rythim						% Radial Shortening AOO Pacing					
No	1	2	3	4	5	6	1	2	3	4	5	6
1	19.6	8.2	7.1	-20.7	25.3	30.9	16.8	4.2	-0.5	-6.0	18.3	29.3
2	36.9	32.4	33.7	24.5	27.9	22.9	54.2	48.9	35.6	31.5	23.2	15.4
3	44.7	40.2	27.4	24.5	24.1	17.3	41.6	42.4	28.6	27.3	26.5	15.2
4	24.7	3.5	4.0	8.43	36.9	32.9	30.9	34.1	22.3	40.9	46.7	17.2
5	29.9	33.5	24.2	30.5	10.9	21.1	31.2	26.8	15.4	21.6	29.0	24.9
6	20.0	29.2	17.8	-2.9	-1.8	1.3	24.3	11.8	0.2	7.8	5.7	1.8
7	35.0	33.1	17.7	30.3	33.5	25.9	59.6	53.7	36.5	28.6	33.7	23.5
8	27.6	29.4	21.0	37.7	41.3	36.2	42.0	37.4	28.8	32.1	41.3	26.4
9	27.9	25.4	13.2	27.2	39.9	22.6	29.1	35.15	13.8	24.65	28.95	28.2
10	52.1	49.4	11.0	20.7	4.2	22.5	35.5	29.0	10.6	12.6	2.4	22.5
11	48.8	53.1	48.5	36.6	16.5	28.9	57.4	34.5	51.3	54.4	39.1	27.8

Left ventricular segments :

1. Anterobasal segment.
2. Anterolateral segment.
3. Anteroapical segment.
4. Apical segment.
5. Inferior segment.
6. Posterobasal segment.

TABLE II-B

LEFT VENTRICULAR REGIONAL WALL MOTION DURING ATRIOVENTRICULAR (DOO) AND VENTRICULAR (VVI) PACING

Case	Left Ventricular Wall Motion % Radial Shortening											
	VVI Pacing						DOO Pacing					
No	1	2	3	4	5	6	1	2	3	4	5	6
1	18.0	10.3	1.1	−10.8	8.3	15.3	1.7	−4.3	−1.5	11.3	40.7	36.4
2	32.6	58.7	33.1	13.9	21.5	9.6	63.1	58.7	56.2	21.1	−2.6	−5.1
3	36.35	39.25	30.55	9.95	9.4	13.45	45.45	34.7	14.1	6.8	5.95	7.65
4	39.0	27.8	−9.2	15.6	55.6	16.6	65.0	69.8	18.8	25.7	24.3	10.3
5	34.2	43.8	29.0	−0.7	10.3	11.4	43.0	49.0	29.1	18.5	−3.9	17.4
6	17.0	−0.9	−4.7	−0.5	−1.8	2.9	32.6	8.2	−1.3	−6.4	−2.9	−3.2
7	29.5	47.3	47.9	24.2	62.0	43.0	59.6	50.3	38.7	12.6	38.3	31.0
8	17.4	20.8	18.7	25.4	19.4	9.8	58.1	58.7	33.5	49.7	27.9	20.2
9	30.3	29.7	10.2	25.0	28.9	34.5	43.7	43.7	16.2	39.0	40.0	38.7
10	37.1	23.7	5.7	15.8	5.7	20.8	62.7	62.5	9.0	−2.5	−24.6	8.9
11	38.3	42.5	24.0	48.4	31.4	29.2	59.4	71.5	55.9	34.9	21.0	12.6

Left ventricular segments :

1. Anterobasal segment.
2. Anterolateral segment.
3. Anteroapical segment.
4. Apical segment.
5. Inferior segment.
6. Posterobasal segment.

TABLE III

THE EVALUATION OF LEFT VENTRICULAR REGIONAL WALL MOTION IN VARIOUS ARTIFICIAL PACING PACING MODALITIES AND NORMAL SINUS RHYTHM

Stimulation Modes	Statistical Analysis For Each Segment 1	2	3	4	5	6
SIN – AOO	5.77 $p > 0.05$	1.86 $p > 0.05$	1.81 $p > 0.05$	5.80 $p > 0.05$	4.09 $p > 0.05$	–1.85 $p > 0.05$
SIN – VVI	–3.40 $p > 0.05$	–0.22 $p > 0.05$	–3.39 $p > 0.05$	–9.58 $\dagger p < 0.10$	–3.60 $p > 0.05$	–5.54 $p > 0.05$
SIN – DOO	14.95 $\dagger p < 0.05$	15.04 $\dagger p < 0.10$	4.17 $p > 0.05$	–5.68 $p > 0.05$	–11.50 $\dagger p < 0.10$	–9.06 $\dagger p < 0.05$
AOO – VVI	–9.18 $\dagger p < 0.05$	–2.08 $p > 0.05$	–5.11 $p > 0.05$	–9.89 $\dagger p < 0.01$	–3.94 $p > 0.05$	–3.70 $p > 0.05$
AOO – DOO	8.55 $\dagger p < 0.10$	13.18 $p > 0.05$	2.45 $p > 0.05$	–5.89 $p > 0.05$	–10.97 $\dagger p < 0.10$	–7.21 $\dagger p < 0.05$
VVI – DOO	17.78 $\dagger p < 0.01$	15.26 $\dagger p < 0.05$	7.56 $p > 0.05$	4.04 $p > 0.05$	–7.91 $p > 0.05$	–3.7 $p > 0.05$

1. Anterobasal segment
2. Anterolateral segment
3. Anteroapical segment
4. Apical segment
5. Inferior segment
6. Posterobasal segment

the other hand, our data showed a 24.9 % increase in cardiac index during atrioventricular pacing as compared to ventricular pacing ($p < 0.01$). Meanwhile, cardiac index values were 43.28 % less in ventricular pacing when we compared them to atrial pacing ($p < 0.01$). Interestingly enough, at a constant pacing rate atrial pacing resulted in a 14.9 % higher cardiac index than atrioventricular pacing with A-V delay slightly less than the patient's normal PR interval ($p < 0.01$).

Left ventricular regional wall motion values in patient's sinus rhythm; atrial, atrioventricular and ventricular pacing are shown in Table II.

As indicated in Table III there were no significant changes in regional wall motion in any segment studied between normal sinus rhythm and atrial pacing ($p > 0.05$). In contrast to this finding, particularly anterobasal ($p < 0.05$), anterolateral ($p < 0.10$), inferior ($p < 0.10$) and posterobasal ($p < 0.10$) segments were found to show contractility changes in DOO pacing as compared to normal sinus rhythm. Contractility changes in these segments were also found when pacing converted from AOO to DOO mode ($p < 0.05$ and $p < 0.10$). Finally, our data demonstrated significant differences in wall motion between DOO and VVI pacing at least in 2 segments, namely in the anterobasal ($p < 0.05$) and apical ($p < 0.10$) segments.

Discussion

The hemodynamic aspects of cardiac pacing have become popular since the first implantation of a permanent pacemaker and there have been many reports on improved hemodynamics by means of artificial pacing. The recent developments in pacemaker technology however, have necessitated the performance of more studies to evaluate the hemodynamic benefits of available systems. Subsequently, the importance of atrial synchrony and beneficial hemodynamic effects of dual chamber pacing were documented.[9]

In earlier studies the effects of ventricular stimulation and the importance of the abnormal stimulation sequence of left ventricular muscle fibers had been suggested.[4, 5] Although a loss of atrial contribution to ventricular filling has been considered as the most important factor for this phenomenon, the development of incoordinate contraction due to abnormal sequence of activation during right ventricular pacing may play a significant role.

The normal electrical activation of the left ventricle initiated from the atrium and traveling through the His-purkinje system via the atrio-

ventricular node provides a relatively synchronous activation of ventricular muscle. In contrast, when stimulation occurs from the ventricles, significant changes arise both in the sequence and rate of electrical activation.[10] Assuming that mechanical activity follows electrical activation after some fixed delay, ventricular pacing should induce significant asynchrony of the onset of mechanical activity.[5] Finally, such mechanical asynchrony might result in hemodynamic sequelae.

In an experimental animal study during atrial pacing, ventricular depolarization was demonstrated to require less than 60 ms for completion but with the ventricular pacing ventricular depolarization was prolonged up to 140 ms due to mainly slower conduction in the ventricular muscle and an abnormal spread of activation throughout the ventricle.[10] The study performed by Boerth, *et al*[8] confirmed these observations and it was suggested that the right ventricular outflow tract pacing might result in an abnormal contraction pattern in the left ventricle that may cause a left ventricular dysfunction.The authors also concluded that any change in the stimulation sequence of myocardial fibers might cause functional derangement even in the presence of the normal inotropic state of the myocardium.

On the other hand Badke, *et al*[5], in a study performed in dogs to evaluate the effects of ventricular pacing on ventricular function, concluded that ventricular pacing produced significant changes in regional myocardial function. This was likely induced by reciprocal interaction of opposing myocardial regions and such interaction might have deleterious effects on the global ventricular function.

Verna, *et al*[6] reported the assessment of regional ventricular contraction by Fourier analysis to study the functional consequences of the abnormal sequence of activation produced by pacing. They demonstrated a loss of synergy during ventricular pacing that produced a slight reduction in left ventricular ejection fraction and end-diastolic volume and this asynchronous contraction was still present during dual chamber pacing, suggesting some impairment of left ventricular function.

Recently, Eiller, *et al*[11] have confirmed the occurence of contraction abnormalities during ventricular pacing and demonstrated a correlation, between contraction abnormalities and perfusion defects in Thallium 201 myocardial scintigrams. The authors have suggested that inhibition of coronary flow by late diastolic contraction of septal and inferoapical segments may be responsible for this abnormal contraction.

In our study we analysed the hemodynamic paramaters and left ventricular regional wall motion through left ventriculograms performed by the technique of digital subtraction angiography. In consistence with the previous reports we demonstrated a 24.9 % increase in cardiac index during DOO pacing ($p < 0.01$) and a 13.28 % increase during AOO pacing ($p < 0.01$) as compared to VVI pacing.[9, 12] When we compared AOO and DOO pacing modes at a constant pacing rate however, we unexpectedly found a 14.9 % decrease of cardiac index during DOO pacing ($p < 0.01$). The unique electrophysiologic difference between these two modes is the traveling of the atrial stimulation to the ventricle via the normal A-V conduction system in AOO pacing. If the ventricular artificial pacing during DOO pacing causes a disturbance in the left ventricular depolarization sequence, it may present itself as contractility changes in the left ventricular regional wall motion. As a matter of fact we could not demonstrate any wall motion difference between AOO pacing and normal sinus rhythm ($p > 0.05$). Meanwhile both VVI and DOO pacing modalities showed contractility changes in the various segments studied ($p < 0.01$ and $p < 0.05$).

These observations have suggested that ventricular stimulation may result in an abnormal depolarization sequence of muscle groups and this may give rise an asynchronous contractility pattern and more importantly to some left ventricular dysfunction.

Our data have brought some more controversy on the concept of the physiologic pacemaker and suggested that atrial synchronous dual chamber pacing is not absolutely physiologic.

Since our study was performed at rest it may not be accurate to predict the figures during exercise. The abovementioned abnormal contractility pattern however, would effect the left ventricular hemodynamics more prominently during exercise.[10]

Additionally, since our study group was comprised of patients with coronary artery disease, the potential of the diseased myocardium to have contractility disturbances may exaggerate the contractility abnormalities observed during ventricular pacing. Thus, the repetition of this study in healthy controls will clarify this controversy.

We evaluated left ventricular hemodynamics and regional wall motion by DSA. This technique has been proved as reliable in demonstrating left ventricular function and regional wall motion and its compatibility with direct contrast angiographic methods and has been reported previously.[13-15] To prevent the systemic toxicity and possible deleterious effects on myocardial function and to omit the difficulties in ob-

taining high quality left ventriculograms, we preferred direct intraventricular low dose diluted contrast injection for digital subtraction ventriculography.[16-18] Additionally, during pacing study each pacing mode was selected randomly to rule out the possible deleterious effects of contrast injection.

In conclusion, according to our data the concept of the physiologic pacemaker should be revised since it must use the normal A-V conduction to establish the most beneficial hemodynamic result. One should however, always take precaution against the presence of an intact A-V conduction.

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Serum Progesterone and Chorionic Gonadotropin Levels in Ectopic Pregnancy

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Summary

Serum beta-human chorionic gonadotropin and progesterone levels were measured in 34 ectopic pregnancies and in 26 normal pregnancies. In the normal pregnancy group, the beta-HCG levels ranged 150 to 6800 mIu/ml with a mean of 1749.80 ± 242.56 mIu/ml. Progesterone levels ranged from 14.5 to 48.6 ng/ml with a mean of 23.75 ± 3.23 ng/ml. In only one woman the serum progesterone level was less than 15 ng/ml. In the ectopic pregnancy group, beta-HCG ranged 45 to 7380 mIu/ml. The mean beta-HCG was 1015.58 ± 365.17 mIu/ml, which is not statistically different from that of the control normal pregnancy group. The progesterone levels in this group was remarkably low, ranging 0.3 to 12.3 ng/ml with a mean of 4.83 ± 1.23 ng/ml. In 31 women serum progesterone levels were less than 10 ng/ml. When the serum progesterone levels of the group are compared, the difference is statistically significant. In both groups, the correlation between the values of beta-HCG and progesterone is not significant.

It was found that, the incorporation of a progesterone assay into the workup of a patient with suspected ectopic pregnancy can be of considerable diagnostic value.

Key Words: Ectopic pregnancy, Progesterone, Beta-human chorionic gonadotropin.

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Introduction

The importance of corpus luteum in the maintenance of pregnancy during the first trimester is controversial.[1-4] It was concluded that corpus luteum is not essential throughout the first trimester.[1] A study by Froweis revealed that abortion followed bilateral oopherectomy before the sixth week in 100 % of the patients, but during the sixth to twelfth weeks bilateral oopherectomy resulted in abortion in only 20 % of the cases.[3]

Ectopic pregnancy is a worldwide epidemic today and its incidence is increasing.[5] It has emerged as one of the most important causes of the maternal death, and its occurence may have a significant detrimental influence on future fertility.[6] Throughout the 1970s the advent of serum beta-human chorionic gonadotropin assay and increased use of ultrasound aided. The clinician in the diagnosis and the management of patients with ectopic pregnancy. Recently, measurements of blood levels of hormones such as progesterone and human chorionic gonadotropin, which are directly involved in the maintenance of early pregnancy has been gaining popularity in evaluating the events during both normal and complicated (such as ectopic pregnancy) pregnancies.[7-10]

The aim of present study was to evaluate the function of the corpus luteum in ectopic pregnancy and to find out the usefulness of serum progesterone determination as a clinical adjunct to the conventional methods of evaluation in the workup of a patient with suspected ectopic pregnancy.

Materials and Methods

During a 10-month period from September 1986 to July 1987, 34 patients with ectopic pregnancy were studied in the Department of Obstetrics and Gynecology, Hacettepe University, School of Medicine. During evaluation, history and physical examination was performed as well as ultrasonograpy. Blood was obtained in each case before surgery for routine studies as well as beta-human chorionic gonadotropin quantation. After surgery was performed and ectopic pregnancy was confirmed intraoperatively and histologically, a progesterone determination was performed on the same sample of blood that was taken before surgery, which had been frozen for later analysis. Control subjects were 26 women with accurate last menstrual dates and uncomplicated pregnancies. Of these women, 14 pregnant women subsequently went on to deliver normalterm, singleton infants. 12 were admitted for therapeutic abortion to Family Planning Clinic. None of them had history of spontaneus abortions.

Progesterone values were determined by using Coat-A-Count^{R} radioimmunoassay kit which is a no-extraction, solid-phase ^{125}I radioimmunoassay designed for the quantitative measurement of progesterone. The minimum sensitivity is 0.05 ng/ml (assay range 0.1 to 50 ng/ml). Values for normal females in the follicular phase ranged from 0.1 to 1.5 ng/ml (N: 28) and the range for luteal phase from 2.5 to 28 ng/ml (N: 22).

The quantitative beta-human chorionic gonadotropin assays were run with a double antibody radioimmunoassay (Amerlex-M, beta-HCG RIA Kit) using ^{125}I-beta-human chorionic gonadotropin.

Statistical evaluations of the results were attained using the Student t test for unpaired samples.

Results

In the control group, the gestational age at the time the serum was sampled ranged from 5 to 8 weeks after the last menstural period with a mean of 6.48 $\pm$ 0.56 weeks. The beta-human chorionic gonadotropin (HCG) levels ranged 150 to 6800 mIu/ml with a mean of 1749.80 $\pm$ 242.56 mIu/ml. Progesterone levels ranged from 14.5 to 48.6 ng/ml with a mean of 23.75 $\pm$ 3.23 ng/ml. In only one woman the serum progesterone level was less than 15.0 ng/ml. In this group, no significant correlation was found between the HCG and progesterone levels. The beta-HCG and progesterone levels in our study are in good agreement with other literature reports on these values in the first trimester.[7, 9]

In the study group, the beta-HCG ranged 45 to 7380 mIu/ml. The mean beta-HCG was 1015.58 $\pm$ 365.17 mIu/ml, which is not statistically different from that of the control group ($P>0.005$). The mean gestational age was 7.57 $\pm$ 0.79 weeks (5.5 to 10.5). The progesterone levels in the ectopic patients were remarkably low, ranging 0.3 t 12.3 ng/ml with a mean of 4.83 $\pm$ 1.23 ng/ml. The progesterone levels 5 of 34 women (14.7 %) were reprasentative of the proliferative phase of the menstural cycle. In 29 of the women, progesterone levels were in the range of the secretory phase of the menstrual cycle. In 31 (91.1 %) women serum progesterone levels were less than 10 ng/ml. When the serum progesterone levels of the two groups is compared, the differance is statistically significant ($P < 0.001$). The correlation between the values of beta-HCG and progesterone in the 34 women is not significant.

Discussion

The source of progesterone production in pregnancy changes from the corpus luteum to the placenta around the ninth week[3], although the

corpus luteum retains its ability to synthesize progesterone throughout pregnancy.[4, 11]

In a patient where ectopic pregnancy is suspected, experience has shown that evaluation using serial beta-HCG determinations, ultrasonography, and/or culdocentesis often does not confer diagnostic information. A single beta-HCG determination, especially in a patient with unknown last menstrual period dates, is often of limited value Although serial beta-HCG determinations may be performed to assess doubling time, this method requires a 36 to 72 hour delay. Ultrasound is helpful if an intrauterine gestational sac is seen. Before six weeks of gestation however, the absence of a gestational sac is nondiagnostic because either an ectopic pregnancy or a normal intrauterine pregnancy (too early to be detected by ultrasound) could be present.[12] Also a pseudogestational sac or a decidual cast that can occur in ectopic pregnancies can be confused with an intrauterine gestational sac. The correlation of HCG levels and ultrasonography in early pregnancy was studied by Kadar *et al.*[13] They stated that absence of the gestational sac in conjuction with the quantitative beta-HCG level above 6500 mIu/ml, suggested an ectopic pregnancy. A beta-HCG less than 6000 mIu/ml, will still render an uncertain diagnosis.

Our results demonstrated low progesterone levels in ectopic pregnancies when compared to normal pregnancies. There are a few other studies which confirm us. Milwidsky *et al.*, reported that all of the 17 ectopic pregnancies they studied, showed progesterone levels of less than 6.9 ng/ml. Recently, another study by the same group showed a mean progesterone level of 2.8 ng/ml in 26 patients with ectopic pregnancies.[14,15] Radwanska *et al.*, found a mean progesterone level of 7.0 ng/ml in 11 patients with ectopic pregnancies.[16] In our study, we found the mean progesterone level to be 4.83 $\pm$ 1.23 ng/ml. The incorporation of a serum progesterone assay in to the workup of a patient with suspected ectopic pregnancy can be a useful adjunct to conventional methods of diagnosis. In our study, 25 of the 26 patients with a normal intrauterine pregnancy of 5 to 8 weeks' gestational period, had progesterone levels greater than 15 ng/ml. The mean progesterone level was 23.75 $\pm$ 3.23 ng/ml in this group of pregnancies. It might be expected that as pregnancy progresses and luteoplacental shift in progesterone synthesis occurs, the progesterone level might decline slightly in normal subjects. Johannson found the lowest progesterone levels in normal pregnancy at nine weeks' gestational age.[3] Even then the mean progesterone level was 16.7 ng/ml. In another study, it was found that 95 % of progesterone levels at nine weeks' of gestation were greater than 10 ng/ml.[17]

In our study, no patient with ectopic pregnancy had progesterone levels greater than 13 ng/ml and 31 of 34 had progesterone levels less than 10 ng/ml. These values are less than those found in normal pregnancies and significantly different from controls at the P: 0.001 level.

In pregnancies which are not developing satisfactorily (presenting as treatened abortion or suspected of missed abortion) and, which are aborted or evacuated, plasma progesterone levels tend to be low, indicating that at that point the pregnancy is already beyond salvaging.[16] Low levels of progesterone correletes well with the adverse outcome of pregnancy especially if the progesterone levels is less than 10 ng/ml.[16] In patients with supporting history and physical findings, beta-HCG determination, ultrasound findings, a progesterone level less than 10 ng/ml suggests an accident of pregnancy (e.g. ectopic pregnancy) and may allow intervention without delay. On the contrary, a normal progesterone level in the patient with elevated risk for ectopic pregnancy would be reassuring. Gerhard and Runnenbaum showed that 94 % of pregnancies, a viable infant had normal levels of progesterone.[17] The finding of a normal progesterone level prevents intervention that may jeopardize a normal, early intrauterine pregnancy.

HCG is luteotropic and its function might be regarded as that of preserving and developing the corpus luteum.[1, 14] The results of our study also call into question the significance of the HCG alone in preserving the corpus luteum. This result confirmed the result of Milwinsky *et al.*[15] In all 34 ectopic pregnancies, significant amounts of HCG were detected in serum, the corpus luteum function, as judged by progesterone production, was greatly disturbed. In the 26 normal pregnancies, HCG values were in the same range and corresponded to the values of normal early pregnancies. This correlation was also shown by others.[14, 18] We found no correlation between HCG and progesterone concentrations. This lack of correlation also exists in the ectopic pregnancies. It seems, therefore, that beta-HCG alone cannot be the only factor in the interaction between corpus luteum and the fetoplacental unit. In ectopic pregnancy the fetoplacental unit appers to be dysfunctional, presumably affected by the adverse conditions at implantation site in the fallopian tube.[19] This pathologic fetoplacental unit probably lacks a factor necessary to preserve the function of the corpus luteum until the fetoplacental unit is mature enough to produce essential hormones independently. Further work is needed in this area.

We concluded that, the incorporation of a progesterone assay into the workup of a patient with a suspected ectopic pregnancy can be of

considerable diagnostic value with appropriate history, physical and ultrasonographic findings and beta-HCG determinations.

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Tendon Transfers of the Foot and Ankle

A Review of Short Term Results of 127 Feet With Poliomyelitis

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Summary

Between the years 1980-1984, 115 children at 6-8 years of age with poliomyelitis were operated on for tendon transfers. A total of 127 tendon transfer operations were performed. Of the 115 patients, 40 were girls (34.78 %), and 75 were boys (65.22 %). Patients were observed for 3 to 7 years; with an average of 5 years. Short term results were evaluated. In 99 patients (77.95 %) excellent, in 24 patients (18.90 %) good results were obtained. Thus, in 123 patients (96.85 %) the surgical outcome was succesful. In 4 patients the results were poor. Various aspects of the technique for tendon transfers in poliomyelitis to achieve succesful results are discussed in this paper.

Key Words: Ankle, foot, poliomyelitis, Tendon transfer.

Introduction

Weakness in muscles in the foot and around the ankle is often encountered in poliomyelitis. Tendon transfers in such patients at least facilitate their rehabilitation. Making rehabilitation more effective has an important role in therapy. Enabling these patients to meet their natural physiological requirements, to lessen their dependence on external

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support or do away with it completely, may provide a permanent solution to a social problem. Poliomyelitis which has been eradicated in many countries still remains a problem in our country.

In our paper, we have especially studied poliomyelitic patients with only ankle and foot involvement. We have investigated the operations performed only at the foot and the ankle and their results. Although in some of our patients lengthening of the achilles tendon, plantar fasciotomy tendolysis and stabilization operations were performed, in our paper the main emphasis is on tendon transfer operations we have performed at the foot and around the ankle which contributed. . In our view, one of the factors to our success is the fact that we have encountered isolated muscular weakness in the majority of our cases. This is an ideal situation for a tendon transfer operation. Because the degree of involvement of paralysis and the benefit to be obtained by the patients are inversely proportional.

Materials and Methods

Various tendon transfer operations were performed on 127 feet involving of 115 patients with sequelae of poliomyelitis at the Clinic of Orthopedics and Traumatology of the Social Security Hospital in Ankara and at the private clinic of Ahmet Örs Foundation Hospital between the years 1980 - 1984. Of the 115 patients 40 were girls (34.78 %), and 75 were boys (65.22 %).

As it is observed in Table I, the transfer of peroneus longus tendon to the anterior of the foot was performed on 69 feet; 21 of which were of girls, and 48 of boys, out of 127 poliomyelitic feet. The transfer of the tendon of Tibialis posterior to the anterior of the foot was made on 12 patients; 9 girls, 3 boys. Transfer of Peroneus to Calcaneus on a total of 24 patients, (4 girls, 20 boys) were performed. The transfer of Tibialis anterior was made on the lateral of the dorsum of the foot on a total of 10 patients, 2 girls, 8 boys. The transfer of Extensor Hallucis Longus on the neck of the first metatarse was performed on a total of 12 patients; 6 girls, 6 boys (Table I).

The youngest age for transfers were six years age in both sexes, and the oldest ages 18 for boys, and 17 for girls. Tendon transfer operations were performed on boys between the age of 10-15, involving 39 feet and on girls in the same age range, involving 28 feet. In this group of patients transfers were made on 15 boys and 5 girls.

The patients were followed for a period of at least for 3 years and, at most for 7 years, (average of 5 years).

TABLE I

CLASSIFICATION OF THE TYPES OF THE TENDON TRANSFER OPERATIONS ACCORDING TO THE PREOPERATIVE DEFORMITY AND SEX

Ppeoperative Type of deformities	Type of Tendon Transfers: Transfers of PL. on to the Anterior *		Transfers of PL. on to the Calcaneus		Transfers of T.A. on to the Laterale **		Transfers of T.P. on to the Anterior ***		Transfers of E.H.L. on to the Metarsal neck ***		Total
	Boy	Girl	Boy	Girl	Boy	Girl	Boy	Girl	Boy	Girl	
Equinus	10	4	–	–	–	–	1	3	–	–	19
Varus	2	3	–	–	8	2	1	6	–	–	22
Valgus	35	12	–	–	–	–	–	–	–	–	47
Calcaneus	–	–	20	4	–	–	–	–	–	–	24
Cavus	1	2	–	–	–	–	–	–	–	–	3
"Cock-up Deformity of Great toe	–	–	–	–	–	–	–	–	6	6	12
Total	48	21	20	4	8	2	3	9	6	6	127

* P.L. : Perenous Longus
** T.A. : Tibialis Anterior
*** T.P. : Tibialis Posterior
**** E.H.L. : Extensor Hallucis Longus

The tendon transfers that are mentioned above can be classified according to the original deformities as follows.

In 19 patients with equinus deformity Peroneus Longus were transferred on to the anterior in 14 cases, and Tibialis Posterior on to the anterior in 5 of them (Table I).

Among 22 patients with varus deformity Peroneus Longus were transferred to the anterior in 5 (Figure 2A, B). Tibialis Anterior on to the lateral in 10 (Figure 1A, B) and Tibialis Posterior on to the anterior in 7 of them (Table I).

Peroneus Longus were transferred on to the anterior in 47 patients with valgus deformity (Table I).

In 24 patients with calcaneus deformity, Peroneus Longus were transferred on to the calcuneus (Table I).

In 3 patients with cavus deformity, Peroneus Longus were transferred on to the anterior (Table I).

12 patients who had "Cock-Up" deformity of the big toe were treated with the transfer of Extensor Hallucis Longus to the neck of first metatarsal bone (Table I).

Subtalar extra-articular arthrodesis were performed in 3 patients with valgus deformity, and in another patient with severe cavus deformity wedge osteotomy was performed before the tendon transfer operation.

The muscle tests of all patients were made by the manual method preoperatively.[1,2] The tendons of muscles with at least power 4, preferably power 5 were transferred because a transferred tendon usually loses one degree from its original power.

All operations were performed under tournique control. Basic principles were used during the tendon transfer operations.[1] In all possible cases, care was taken to make the transfer of tendons by making them pass under the deep fascia, and follow a direct course from its original site.

As a surgical technique, tendons were kept in minimally traumatized form, muscular and neural contents of the tendons were not damaged. At the moment of transfer, in order not to damage the tendon, a wet plug was always used. In the transfer of the tendon, preferance was given to make the fixture always inside the bone, and the pull-out technique was mostly utilized. With this technique, the tendon can be pulled from the insertio of the muscle with the desired tension, and it can be fixed to the bone under suitable tension. The pull-out fixture was generally kept for isx weeks.

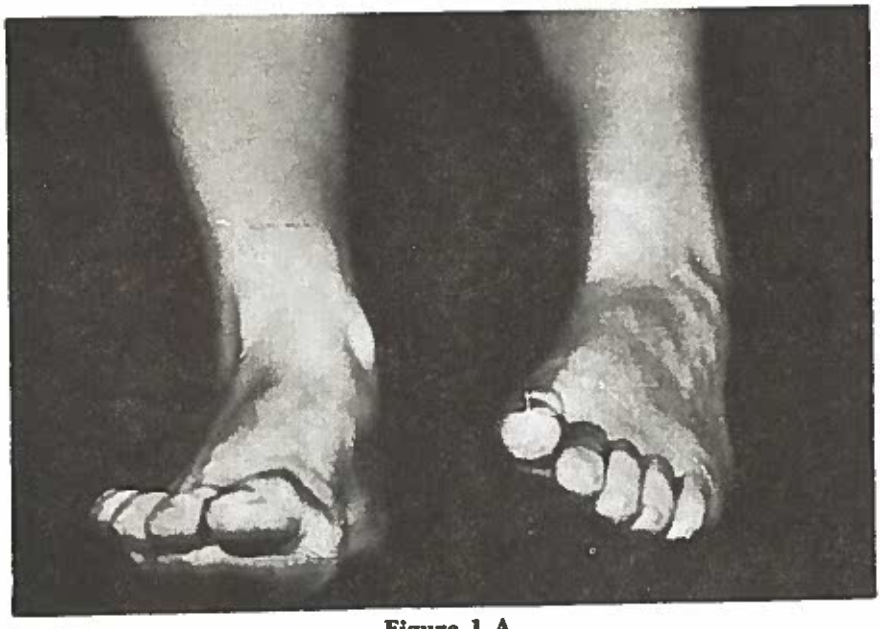

Figure 1 A

Preoperative photograph shows varus deformity of the left foot and ankle in a fourteen year old girl, who had the sequela of the Poliomyelitis Anterior.

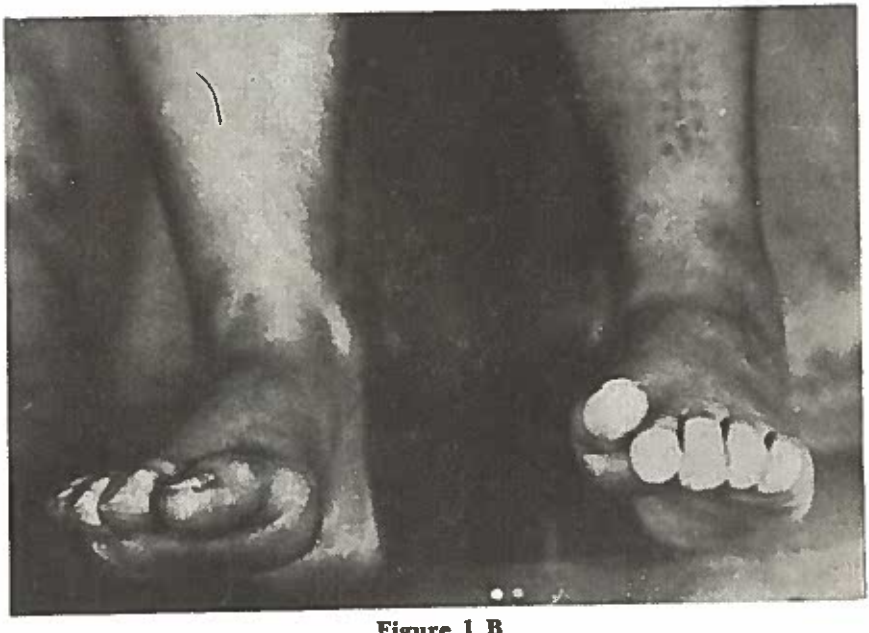

Figure 1 B

Post operative photograph of the same patient (seven years later). The Tibialis Anterior Tendon was transferred on to the lateral of the dorsum of the foot. The functioning transfer is rated good clinically on the basis of manual testing.

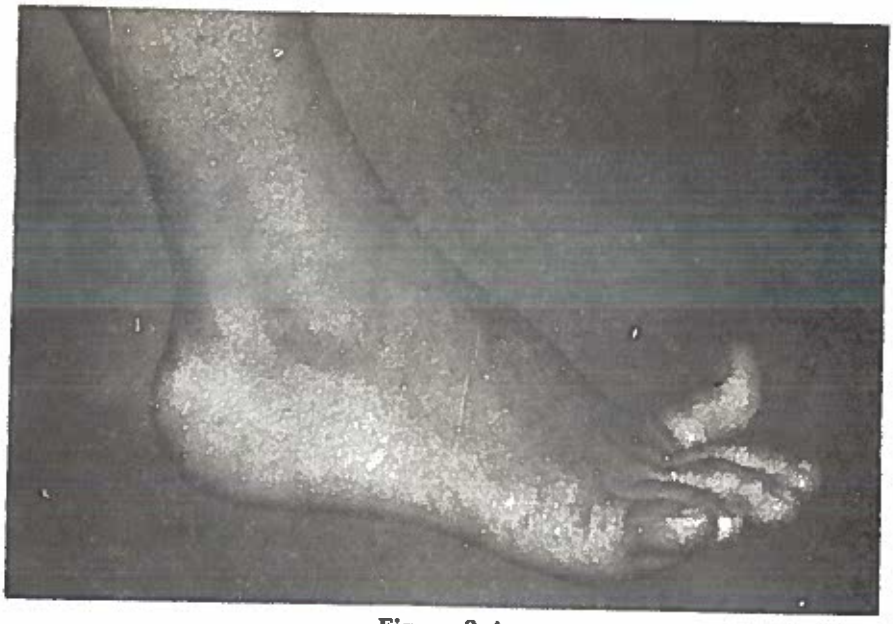

Figure 2 A
Preoperative photograph shows equinus deformity of the right foot and ankle in a 16 year old girl, who had the sequela of the Poliomyelitis Anterior.

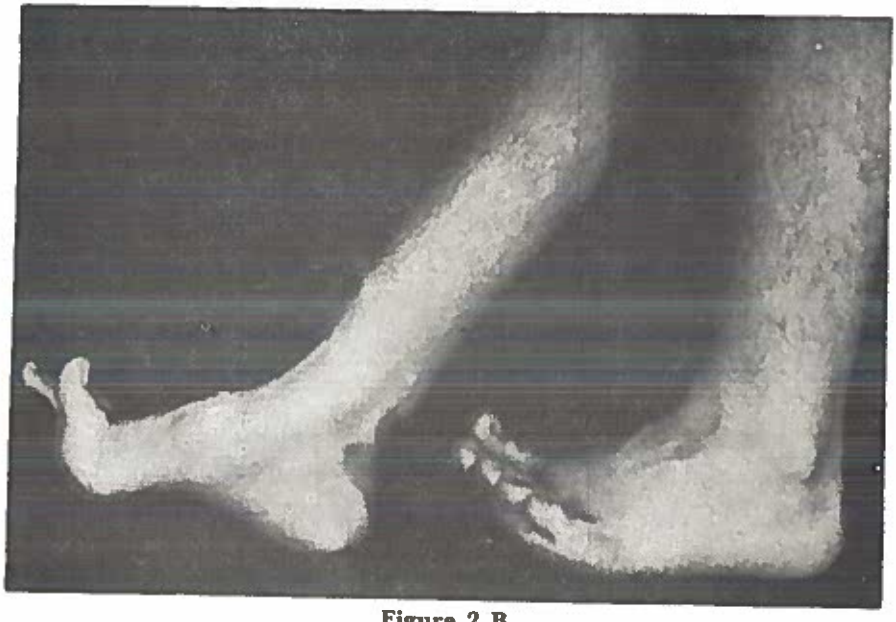

Figure 2 B
Postoperative photograph of the same patients (three years later). The Peroneus Longus Tendon was transferred on to the second cuneiform. The functioning transfer is rated good clinically on the basis of manual testing.

Following the transfer, an underknee Paris cast was applied to the foot, and was kept on for an average of six weeks. At the end of this period, the cast was removed, and the pull-out silk sutures were taken away. Following the removal of the cast, splint was applied only at night for a fortnight, and in the meantime active exercises were started. At this period, exercises were always started under the control of a physiotherapist, and maintained by her as long as it was necessary. Afterwards, exercises were continued under the care of the patient's family.

We have taken special care to enable the patients to return to their normal gait following the tendon transfers. For this purpose, we used appropriate walking apparatus and splints where needed (Figure 3).

Figure 3
The view of the child with walking apparatus.

Results

When the patients were asked to present themselves for the final check of this study, they were categorized into three groups considering the function and power of the transferred tendon at its new site.

1. In the first group, if the transferred tendon is adequate in its new site functionally, and as regard to its power, i.e. if it is at power 4, it is considered excellent.

2. In the second group, if the transferred tendon can perform its functions in its new site, but if the force is not adequate, i.e. it is at power 3, it is considered good.

3. In the third group, if the transferred tendon is inefficient in its new site both functionally, and as regards to power, i.e. if it is at power 1 or 2, it is regarded as poor.

Of the transplanted 127 feet, the results in 99 were excellent (77.95 %), in 24 good (18.90 %) while in four of them the results were poor (3.15 %) (Table II).

The best results were obtained in the transfer of the tendon of Extensor Hallucis Longus to the base of the metatarsal bone (83.33 %) while the worst result was obtained in the case where the tendon of Tibialis Posterior was transferred to the anterior (12.50 %) (Table II).

TABLE II

THE RESULTS OF THE TENDON TRANSFER OPERATIONS

Transferred Tendon	The number of Transferred Tendons	Excellent		Good		Bad	
		No of Cases	%	No of Cases	%	No of Cases	%
* P.L. to the Anterior	69	57	82.60	10	14.49	2	2.89
* P.L. to the Calcaneus	24	17	70.00	7	30.00	–	0.00
** T.A. to the Lateral	10	8	80.00	2	20.00	–	0.00
**** T.P. to the Anterior	16	10	62.50	4	25.00	2	12.50
*** E.H.L. to the first metatarsal neck	12	10	83.33	2	16.66	–	0.00
Total	127	99	77.95	24	18.90	4	3.15

* P.L. : Peroneus Longus
** T.A. : Tibialis Anterior
***E.H.L. : Extensor Hallucis Longus
**** T.P. : Tibialis Posterior

In one of the two patients in whom the tendon of Tibialis posterior was transferred to the anterior, maceration and superficial infection developed at the site of the fixture button placed on the foot sole, due to extreme pressure. While in the other patient we noticed that the sutures

were broken when we removed the cast post-operatively after 6 weeks. For these reasons, the results in these two patients turned out to be poor.

In two patients with the transfer of peroneus longus to the anterior the results were also graded as poor. In one of them we suspected that the fixing suture was broken. While in the latter, there was neither breakage in the suture nor infection. We account for the inadequency of power in the tendon as a probable mistake we may have made in the method we applied.

Discussion

The aim of tendon transfers is to augment the functions of the paralyzed muscle or muscles by exchanging them with tendons which will supply them with active motor force; to prevent the deformity which a paralyzed muscle will bring about and to contribute to stability by providing a better muscular balance.

The principles of tendon transfers have been laid down by many authorities.[1-3] We want to underline the importance of some of them according to the results.

The first important thing to be considered is the careful and correct determination of muscle power, and the detailed establishment of all the bone deformities. Tendon transfer operations performed on the foot and the ankle should be applied on patients whose knee and buttock muscles are functionally good or on those in whom those regions have been strengthened as required. For the patients whose knee and bottock muscles are paralyzed, even if the functions of the foot and the ankle are restored, it will not provide much use as regards to gait.[4] During the preoperative period it should definitely be discussed whether the patient is intelligent and cooperative enough for this operation, and whether the family members are concious of this event.[5]

It is preferable to delay the operation until the age of six.[2, 3, 6] As we did on four of our cases, bone deformities should be corrected prior to tendon transfers.[2, 3] While stabilizing operations for the correction of bone deformities are being planned, the age of bone maturation should be taken into account.[7] A period of physiotherapy should follow the correction of a fixed deformity before a transfer is planned.[8]

In the meantime, the effect of tendon transfer on the balance of other muscles of the foot must definitely be taken into account. It is imperative that tendon to be transferred should be able to perform the functions spontenously. Although one degree of power loss during the transfer was

expected, in a number of cases, only less than one degree of power loss was identified unexpectedly. In all of our cases we preferred to use synergic muscles on their antogonists.

Our follow up studies and postoperative evaluations have shown that the most succesful tendon transfers proved to be the ones in which the operation was performed because of the weakness of a single, isolated muscle. It is very well known that the more widespread the paralysis is, the less the patient will benefit from the surgical treatment.[2, 3, 9] The best results obtained in the transfer of Extensor Hallucis Longus to the neck of the first metatarsal bone are due to this concept (Table II). As well as the transfer of Extensor Hallucis Longus, the anterior transfer of Peroneus Longus appeared to present good results.[10, 11] A combination of technology and experience rendered good results.The antero-lateral transfer of the Tibialis Anterior also seemed to be successful as other authors mentioned.[5]

In our opinion the transfer should be made not only to a certain site but to the best site according to the individual case.

First of all we evaluated our patients according to the strength of the transferred tendon. It was higher than we have expected. Our second criteria was the gait, and most of the patients who were graded as good or excellent appeared to walk with a heel-to-toe gait.[5]

According to the results we have obtained from our study we have realized that tendon transfers have quite a high degree of success in those where a suitable surgical technique and planning has been applied. This can be verified by adequate manual muscular test and correct operative indications.

One final important point is that a defect during the performance of testing the operation (such as the tendon not being of sufficient tension or the breaking of the suture) should be corrected instantly.

In conclusion, we feel that these operations have advantages both to the patients and to their environment. It makes the rehabilitation of these patients easier and decreases their depedence on a certain apparatus or they can even get rid of the apparatus completely. For these reasons, tendon transfer operations have phychological benefits for the patients as well as social value.

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Reconstruction of Lower Leg Tissue Defects with Fasciocutaneous Flaps

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Summary

Four cross-leg fasciocutaneous flaps comprising skin subcutaneous fat and deep fascia have been succesfully used to cover various defects in the lower extremity. The hospitalization period was between 1 to 1,5 months, and all of the defects healed completely.

Key Words: Fasciocutaneous flap.

Introduction

Skin defects below the knee present many difficult problems, particularly when combined with fractures, osteitis, ostecmyelitis and so forth. Grafts and local skin flaps, useful elswhere, have limited success in this area.[1] In 1980 Ponten[2] first described the use of the fasciocutaneous flap in the lower leg, where he proved its reliability in a series of 23 patients. This discovery that undelayed fasciocutaneous flaps could be used with confidence to cover exposed bone and tendons, not only has reduced the time and cost of treatment, but also provided the surgeon with a safe and effective new technique.

The fasciocutaneous flap is very useful in the repair of soft tissue defects on the lower leg. It is easy to design and construct long flaps that are safe because of good circulation.

Material and Methods

Between April 1984 - May 1986 four patients with lower leg defects underwent surgical repair at Hacettepe University Medical School,

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Department of Plastic and Reconstructive Surgery. The youngest patient was 17, and the oldest 28, the average being 24 years.

Three defects to be covered were on the heel and one on the ankle.

The flaps were planned on the opposite leg with the base always sited proximally. The incision was carried right through the skin, subcutaneous tissue and fascia, and flaps comprising of skin fat and fascia over the gastrocnemius muscle were raised. The length of the flaps were between 15-22 cm. The base varied from 5 to 8 cm and the length/width ratio of the average flap was 3/1.

The skin surrounding the defect was excised and the edges of the wound undermined. The fasciocutaneous flap was rotated into position and the fascia was sutured beneath the undermined skin. The secondary defect was covered immediately with a split-skin graft. 15 days after the first operation the base of the flaps were divided and the flaps were transplanted to the defects.

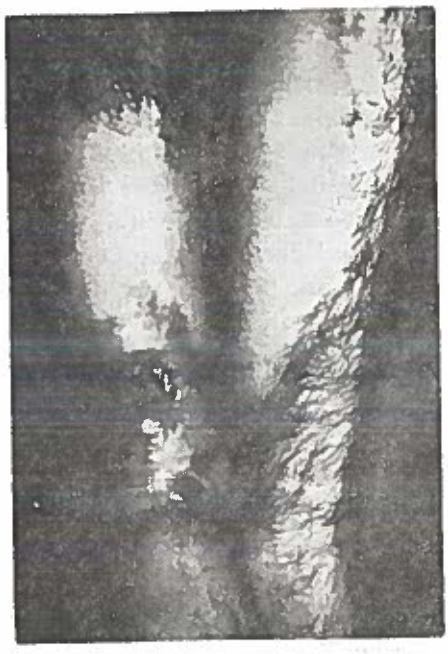

Figure 1
An open wound complicated with osteomyelitis.

Figure 2
The cross-leg fasciocutaneous flap in position.

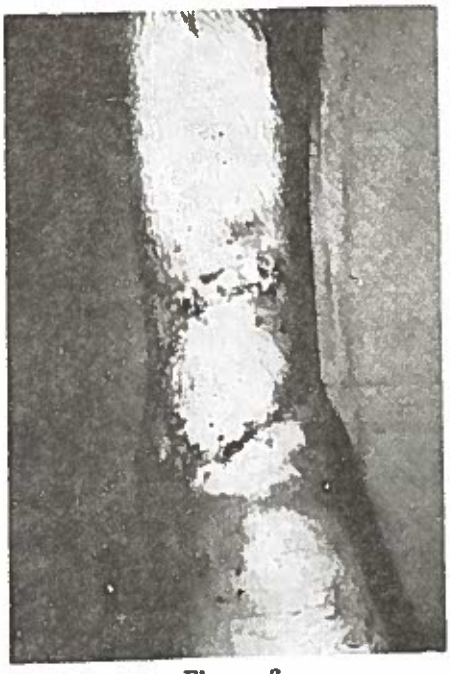

Figure 3
Appearance of the same patient 6 months after the operation.

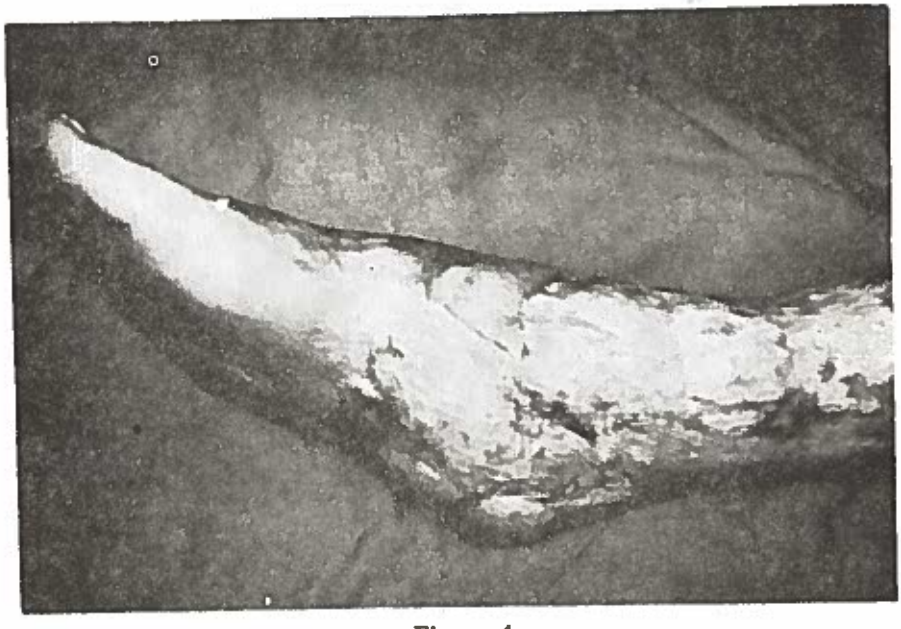

Figure 4
This patient was unable to walk because of the tissue loss on her heel.

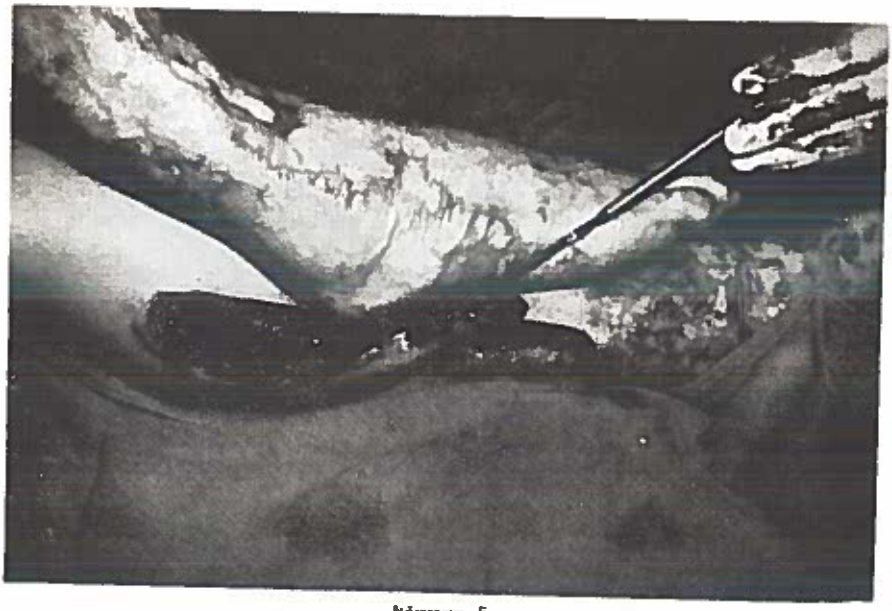

Figure 5
The cross-leg fasciocutaneous flap is transferred to the defect.

Figure 6
Appearance of the same patient 6 months after surgery.

Results

In three cases the flap healed completely. In one case necrosis occurred on the tip of the flap. The damage was superficial, and when the necrotic skin was excised there was viable vital fat and fascia underneath. Granulation tissue formed quickly and the defect was succesfully grafted.

Considering the large areas to be covered and the complicating problems, these results seem satisfactory (Figure 1-6).

Discussion

After its description by Ponten in 1980 several clinics have reported their experience with fasciocutaneous flaps.[3, 4]

The inclusion of the deep fascia in flaps designed in the lower extremity appears to augment flap viability. Dissection of the flap is simple. Since the underlying muscle is left intact, there is no question of functional impairment, which is an important consideration when myocutaneous or muscle flaps are used. Moreover, in the absence of muscle, the flaps are far less bulky, and transposition of a fasciocutaneous flap is considerably easier than transposition of a myocutaneous flap.

There is very little bleeding from the dissected surfaces of these flaps because of the plane between the fascia and muscle is relatively avascular and can be opened up with ease. The exposed muscle accepts a skin graft readily. The contour defect is usually not so obvious that it is left following the use of a myocutaneous flap.

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Fragile Site on Chromosome 16

Güven Lüleci, Ph.D.* / Aynur Acar, Ph.D. / Gülseren Bağcı, Ph.D.*****

Summary

The importance of genetic risks associated with the fragile site on chromosome 16 is not yet fully understood. We found a fragile site on chromosome 16q22 in a woman who had recurrent abortions in the first trimester. The fragile site was in general mono or dichromatid breaks and fragments. Further investigations of family members revealed that her two sisters, two brothers and mother had normal karyotypes, but her youngest brother had a fragile site at 16q22.

Key Words: Fragile site, chromosome 16.

Introduction

For the first time, Dekaban has reported the fragile site on the long arm of a C group chromosome in 1965.[1] Then several fragile sites have been recognized and documented on human chromosoms.[2] These include 2q11, 2q13, 3p14, 6p23, 6q26, 7p11, 8q22, 9p21, 9q31, 10q23, 10q25, 11q23, 12q13, 16q23, 17p12, 20p11, Xq26, Xq27. In addition many chromosome breaks and gaps can be induced by the addition of certain chemicals.[3-6] Hereditary fragile site chromosome 16q22 is inherited by the simple Mendelian dominant transmission.[7] The significance of fragile chromosome 16q22 has not yet been explained. Therefore, we wish to report a new family having a fragile site at 16q22.

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Materials and Methods

A couple was referred to our department because of infertility due to repeated abortions in the first trimester. The wife was gyneocologically normal. The couple were not related, were married for 8 years and had no children. They were not taking any drugs, they had not been exposed to ionized irradiation and they had no clinical signs of any disease known to cause spontaneous chromosomal breakage syndrome.

Peripheral blood for routine cytogenetic studies was cultured in Mc Coys 5A supplemented with 20 % fetal calf serum[8] The chromosomes were banded by Trypsin-Giemsa technique.[9] A few months later, cytogenetic analysis was repeated in the female, two brothers, two sisters and her mother. The patient's father was not alive.

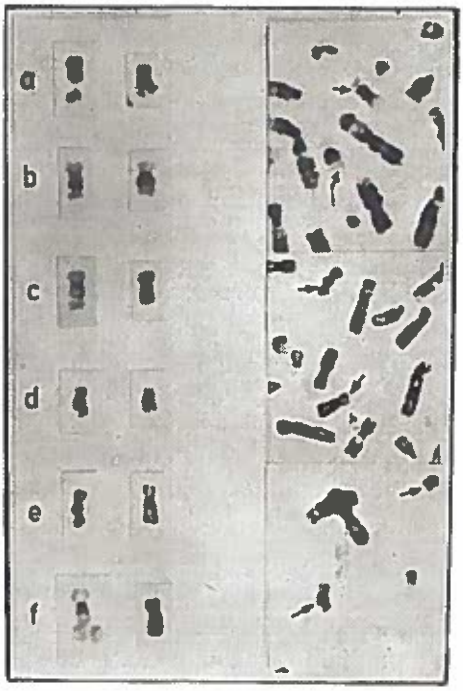

Figure 1
Hereditary fragile site on chromosome 16q22. a) Fragment, b-c) dichromatid break, d-e) monochromatid break, f) triradial configuration.

Results

Cytogenetic analysis was carried out on this patient who was referred to our laboratory because of four recurrent abortions in the first trimester. The patient presented fragility 16q22 in forms of mono-dichro-

matid breaks and fragments in the cells examined. There were no other chromosomal abnormalites. A few months later the cytogenetic analysis was repeated in this patient. The fragile site at 16q22 was constantly observed in 40 % of the cells. Dichromatid breaks (26 %), monochromatid breaks (9 %) fragments (4 %) and triradial figure (1 %) were observed (Figure 1). The patient's family were also examined to investigate the possible inheritance of fragile site 16q22. Her sisters and two brothers were married and had healthy children (Figure 2). But a fragile site on chromosome 16q22 in 38 % of cells as monodichromatid breaks (34 %), and fragments (4 %) was found in her youngest brother.[17]

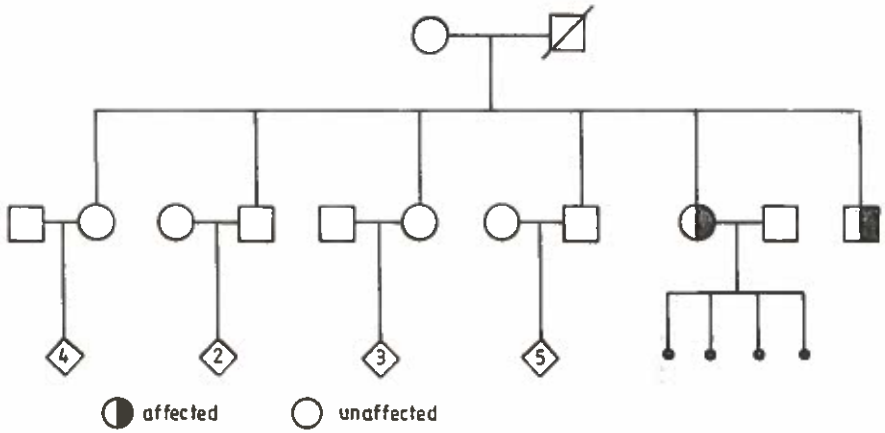

Figure 2
Pedigree of family.

Discussion

Fragile X chromosome with mental retardation is known to be clinically important and has been reported by many researchers.[10, 11] The origin and clinical importance of autosomal fragile sites however, is uncertain.[2] Various fragile sites on chromosome 16 of this group were identified.[12, 13, 14] Magenis *et al.* examined several generations of a large family of 298 and observed a fragile site on chromosome 16 in 30 of them.[7] Hereditary transmission was possibly autosomal dominant. 14 couples with the trait had children with major chromosomal abnormalities. Abortion rates for carriers were more frequent than unaffected couples. Following studies demonstrated fragile 16q21-22 in lymphocyte cultures of some aborting women.[13, 15]

In our case, fragile 16q22 was observed in a 30 years old woman who had first trimester abortions, and her 17 years old brother also had the same defect. Both the patient and her brother were healthy. The fact that

fragility was found only in 2 of 5 children of the family and that the mother had a normal karyotype suggests that the father was responsible for the Mendelian transmission. Although the clinical significance of 16q 21-22 is uncertain, chromosomal abnormalities were reported by different scholars in some children of carrier cases.[7, 13, 15, 16, 17] Moreover, Hecht's study an abortions and stillbirths has revealed a high proportion of fragile sites and chromosome rearrangements in the parents' chromosomes.[18] Similarly, recurrent abortion in our case might be due to these chromosome anomalies seriously affecting the fetus. The bachelor brother will be followed for possible anomalies in his siblings.

This study reports a fragile 16q22 for the first time in Turkey and supports the findings of other authors about the relationship between spontaneous abortion and this fragility. Reports of more cases are required to be able to substantiate if fragile 16q21-22 leads to spontaneous abortions and *de nova* structural chromosome changes. When such an association is established, prenatal diagnosis can be suggested to carrier parents.

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A Case Report of Typical Turner Syndrome with Rare Karyotype

Güven Lüleci, Ph.D.* / Gülseren Bağcı, Ph.D. / İsmet Aydın, Ph.D.*****

Summary

Sex chromatin analysis was found to be 0 % negative in a 16 year old case with a typical Turner Syndrome. By performing chromosome analysis, 45 XO/46X,r(X) karyotype, rarely reported in the literature were observed. The patient was treated by cyclic Estrogen therapy. She had menstruation and grew 5 cm taller. In turner syndrome, in addition to the sex chromatine determination, it is emphasized that chromosome analysis must also be performed.

Key Words: Turner syndrome, chromosome abnormalities.

Introduction

In 1938, Turner described a syndrome which is characterized by dwarfism, sexual infantilism, webbing of the neck and many other congenital abnormalities.[1] After development of suitable techniques for chromosome constitution, Ford *et al* found that this disorder has only one X chromosome on their karyotype.[2] Afterwards, many varients of Gonadal Dysgenesis have been reported.[3, 4] We recently examined a 16 year-old phenotypic female with this syndrome who has a rare chromosomal abnormality.

Case Report

R.B., a 16 year-old girl, was born after an uncomplicated pregnancy. At birth, her mother was 23 and father 27. When she was 13, her parents

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had noticed short stature and sexual infantilism. On physical examination the patient appeared well except for shortness. Her height was 125 cm and her weight was 42 kg. There was a short webbed neck with a low hairline in the back and shieldlike chest with widely separated nipples. Duffness over the dorsum finger and cubitus valgus were the other abnormalities. She was sexually infantile except for sparse pubic and axillary hair. She also was amenorrhoea. There were no cardiologic defects.

The girl was referred to our laboratory by a physician. First, sex chromatine analysis of 100 cells was performed. The method of Moorhead *et al* was used to examine the chromosomes.[5] Lymphocytes were cultured in Mc Coy's 5A supplemented with 15 % fetal serum. The chromosomes were examined by Trypsin-Giemsa banding and C banding.[6, 7] Serum hormon levels were measured by RIA technique using Amersham kit.

Results

The patient was referred to our laboratory with prediagnosis of Turner syndrome to determine chromatin constitution. Sex chromatin analysis was 0 % negative. Since many different karyotypes for ovarian dysgenesis were found, chromosome analysis was performed. In addition to the 45 X chromosomal constitution, 46,X,r(X) karyotype was observed in 90 of 100 cells examined by the Giemsa banding method. At the same time, there was 2 % enderoduplication and rarely breaks and gaps on different chromosomes. By using C-banding, it was shown that the ring chromosome had centromer and was very small in size (Figure 1). The chromosomes of the patient's family were analyzed. No abnormality was found. Results of endocrinological examinations are summarized in Table I. The patient was treated by cyclic Estrogen therapy. One year later, she had menstruation and grew 5 cm taller.

TABLE I

RESULTS OF ENDOCRINOLOGICAL EXAMINATIONS

Tests	Examined	Normal
T_3	1,34 ng/ml	0,8 – 2ng/ml
T_4	9 mg/dl	4,5 – 11,5 mg/dl
TSH	3,4 m/u/ml	0,5 – 5 m/u/ml
FSH	64 m/u/ml	1,7 – 8,5 m/u/ml
LH	2,9 m/u/ml	2 – 15,4 m/u/ml
Ostradiol	14,5 pg/ml	20 – 120 pg/ml
Progesteron	0,84 ng/ml	0,2 – 1 pg/ml

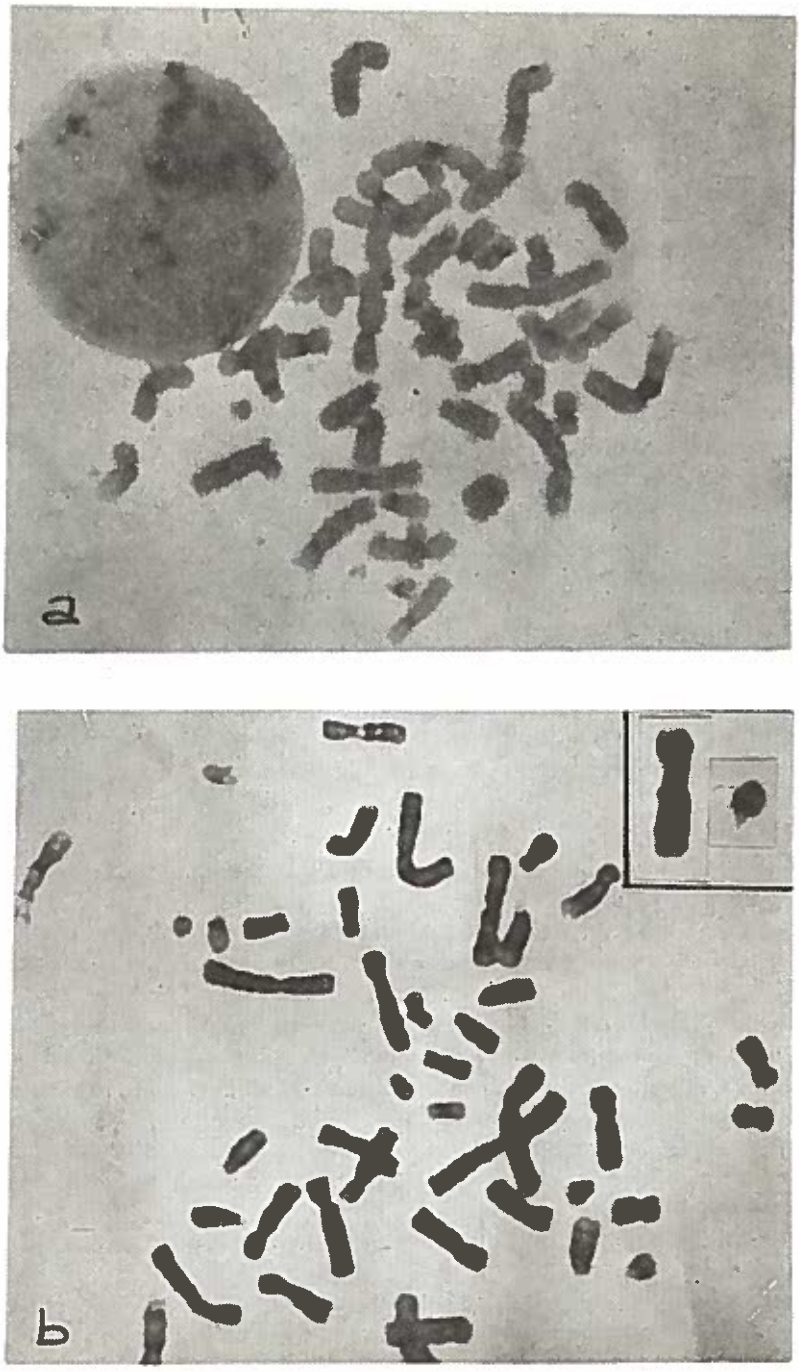

Figure 1
Ring chromosome of the x in the case. a) C-banding b) Giemsa-banding.

Discussion

In addition to the observations made in the Turner syndrome which is cytogenetically defined as 45,X0, different karyotypes were observed in the recent years.[3, 4-8] These patients have no sex chromatin. When chromosome analysis was performed, different mosaicism patterns, ring and isochromosomal type abnormalities can be seen. Clinical findings vary according to the chromosomal differentiation. Therefore, sex chromatin analysis alone is not sufficient to establish the diagnosis. In our case, sex chromatin was negative, but chromosome analysis showed additional X ring chromosome in 90 % of lymphocytes. As illustrated in Figure 1, the ring was very small in size. From reports in the literature, the morphological appearance of the rings varies with the degree of metaphase contraction in the individual cell.[3-8] In our case the ring was the same size in all cells. The positive response to the cyclic hormone therapy and growing up may be a result of X ring chromosome. This explanation however, cannot be proved by available methods. There was 2 % enderoduplication but no sign of malignancy appeared.

Although the patient is clinically a typical Turner syndrome in respect to the findings of 45,X0/46,X,r(X) karyotype, it is cytogenetically original. In Turner syndrome patients, in addition to the sex chromatin determination, chromosome analysis must also be performed. Different karyotypical findings may give variable phenotypic expression.

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Sex Cord (Gonadal Stromal) Tumours of the Testis

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Summary

Sex cord/stromal (gonadal stromal) tumours of the testis constitute 20 % of childhood testicular neoplasms. Half of these are incompletely differentiated forms and malignant behaviour is unusual. We herein describe an additional case of gonadal stromal testicular tumor with a focus on Sertoli cell differentiation in a fourteen-month old boy.

Key Words: Childhood testicular tumours, Gonadal-stromal tumour, Sertoli cell carcinoma, sex cord/stromal tumour.

Introduction

Whilst approximately 93 % of all testicular neoplasms in adults are of germ cell origin; sex cord/stromal tumours constitute 20 % of all testicular tumours in children.[1,2] Mostofi and Price[3] regard Leydig and Sertoli cell types as specialized gonadal stromal cells and accordingly use the term "gonadal stromal tumour". Marshall *et. al.*[4] on the other hand, prefer the term "sex cord-stromal tumour" to point out the embryologic origin and epithelial shape of Sertoli and Leydig cells.

Endocrinological manifestations such as precoccious puberty or gynaecomastia are more common with Leydig cell tumours[2,4], but may also be observed in Sertoli cell tumours.[5] Only 10 % of Leydig and

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Sertoli cell tumours have been reported to be malignant.[4, 5] The criteria for malignancy have not been clearly defined for Sertoli cell tumours[1], but according to Godec[5], increased mitotic figures, cellular pleomorphism and poor differentiation with scanty tubul formation suggest malignant behavior.

Case Report

A fourteen month old boy presented with a mass in the right hemiscrotum. The parents apparently observed an increase in size of the mass which was first noticed at 8 month of age. On physical examination, the right testicle was firm and tender measuring 5 x 3,5 x 2,5 cm. The boy was otherwise healthy with no evidence of endocrinological abnormality. Chest and abdominal X-rays, liver and kidney function tests, α-fetoprotein (AFP), β-human chorionic gonadotropin (β HCG), testosteron and oestradiol levels were within normal limits.

A right inguinal orchiectomy was performed. Grossly, the tumour was whitish-yellow and firm in consistency with a maximum diameter of 4 cm (Figure 1). The margins were indistinct and the surviving parenchyma occupied only a small part.

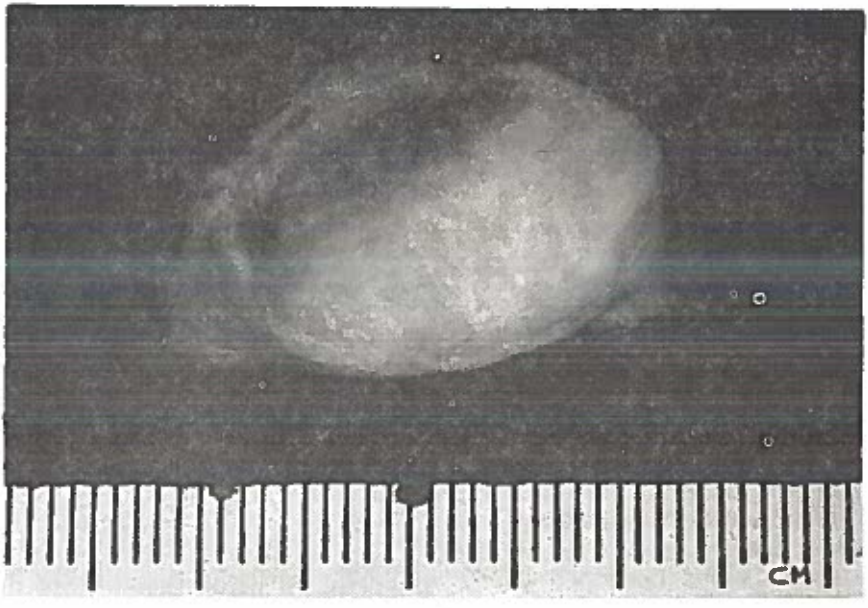

Figure 1
Gross appearance of the tumor. Note lack of encapsulation.

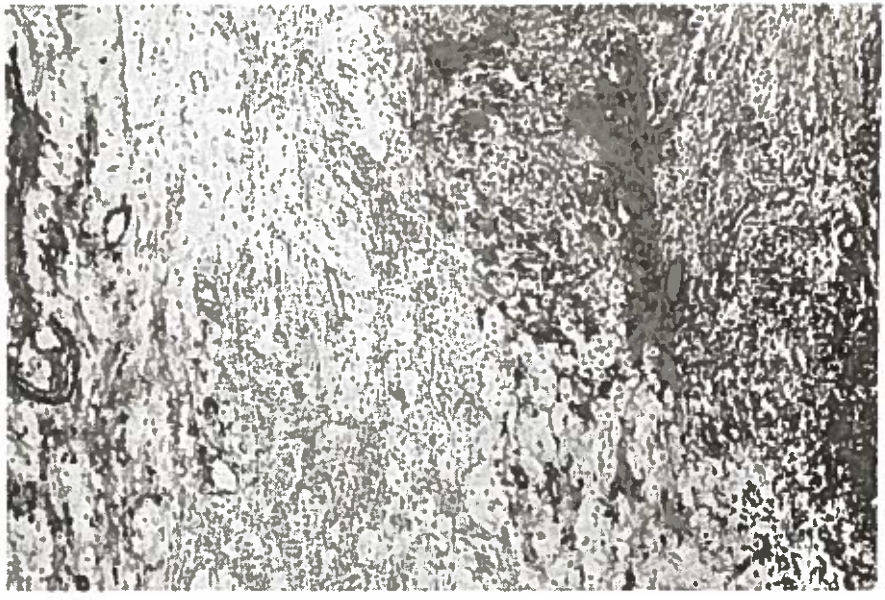

Figure 2

Low-power view of an area showing vacuolated cells of stromal origin.

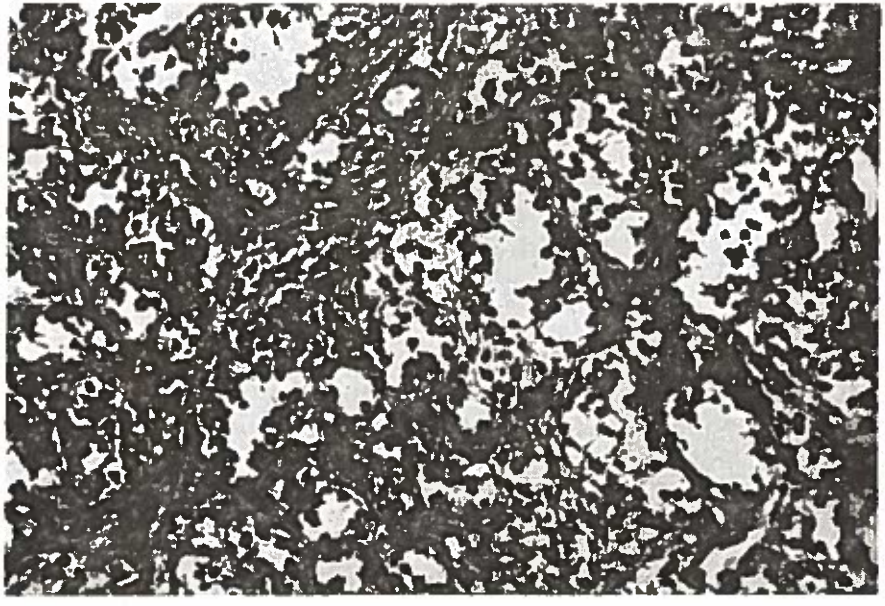

Figure 3

High-power view of tubular structures which were not a prominent feature.

Histologically, the tumour was found to infiltrate the adjacent parenchyma diffusely and contained cells with small oval nuclei and little cytoplasm as well as oval-slender fibroblast-like cells. There were also large groups of vacuolated cells with small round nuclei and large, pale cytoplasm (Figure 2). The former cells showed distinct tubular orientation in some fields and this feature was considered as evidence of Sertoli cell differentiation (Figure 3). Gomori's reticulum and von Gieson stains disclosed abundant reticulin fibers and collagen respectively. No necrosis or cystic degeneration were seen and mitoses were very infrequent. The epididymis and tunica were found to be free of tumor.

Discussion

Together with the present case, there have been some 86 cases of testicular sex cord/stromal tumours reported.[2, 5, 6] Ten of these occurred in newborns.[7] Malignant behavior was noted in 11 cases only one of which was prepubertal.[5]

Stemming partly from our limited knowledge on the origin and biological behaviour of this rare group of neoplasms, there still exists some confusion on the nomenculature. Godec[5] uses Sertoli cell tumour and gonadal stromal tumour synonymously, whereas White and Mc Carthy[6] regard Leydig cell tumors as a separate group from gonadal stromal tumours. According to the WHO classification, however, Leydig and Sertoli cell tumours are well-differentiated forms of sex cord/stromal tumours.[5] The incompletely differentiated forms may be with or without focal differentiation.[3]

Excluding some endocrinological manifestations seen more frequently in Leydig cell tumours, no clinical clue to the diagnosis has been described.[4] Gynaecomastia generally heralded malignant behaviour, irrespective of the histologic type.[5, 6] No formulation could be made to determine the malignancy of sex cord/stromal tumours histologically, but certain features such as pleomorphism, high mitotic rate and invasion might help in differentiation.[1, 6]

Immunohistochemical profile of a newborn[7] and a 4 month old boy[1] showed no positivity for AFP, carcinoembryonic antigen (CEA) and β HCG in the former and no positivity for AFP, CEA and oestradiol in the latter. Although we could not carry out immunohistochemical studies in this case, the blood levels of tumor markers and of sex hormones were within normal limits.

Orchiectomy alone is regarded as sufficient to cure all the patients without local invasion and metastases[1, 5], and almost all the metastases

occur within 5 months after the operation.[1] Our follow-up period is now one year and the patient is completely well and disease-free. Although these tumors are generally benign, we recommend regular follow-up just as we do in the case of the germ cell tumor of the testis.

Acknowledgement: The authors are grateful to Dr. R.E. Scully, Massachussets, USA for reviewing the slides.

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Bilateral Microsurgical Salpingostomy

A Case with Recurrent Tubal Pregnancy and its Successful Outcome

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Summary

A case with recurrent ectopic pregnancy in the contralateral fallopian tubes who was treated by microsurgical salpingostomy twice on each side, is presented. Following bilateral salpingostomy, a normal intrauterine pregnancy was achieved.

The purpose of this report is to discuss the surgical management of tubal pregnancy with emphasis on the preservation of fertility. This case report supports linear salpingostomy as the optimal treatment for early ectopic pregnancy.

Key Words: Ectopic pregnancy, microsurgical salpingostomy, preservation of fertility.

Introduction

Approximately once in every 100 pregnancies, the gestational sac attaches outside the uterine cavity, mostly within the fallopian tube. Ectopic pregnancy tends to recur in one third of the patients. The classical approach to the management of ectopic pregnancy has been the removal of the tube which may result in the removal of both fallopian tubes in a young woman with recurrent ectopic pregnancy. The overall viable pregnancy rate following salpingectomy would be around 30 % at best.[1] Conservative surgical procedures utilizing microsurgical techniques rather than salpingectomy, have improved fertility with success rates varying between 40-72 %.[2,3]

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In this report, an infertile young woman with recurrent ectopic pregnancy in opposite tubes who was treated by salpingostomy each time, is presented. She currently has an ongoing normal 38 weeks' intrauterine pregnancy. This report supports the view that preservation of the involved tube should be considered when fertility is desired by the patient.

Case Report

S.A (record no. 1756738), a 25-year-old married woman presented with primary infertility on February 22, 1985. She also complained of oligo-amenorrhea and hirsutism for more than 4 years. Initial infertility evaluation showed normal semen analyses and bilateral tubal patency associated with a normal shaped uterus on the hysterosalpingogram. Hormonal profile was compatible with anovulation and ovarian hyperandrogenism was indicated by high serum lteinizing hormone (LH) and testosterone levels. She was somewhat obese and had clinical features of moderate hirsutism. It was suggested that she should have an ovarian wedge resection after she failed to respond to Clomiphene Citrate and Bromocryptine elsewhere. Clomiphene (Clomid-Merrell) was started at a dose of 50 mg per day between days 3 through 7 with human chorionic gonadotropin (Pregnyl-Organon), 10 000 units intramuscularly on day 14 of the cycle. Upon failure to respond to Clomiphene, as indicated by no response during 6 months of treatment with increasing amounts of Clomiphene (up to 200 mg/day for 5 days) Human Menopausal Gonapotropin (Humegon-Organon) was started to induce ovulation.

HMG injections were administered on an individualized base, monitoring of the ovarian follicles with serial ultrasonography. HCG was given when dominant follicle was visualized ultrasonographically and ovulation occurred as confirmed by repeat ultrasonography and elevation of basal body temperature. She conceived in the first treatment cycle but started having vaginal spotting soon after delay in the menstrual period. The suboptimal increase in serial serum β-HCG levels and the absence of gestational sac in the uterine cavity suggested the possibility of an early ectopic pregnancy. Laparoscopy done under general anesthesia on May 8, 1985, revealed an intact, unruptured ectopic pregnancy, 4x2 cm in dimensions in the ampulla of the right tube. A linear incision was made on the antimesenteric border of the tube using micro-unipolar cautery and the products of the pregnancy was expelled by gentle massage. No special effort was used to remove all placental tissue from the implantation site and micro-bipolar cautery was utilized for hemostasis. The pelvic cavity was lavaged thoroughly with saline solution that included heparin. Microsurgical linear salpingostomy was performed and the

patient was discharged on the 6th post-operative day following an uneventful recuperation period. The hysterosalpingogram, 3 months after surgery revealed tubal patency of the involved fallopian tube.

Since oligo-amenorrhea and infertility problems continued, the patient applied to the Infertility clinic for the second time. A second course of HMG-HCG regimen was given and ovulation was achieved in the same cycle. Basal body temperature remained elevated, serum B-HCG level increased and vaginal spotting started again, identical with the first unfortunate clinical picture. On January 4, 1986 she underwent another laparoscopy and microsurgical linear salpingostomy, this time for a recurrent tubal pregnancy, measuring 3x2 cm in the ampulla of the left tube. The opposite tube which was operated previously looked completely healthy. Upon discharge from the hospital, she was re-evaluated hormonally and the serum testosterone level was found to have further increase associated with changes compatible with the ongoing status of anovulation. Nightly doses of Dexamethasone, 0.5 mg/day successfully suppressed serum androgen levels, but anovulation continued. In November 1986, ovulation induction was attempted by the same treatment regimen, this time including Dexamethasone. She has finally conceived in the uterus and the pregnancy is continuing normally in the third trimester.

Discussion

Ectopic pregnancies have shown an increasing trend during the past decade. Pelvic tuberculosis may play additional role in the development of tubal pregnancy, especially in developing countries like ours'. The gynecologists who deal with infertile patients should suspect the possibility of ectopic pregnancy, even more since it was previously shown that the incidence of ectopic gestation among the infertile patient population is around 10 %.[4]

Selection of the proper operative technique is the single most important issue in the surgical management of tubal pregnancy. The rationale for conservation of the tube is that in more than 50 % of the patients the opposite tube is abnormal[5], 10 to 50 % will have another tubal gestation, often in the contralateral tube.

With improved methods of early diagnosis of ectopic pregnancy by means of the rapid, sensitive B-subunit of human chorionic gonadotropin pregnancy test, ultrasound with better resolution, and the wide application of diagnostic laparoscopy, the gynecologists must be prepared to deal with a higher proportion of unruptured ectopic pregnancies.

A patient who undergoes a conservative surgical procedure should fulfill the following criteria; she must be at a reproductive age and desire further pregnancies; the tube must be unruptured; she must be surgically stable; and her surgeon should be able to utilize the microsurgical technique. Among various methods of conservative surgery for ectopic pregnancy, salpingostomy seems to be the preferred technique. The effectiveness of linear salpingostomy for ectopic pregnancy can be analyzed better by evaluating patients with only one fallopian tube. Results of the review of at least 10 cases in the literature are summarized in Table I.

Conservative operations in selected cases of tubal pregnancy seem feasible and safe and do not further impair tubal function. Since intrauterine pregnancy is more apt to occur than is repeat ectopic pregnancy, it seems logical that the involved tube should be saved whenever fertility is desired.

TABLE I

THE RESULTS OF CONSERVATIVE SURGERY FOR TREATMENT OF ECTOPIC PREGNANCY IN PATIENTS WITH ONLY ONE FALLOPIAN TUBE

Reference	No. of Patients	Intrauterine pregnancies (%)	Repeat ectopic pregnancies (%)
DeCherney[3]	15	53	20
Suchet[6]	14	57	15
Vallez[7]	13	100	0
Caffier[8]	10	40	10
Jarvinen[2]	10	60	30

In our series of 16 cases of linear salpingostomy, which is in the process of publication, the viable pregnancy rate and recurrent ectopic pregnancy rate are 50 % and 12 % respectively. A more conservative approach, nonsurgical treatment of ectopic pregnancy with methotrexate, is currently being utilized by the author and the results will be published as a seperate report, elsewhere.

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Inclusion-Body Myopathy

New Clinical Forms and a Review of the Literature

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Summary

We analyzed 56 cases of biopsy-proven inclusion-body myopathy (IBM), 13 of which composed our series collected in 15 years. The remaining 43 represented the total number of reported cases until 1986. Electromyographic and histopathologic findings were consistent with previously described patterns, but the clinical presentation of our cases was unusually heterogenous. 61 % of them showed limb-girdle muscle weakness, while in 15.4 % distal weakness was noted. Three of our patients (23.1 %) presented with syndromes not previously associated with IBM. One patient had oculopharyngeal involvement, the second showed scapulohumeroperoneal pattern, and the last presented with scapuloperoneal weakness. This is the first report of IBM presenting with these three syndromes which are classically seen in the adult forms of muscular dystrophy or spinal muscular atrophies. IBM should be considered in the differential diagnosis of patients presenting with oculopharyngeal, scapulohumeral or scapuloperoneal syndromes.

Key Words: Inclusion-body myopathy (IBM), Oculopharyngeal, scapuloperoneal, scapulohumeral, basophilic-lined vacuoles, filamentous inclusions.

Introduction

Inclusion body myopathy (IBM) is a chronic progressive disease of the skeletal muscle which was first described by Chou in 1967.[1] The term IBM was coined by Yunis and Samaha in 1971.[2] This disease is considered to be a variety of idiopathic inflammatory myopathy. It is morphologically characterized by basophilic-lined vacuoles containing polymorphic osmophilic whorls and intracytoplasmic or intranuclear filamentous inclusions.[3] (Figure 1, 2)

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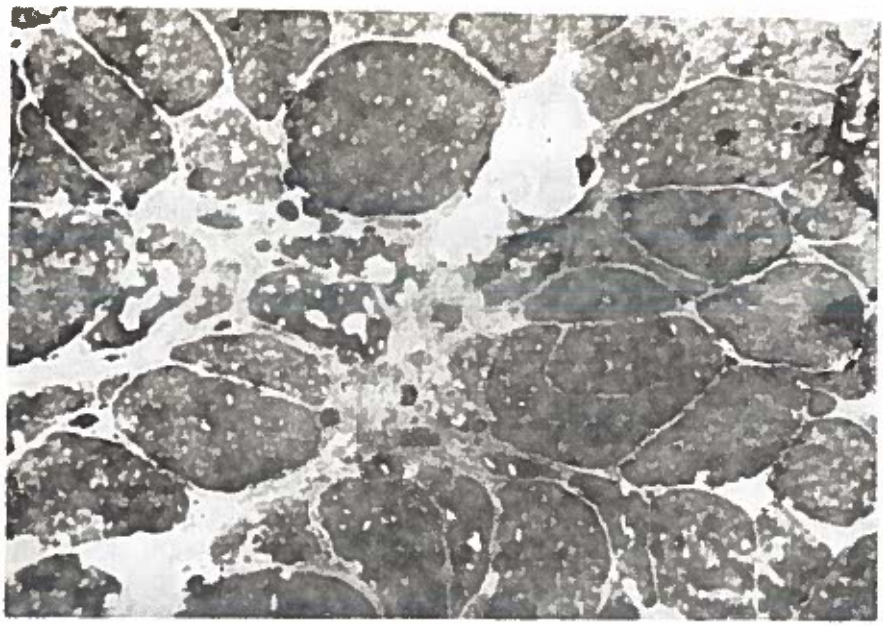

Figure 1
Horizontal section of striated muscle shows degenerated fibers containing vacuoles which are surrounded by normal muscle fibers (x400).

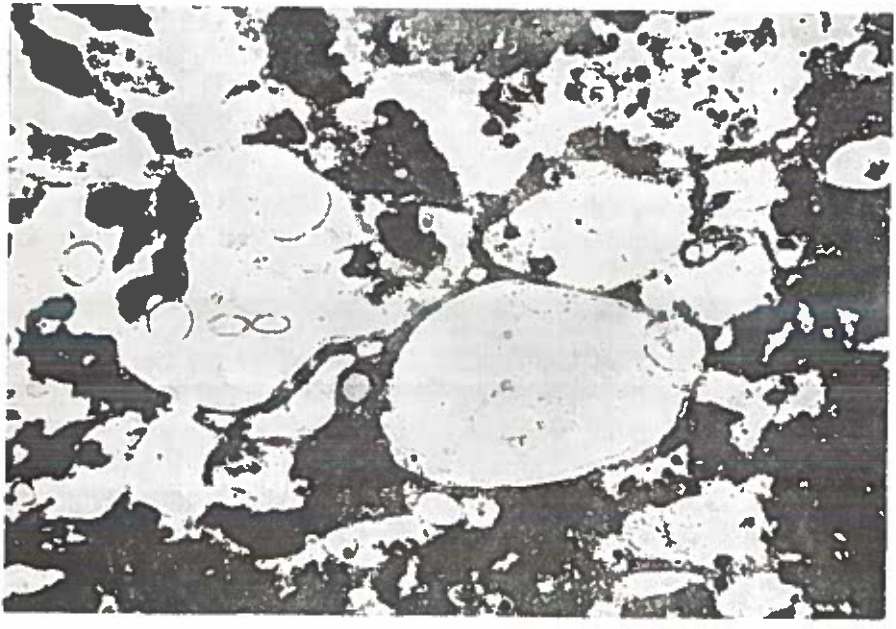

Figure 2
Electron microscopic examination of striated muscle shows intracellular and intracytoplasmic inclusions.

TABLE I

OUTLINE OF CLINICAL AND LABORATORY FINDINGS IN 56 PATIENTS WITH INCLUSION-BODY MYOPATHY

1. SEX: Males: 37 (66.1 %) Females: 19 (33.9 %)
2. AGE: Range: 22-85 years, Mean: 54.5 years (Males: 54.6, Females: 53.8)
3. DURATION OF SYMPTOMS: Range: 1 month – 27 years
   Mean: 5.5 years
4. DISTRIBUTION OF WEAKNESS:
   - Legs > arms : 25 (44.7 %)
   - Legs = arms : 21 (37.5 %)
   - } (82.2 %)
   - Arms > legs : 4 (7.1 %)
   - Legs only : 4 (7.1 %)
   - Arms only : 2 (3.6 %)
   - Proximal > distal : 28 (50.0 %)
   - Proximal = distal : 18 (32.1 %)
   - } (82.1 %)
   - Distal > proximal : 10 (17.9 %)
5. OTHER SYMPTOMS: Myalgia: 6 (11.8 %) Dysphagia : 8 (14.3 %)
6. DEEP TENDON REFLEXES (in 29 cases) : Depressed : 13 (44.8 %)
   Absent : 9 (31.1 %)
   Normal : 7 (24.1 %)
7. SERUM CPK (in 49 cases): Increased : 36 (73.5 %) Normal: 13 (26.5 %)
8. INFLAMMATORY CELLS (by biopsy in 47 cases):
   Positive: 31 (66 %) Negative: 15 (34 %)
9. A. EMG PATTERNS (in 43 cases):
   - Myopathic (including 3 myotonic as well) : 26 (60.5 %)
   - Myopathic + Neurogenic : 11 (25.6 %) } 42 (97.7 %)
   - Neurogenic : 5 (11.6 %)
   - Normal : 1 (2.3 %)
   - Fibrillations in 26 myopathic-pattern EMGs : 20 (77 %)
   - Fibrillations in 11 myopathic + neurogenic-pattern EMGs : 4 (36 %)
   - Fibrillations in 5 neurogenic-pattern EMGs : 4 (80 %)
   - Clinical pattern in 11 cases with myopathic-neurogenic pattern EMGs:
     a. 8 cases with equal involvement of arms & legs (72 %)
     b. 5 cases with predominantly distal involvement (45 %)
     c. 4 cases with predominantly proximal involvement (36 %)

   B. Single-fiber EMG (in 7 cases): MFD and MCD : increased in 7 (100 %)
   MCD (range) : 66-120 μsec
   MCD (mean) : 83.7 μsec

   C. NCV (performed in 9 cases): Mildly slow : 4 (45 %)
   Normal : 5 (55 %)
10. STEROID TREATMENT (in 24 cases):
    - No change : 15 (62.5 %)
    - Mild to moderate improvement : 6 (25.0 %)
    - Worsening : 3 (12.5 %)

TABLE II
CLINICAL AND LABORATORY FEATURES IN AUTHORS' 13 CASES

No of Cases	Sex Age	Duration of Symptoms	Clinical Form	Distribution of Weakness	Deep Tendon Reflexes	Serum CPK	E.M.G.	Inflammatory Cells
1	M/38	1 year	Scapuloperoneal	legs > arms proximal = distal	Depressed	No Information	Myopathic	–
2	M/60	1 year	Limb-girdle	legs > arms proximal > distal	Normal	↑	No Information	–
3	F/63	3 years	Limb-girdle	legs > arms proximal > distal	No Information	↑	Myopathic	–
4	F/44	1 year	Limb-girdle	legs > arms proximal > distal myalgia	Normal	↑	Normal	+
5	M/62	5 years	Limb-girdle	legs only proximal only	Depressed	↑	Myopathic Neurogenic	+
6	M/56	3 years	Oculopharyngeal	legs > arms proximal = distal ptosis, dysphagia	Absent	No Information	No Information	–
7	F/85	1 month	Distal	legs > arms proximal < distal	Absent	No information	Neurogenic	–
8	M/62	6 months	Limb-girdle	legs > arms proximal > distal	Depressed	↑	Myopathic	+
9	M/40	2 years	Limb-girdle	legs = arms proximal only	No information	↑	Myopathic Neurogenic	+
10	M/76	10 years	Distal	legs > arms proximal = distal	Absent	↑	Myopathic Neurogenic	+
11	F/43	3 years	Scapulohumero-peroneal	legs > arms proximal < distal	Absent	No information	No information	+
12	M/48	6 months	Limb-girdle	legs = arms proximal = distal dysphagia	Normal	↑	Myopathic Neurogenic	–
13	M/61	1 year	Limb-girdle	legs = arms proximal > distal dysphagia	Depressed	↑	No information	+

The purpose of this paper is to review all the reported cases in the literature together with the authors' 13 cases in order to analyze the various features of the disease and to describe new clinical and electrophysiologic characteristics in the light of current literature.[2-15] (Table I). From the analysis of our own cases, we concluded that IBM may present with quite diverse clinical manifestations, often in the form of various adult-onset muscular dystrophies, such as limb-girdle, distal, scapulohumeral, scapuloperoneal (or scapulohumeroperoneal), and oculopharyngeal types (Table II). However, the main symptoms are chronic and progressive limb weakness, which is usually more prominent in the lower extremities than in the upper and affecting the proximal muscles more so than the distal ones. Eight of our 13 cases (61.5 %) presented with limb-girdle weakness which is the most common clinical form. We have also seen cases with scapulohumeral, scapuloperoneal (or a combination of these two), oculopharyngeal or distal muscle involvement. Here we describe 4 of our 13 cases, 3 of which represents one of the new clinical forms mentioned above.

Materials and Methods

Case 1 : Limb-girdle type: R.N., a 62-year-old white male patient, was first seen in December 1985 with the complaint of leg weakness. He was unable to hold up his weight with his knees bent. He had also fallen several times and had difficulty arising from a sitting position and climbing stairs.

Neurological examination revealed normal mental status and intact cranial nerves. Motor system testing revealed 4+ /5 strength in left shoulder abductors and in the left biceps muscle. The left quadriceps muscle was also 4+ /5, as well as the hip flexors bilaterally. Marked quadriceps wasting was noted on both sides. Deep tendon reflexes were hypoactive and symmetrical in the upper extremities. The left knee jerk was absent, and the right knee jerk was +1. Ankle jerks were +1 bilaterally. Neurological examination was otherwise normal.

Case 2 : Scapulohumeroperoneal type: S.P., a 43-year-old white female patient with a family history of progressive muscular dystrophy in her mother and grandmother, complained of difficulty walking and getting up from a sitting position for the past 3 years. She was also noted to have foot-drop for one year.

Neurological examination revealed normal mental status and intact cranial nerves. Motor system testing showed minimal weakness in neck flexors (5-/5). There was mild scapular winging bilaterally. Biceps and triceps mucles, wrist extensors, and anterior tibialis muscles showed mar-

ked weakness bilaterally (0-1+/5). Iliopsoas and gluteus muscles were minimally weak (5-/5). Deep tendon reflexes were absent in both lower extremities. There was decreased pin-prick sensation in the lower extremities of the "stocking" type.

Case 3 : Oculopharyngeal type: R.W., a 56-year-old black male patient, had noted progressive right arm and left leg weakness for the past 3 years which was manifested by gait difficulty. He also had complained of left upper arm weakness for several months and drooping of the left eyelid for many years but with no recent progression. He had intermittent dysphagia. Family history was positive for "myasthenia" in son and daughter.

Neurological examination revealed normal mental status. Extraocular movements were full with subjective diplopia on lateral gaze bilaterally. There was ptosis on the left. The right orbicularis oculi muscle was weak, and the sternocleidomastoid and trapezius muscles were weak bilaterally. The patient's tongue protruded to the left. Motor system testing revealed wasting in both intrinsic hand muscles with 3+/5 strength. Marked wasting was noted in the left gastrocnemius and anterior tibialis muscles, and moderate wasting in the left hamstring and quadriceps muscles. The right gastrocnemius, anterior tibialis, as well as the right biceps and forearm muscles also showed some degree of atrophy. Deep tendon reflexes were all absent except for the right knee jerk. Other neurological findings were normal.

Case 4 : Distal type: F.B., an 85-year-old black female patient, was seen for weight loss and inability to ambulate for the preceding 6 months.

Neurological examination showed 4+ to 5-/5 weakness in the upper extremities. Lower extremity testing revealed 4+/5 weakness in the right hip flexors. Anterior tibialis muscle strength was 4+/5 on the right and 3+/5 on the left. The patient was areflexic in the lower extremities. Biceps and triceps reflexes were also absent.

Nerve conduction studies and nerve biopsy showed evidence of mild distal and predominantly sensory peripheral neuropathy.

In all these cases, and in the remaining 9, IBM was confirmed by electron microscopy. EMG was done in all patients.

Results

After analyzing the 43 cases reported in the literature and the 13 cases analyzed in the present study, the following conclusions are made:

The onset of IBM may occur between the ages of 22 and 85 years, with a mean age of 54.5 years. 50 % of the patients were over 60 years of age (Figure 3). Therefore, although it seems clear that there is a wide distribution in the age of onset, it can also be stated that IBM is primarily a disease of late adulthood. Two-thirds of the patients were male (66.1 %). The duration of symptoms ranged widely between one month and 27 years, with more than 65 % of the cases presenting with a symptom duration of from one month to 2 years or from 6 to 10 years (Figure 4). The mean duration of symptoms was 5.5 years.

Motor weakness began gradually and proceeded progressively involving primarily or exclusively the lower extremities in 29 of 56 cases (51.8 %) and the upper extremities in only 6 cases (10.7 %). In 21 of the 56 cases the weakness was diffuse and almost equal in degree in the upper and lower extremities (Figure 5).

The mode of limb involvement was also variable. Proximal predominance was noted in half of the cases (50 %), whereas distal muscles were affected more in only 10 cases (17.9 %). In 18 cases (32.1 %), proximal and distal muscle weakness was evenly distributed (Figure 6).

8 out of our 13 cases (61.5 %) showed a clinical pattern of the limb-girdle type. The remaining 5 cases presented with distal (2), scapuloperoneal (1), scapulohumeroperoneal (1), and oculopharyngeal (1) types (Figure 7).

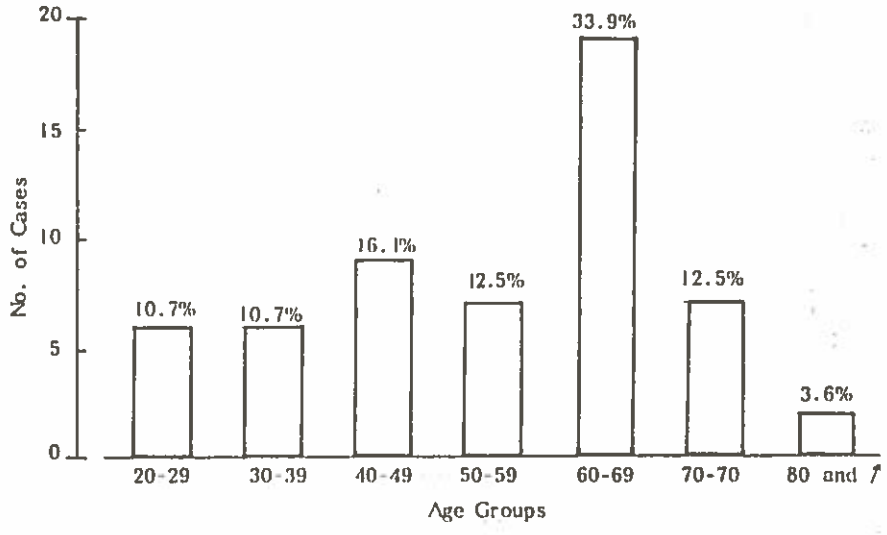

Figure 3
Distribution of cases according to age groups.

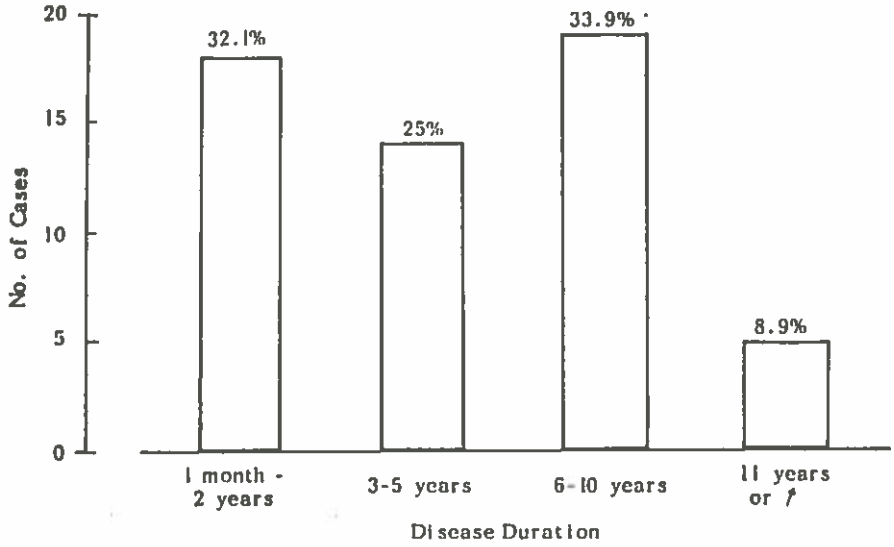

Figure 4
Distribution of cases according to symptom duration when first seen.

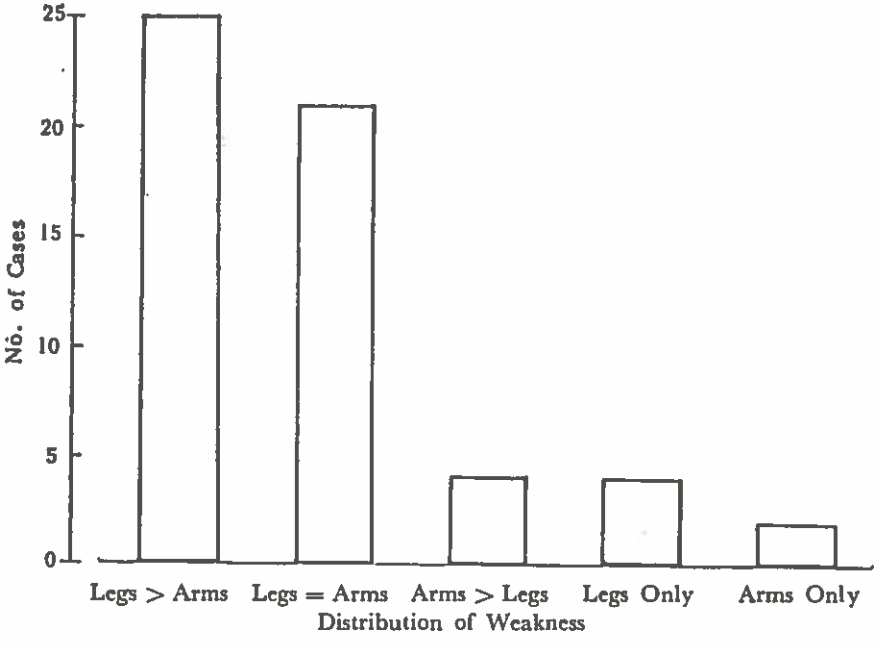

Figure 5
Distribution of weakness between upper and lower limbs.

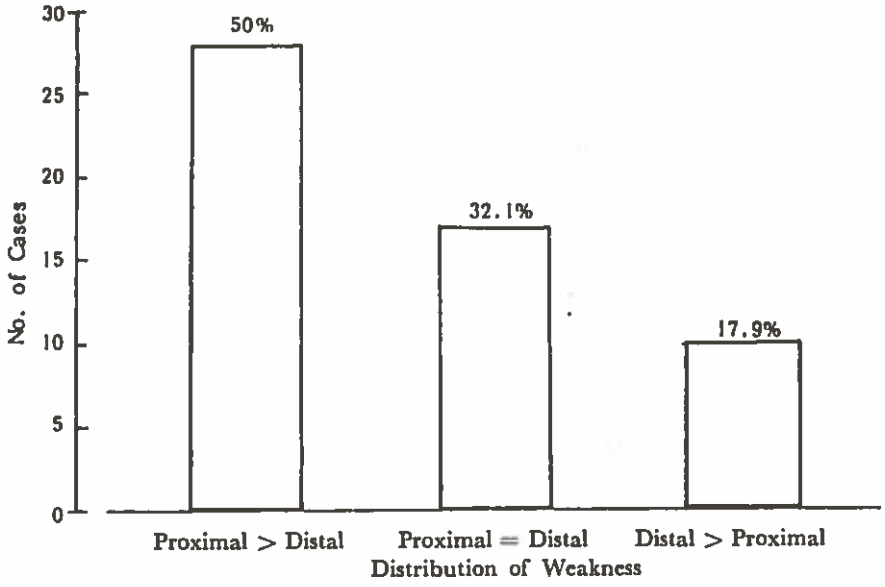

Figure 6
Distribution of weakness between proximal and distal limb muscles.

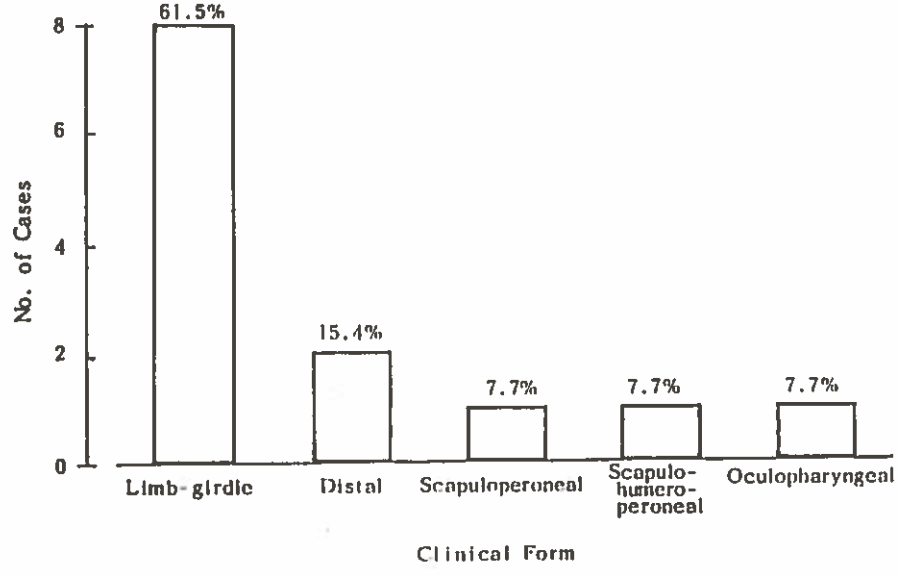

Figure 7
Distribution of authors' cases according to clinical presentation.

Myalgia was noted in only 6 cases (11.8 %) and dysphagia in 8 (14.3 %), both of which are known to be unusual symptoms in IBM.

Deep tendon reflexes were either depressed or absent in three quarters of the cases (75.9 %) and normal in the remainder.

An electromyogram was done in 43 of 56 cases, 26 of which showed pure myopathic changes (60.5 %), whereas 11 cases (25.6 %) revealed an association of myopathic and neurogenic findings. Only 5 cases (11.6%) showed electromyographic evidence of pure neurogenic disorder, and only one patient had a normal EMG. The highest percentage of fibrillations was found in cases with pure neurogenic findings (80 %), followed by 77 % in cases with pure myopathic changes (Figure 8).

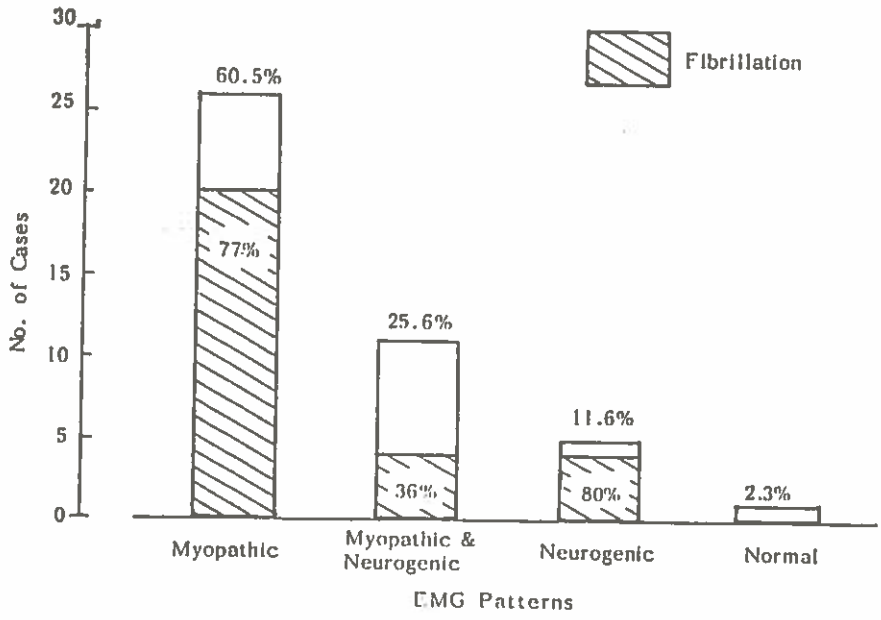

Figure 8
Electromyographic patterns.

Nerve conduction studies were done in only 9 cases, 4 of which showed mild slowing. The SFEMG was performed in 7 cases, all of which showed increased muscle fiber density and increased jitter.

The clinical pattern in 26 cases with pure myopathic EMG findings was that of predominant involvement of the legs and proximal limb muscles, as opposed to the arms and distal limb muscles. The clinical pattern in 11 cases with mixed myopathic-neurogenic electromyographic findings was that of an equal involvement of the upper and lower ex-

tremities in 8 cases (72 %), whereas in 5 cases with pure neurogenic electromyographic findings upper and lower limbs were affected approximately to the same degree.

Serum CPK was increased in nearly three-quarters of the cases (73.5 %) to varying degrees, ranging from twice to 15 times normal.

Muscle biopsy showed evidence of inflammatory myopathy in two-thirds of the cases (66 %).

Steroid treatment was ineffective in the majority of cases in the whole series (62.5 %). However, mild to moderate improvement was noted in one-fourth of the patients. Only 12.5 % of the patients became worse following this mode of therapy.

Discussion

IBM is known as a rare form of inflammatory myopathy which is clinically different from polymyositis and dermatomyositis in several aspects. It has a relatively benign and protracted course. It lacks the features of collagen vascular disease, as opposed to dermatomyositis and polymyositis. However, there was one case of IBM reported by Chad *et al.* which was associated with Sjogren's syndrome.[16]

IBM is clinically characterized by chronic progressive and usually painless weakness of the limb muscles, affecting the lower limbs more than the upper ones.[6] Dysphagia, like myalgia, is an infrequent symptom. Males are affected more than females. Although the disease may begin at any age from the third decade on, its usual onset is around the age of 60. Proximal muscles are more involved than distal muscles, but in a considerable number of cases the weakness is distributed evenly. Deep tendon reflexes are usually diminished or absent. Most cases show mild to moderate elevation of serum CPK. IBM is notoriously resistant to corticosteroid therapy in the majority of cases.[4, 17] There are occasional patients however, who may benefit from this form of treatment.

It is important to differentiate this disorder from polymyositis and dermatomyositis, the prognosis and treatment of which are quite different

Morphologically, electron-microscopic demonstration of abnormal filaments in the muscle cells is necessary for definite diagnosis, but IBM may be suspected from the findings in light microscopy of numerous hematoxylinophilic granules in lined vacuoles of the muscle cells which correspond to whorls of cytomembranes.[4]

Although IBM is generally regarded as an idiopathic inflammatory myopathy, some authors have pointed to a neurogenic origin, both

electrophysiologically and morphologically.[3] Grouped fiber atrophy and angular fibers were observed in several cases, which is a pattern seen after denervation. The type grouping pattern seen after reinnervation was not encountered. Target cells, which occur only in neurogenic disease, were seen in only one case. Fiber splitting, which is more common in neurogenic diseases, has also been described in IBM. Such morphologic features also favor a neurogenic origin and argue against a pure myopathic disorder in some cases.

In general, IBM is morphogically characterized by basophilic-lined vacuoles which contain polymorphic osmophilic whorls and intracytoplasmic or intranuclear filamentous inclusions in the skeletal muscle.[3,4] The nuclear inclusions are distinctly fibrillar and thus different from the inclusions of known viral diseases. There is usually a mild to moderate amount of inflammatory cells and mitochondrial abnormalities.

The prerequisite for diagnosis of IBM is the ultrastructural presence of intracytoplasmic masses of abnormal filaments as described above. These are more abundant in the cytoplasm than in the nucleus. They may be viral products, but virus isolation has not yet been successful, except for one case in the literature which was shown to be an adenovirus type[2, 18]

The morphological abnormalities described may be shown with light microscopy, but most authors believe that electron-microscopic studies should be done, especially for the demonstration of abnormal filamentous inclusions.

The basophilic-lined vacuoles seen in IBM have also been observed in oculopharyngeal and limb-girdle dystrophies, as well as in the distal, scapulohumeral, and scapuloperoneal forms of adult-onset myopathies. This suggest that IBM may present with various clinical myopathic patterns, the limb-girdle type being the most common.

Electrophysiologically, all cases have shown a mixed pattern of myopathic and neurogenic electromyographic findings. Neurogenic EMG findings may be secondary to denervation of the intramuscular nerve endings. This explanation does not militate against the proposed and widely accepted myopathic origin of the disease. Mild slowing of the nerve conduction velocities in some cases could also be secondary to neurogenic involvement, rather than a serapate peripheral nerve disorder. On the other hand, SFEMG, which was done in 7 cases, does not seem to shed light upon the myophatic or neurogenic nature of IBM, because increased muscle fiber density and high jitter values are signs of denervation as well as of a myopathic process.[3]

From analysis of the author's 13 cases, it is concluded that IBM may present with various forms of adult-onset muscular dystrophies, among which the limb-girdle type is the most common. IBM should, therefore, be ruled out in patients who may present with scapulohumeral, scapuloperoneal, oculopharyngeal, and distal myopathies. Although this can be accomplished by light microscopic study of skeletal muscle tissue, definite diagnosis rest upon electron-microscopic demonstration of intracytoplasmic masses of abnormal filaments.

Electrophysiological and morphological studies of all reported cases show that IBM is a primary disease of the skeletal muscle, usually with inflammatory changes and occasionally with neurogenic features as well. The 5 pure neurogenic cases may represent a rare and distinct form of this disease because 4 of these showed no evidence of inflammatory cells on the muscle biopsy and only 2 had mildly elevated serum CPK values. This implies a bimodal pathological spectrum of IBM.

From the electrophysiological viewpoint, the highest percentage of fibrillations in the EMG was seen in the pure neurogenic group (80 %), followed by the pure myopathic cases (77 %). Interestingly, in the mixed group with both myopathic and neurogenic features, fibrillations were found in only 36 % of the cases. This could be due to the longer duration of symptoms of patients in this group (averaging over 2 years).

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Ultrastructural Changes in the Rat Submandibular Gland Acinar Cells Produced by Isoprotorenol

Esin Aşan, Dt. Ph. D.* / Meserret Cumhur, Dt. Ph. D.**

Summary

Electron microscopic alterations of rat submandibulary gland acinar cells were studied after stimulation of secretion by a sympathomimetic amine, namely isoprotorenol. Ultrastructurally the most significant alteration was seen on the granule substructure reconstituted after IPR. These changes were interpreted as a reflection of accelerated synthesis of some secretory molecules. Additionally, previously unreported organization of golgi membranes and new granule formation were also discussed.

Key Words: Seromucous secretory granules isoprotorenol (IPR), golgi area.

Introduction

Isoprotorenol has been extensively used in studies concerning the secretory dynamics of various exocrine glands. There have been some conflicting results, however, on the effect of isoprotorenol in rat submandibular gland.[1,6] Submandibular gland of the rat is significantly different from the usual structure of mammalian salivary glands. Normally secretory end pieces or alveoli of salivary glands consist of either serous mucous, or seromucous cells (mixed gland). In the mixed glands serous and mucous cells are found together and some mucous cells are capped by serous demilunes. Yet, from a structural point of view in the submandibular gland secretory end piece consists of one type of cell resembling the mucous cell. These special mucous cells can be considered

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as seromucous cells (but not in the form of demilune) as far as the chemical nature of the secretory product is concerned.[7-9] The aim of this study is to observe the seromucous granule formation after stimulation of secretion at the ultrastructural level. An attempt also has been made to see whether IPR alters the composition of granule content, which corresponds to the fine structural changes in granule morphology.

Materials and Methods

Male Swiss albino rats weighing between 200-250 gr. were fasted 24 hrs. prior to the experiment. Water was available ad libitum. Dl (isoprotorenol) Hcl, (sigma) at a dose of 25 mg/kg. was injected intraperitoneally in % 0.9 saline. Control group animals received only % 0.9 saline. Animals were killed 24 hours after injection, their submandibulary glands were rapidly removed and processed for electron microscopy. Tissue samples were first fixed in % 2,5 gluteraldehyde and % 1 acrolein solution in M/15 phosphate buffer (pH 7.2) and then postfixed with % 1 osmiyum tetroxide in the same bufffer. After dehydration in ethanol, tissue slices were embedded in araldite. Contrasted thin sections were examined with a ZEISS EM 9S2 electron microscope.

Results

Controls Glands: Acinar cells were filled with characteristic pale secretory granules. Rough endoplasmic reticulum and the nucleus were located basally. Secretory granules were generally pale, oval or round structures with a fibrillary secretory material resulting in a flocculent appearance.Granule membranes were usually well preserved.The granules, however, tended to fuse forming large secretory vacuoles (Figures 1,2).

Three types of secretory granules were observed according to their electron density and intragranular substructure. Most of the granules contained unevenly distributed fine filaments. Filaments tended to concentrate in some regions of the granule. The rest of the granule matrix appeared unstructured. In some granules intra granular filamanteous structures were more prominent and condensed. These filaments came together very tightly to form relatively denser secretory granules. Granules of this type were usually found in the vicinty of the golgi complex. Very few granules possessed tubular or vesicular membraneous structures. The orientation of tubuler structures were random and appeared as different profiles according to the plane of sections. In some granules, tubular structures completely filled the granule while in other cases these structures were found to be located at the center of the granule or pushed towards one side. The remaining granule matrix was in the form of filaments (Figure 3).

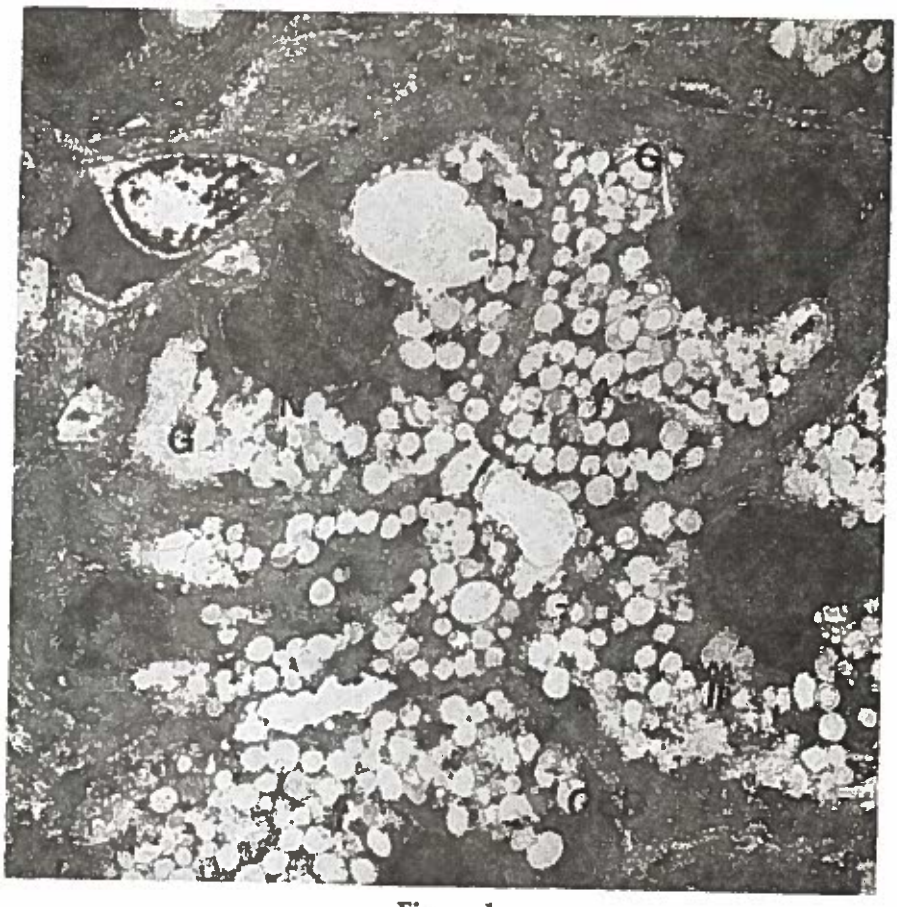

Figure 1

Acinar cells from the control group. Cells are filled with secretory granules with different density and internal structure. Large pale granules (arrow) are abundant. Denser granules containing tubular structure are also seen (double arrows) RER: Rough endoplasmic reticulum, N: Nucleus, M: Mitochondria, L: Lysosomes. x 6600.

In the fasting control group, secretory granule formation was also detected in some acini. In such an acini the golgi complex was quite extensive and had a different structure from those seen elsewhere. It consisted of very few parallel golgi stacks located very near to the rough endoplasmic reticulum, while many tubules and vesicles were scattered randomly in a large area (Figure 2). Dense granules containing tightly packed filaments were seen in the vicinity of the golgi complex.

These newly formed granules were incompletely surrounded by membranes (Figure 2). Small lipid droplets and very few lysosomal structures were also encountered in the control group. Large, long, irregularly shaped mitochondria with well developed cristae were especially abundant along the lateral cell boundries of the acinar cells.

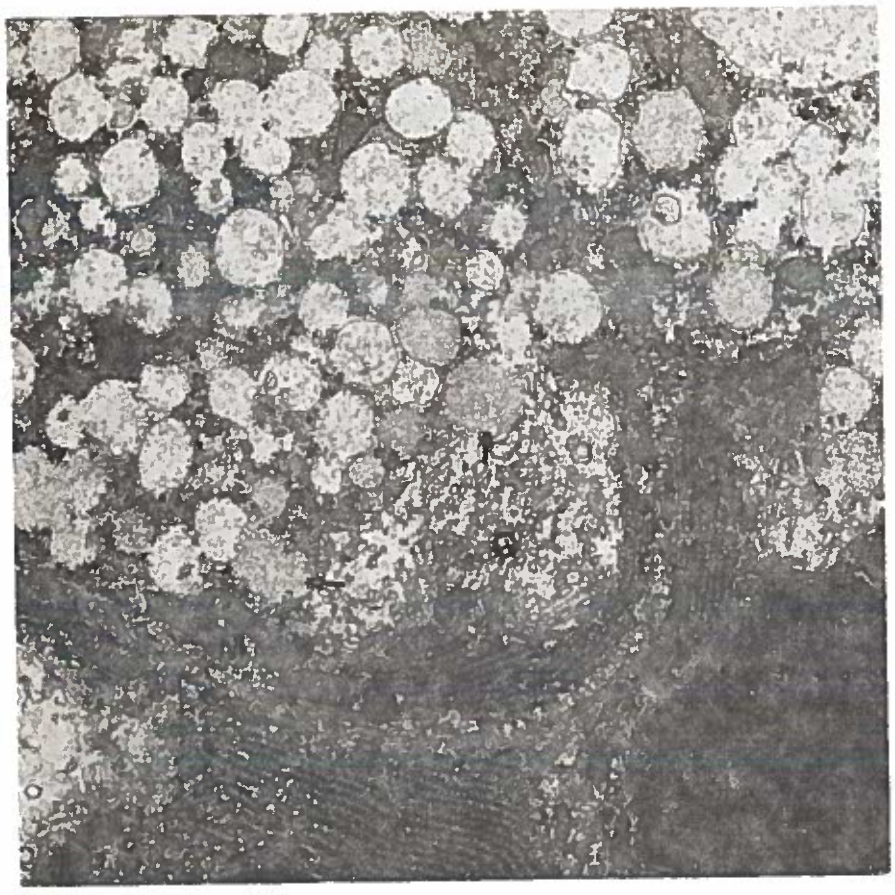

Figure 2

A portion of an acinar cell from control group showing parallelly arranged rough endoplasmic reticulum tubuli and adjacent golgi complex. Granules containing condensed fibrillar material are seen in the vicinity of the golgi complex (arrow). G: Golgi complex, M: Mitochondria, RER: Rough endoplasmic reticulum. x 14100.

Twenty four hours after IPR injection acinar cells were again filled with secretory granules. Most of these newly formed granules were much more electron dense than those of the control group. Pale granules containing loosely arranged fine filaments were rare, while relatively denser granules with densely packed filaments and those possesing tubular structures were predominant (Figure 4). The general overally preservation of the tissue was sufficent as judged by mitochondrial morphology, but the granule membranes were usually incomplete. The granules were often fused with each other and their contents became confluent (Figures 4,5).

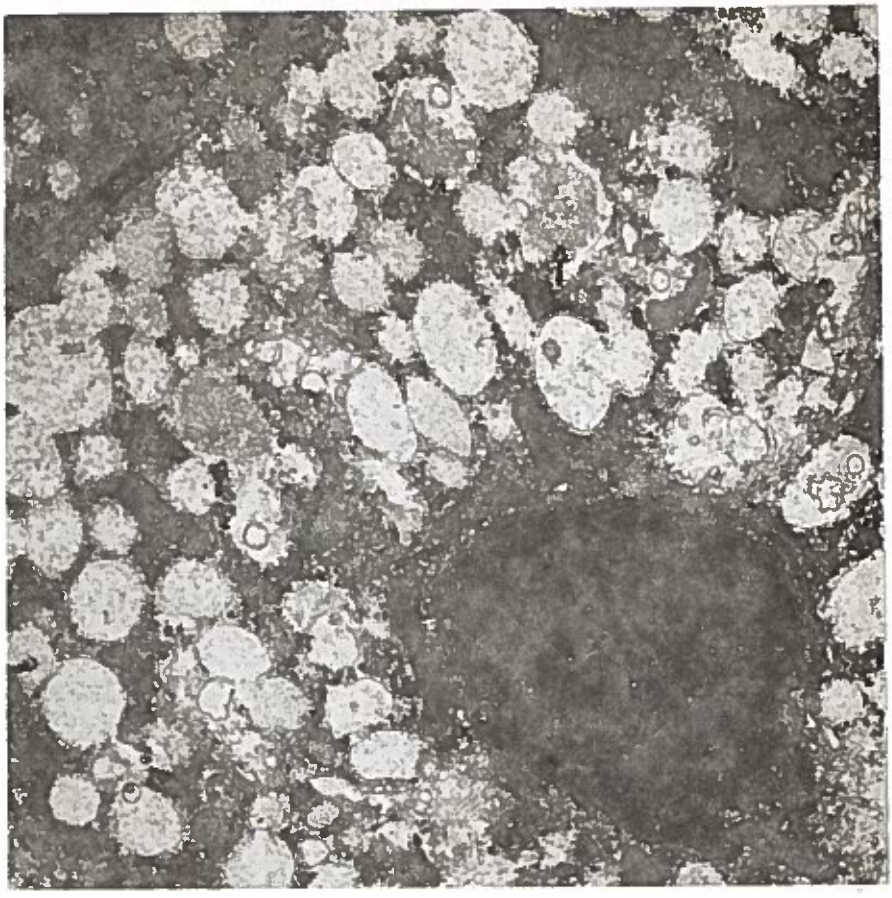

Figure 3

An acinar cell from the control group. Large pale granules are numerous. Granules with tubular structures (arrow) are also seen. x 14100.

There was no significant alteration in the development of rough endoplasmic reticulum. The parallel cisternae of rough endoplasmic reticulum, located in the basal region of acinar cells, were seen as was the case in the control group. The only notable difference was the extensive development of Golgi complex, indicating that cells were very active in packing the secretory product into secretory granules. Most of the cells contained an extremelly large golgi area adjacent to the rough endoplasmic reticulum (Figures 4,5,6,7,8).

These large golgi areas were mainly composed of short, often curved tubules, and associated vesicles with very few golgi stacks. These short tubules appeared to be originating from the golgi membranes. The

Figure 4

Parts of the two adjacent acini after IPR. Large pale granules are rare. Relatively small, denser ones are abundant. RER: Rough endoplasmic reticulum, N: Nucleus, M: Mitochondria x 6600.

vesicles represented the cross sections of tubules (Figure 6). The structure of the golgi complex was so different from the usual architecture of these organelles that the term "golgi area" was found to be more appropriate to designate it.

In some cells we have observed atypical or unusual arrangement of these tubular structures. Tubules were helically arranged around a homogeneous moderately dense material to form a large structure resembling a secretory granule. These unusual structures were often found to be located within the area of the rough endoplasmic reticulum (Figures 7,8).

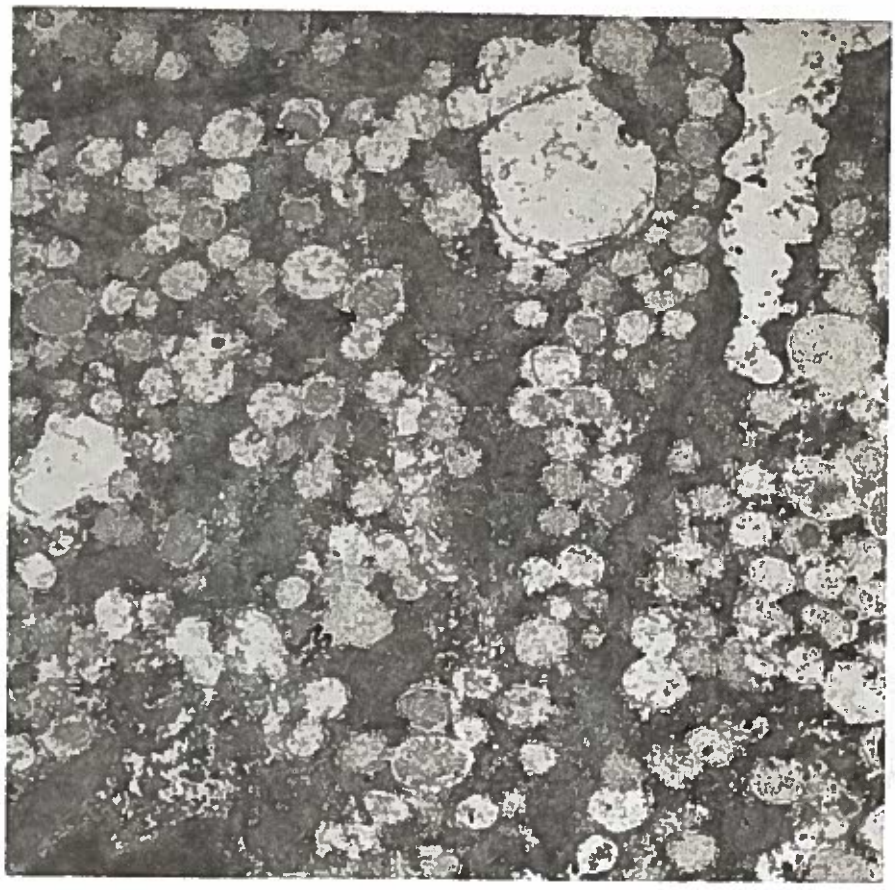

Figure 5
Details of the apical portions of two adjacent acinar cells after IPR. Granules containing tubulo-vesicular structures are numerous (arrow). Lateral cell boundaries are extensively interdigitated. Mitochondria, junctional complexes, and granule membranes are well preserved x 14100.

Discussion

The secretory cycle of exocrine glands can be divided into three stages. The first stage is discharge of accumulated secretory material by exocytosis. The intermediate stage is characterized by reorganization of cell organelles and adaptation of cells to new conditions and the third stage is formation and accumulation of new secretory granules.[1]

Isoprotorenol (IPR) is one of the most active symathomimetic amines widely used in studies dealing with the secretory cycle of exocrine glands. IPR increases the flow of saliva less effectively than acute parasympathomimetic stimulation. Its main effect is to induce the protein

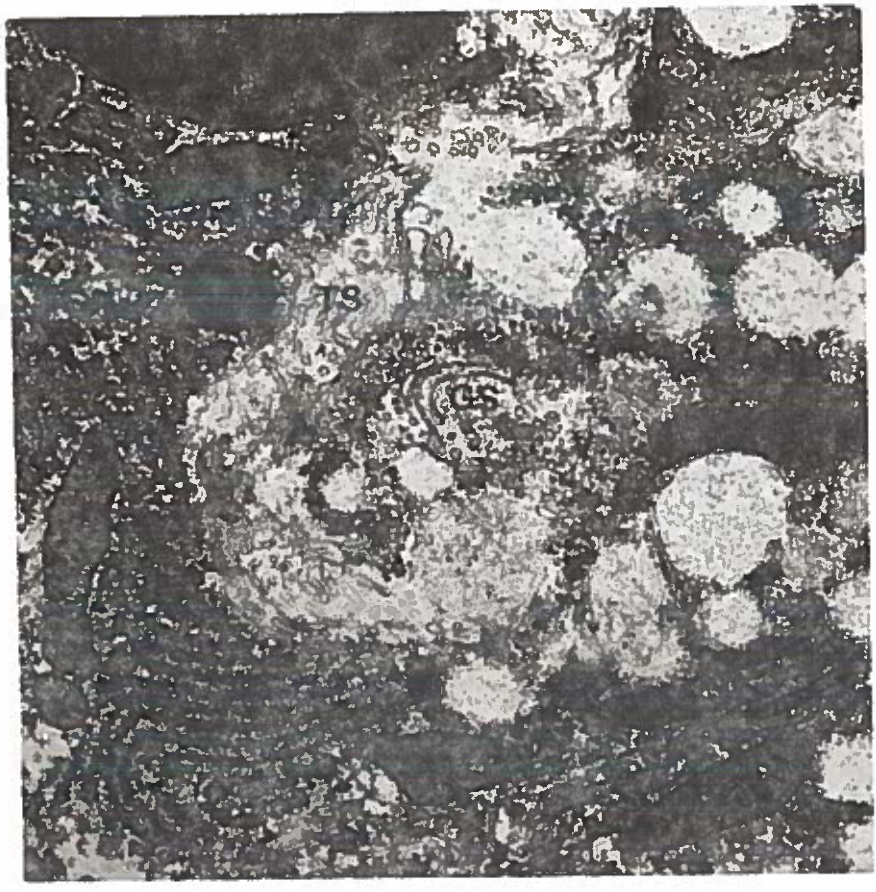

Figure 6

Electron micrograph of a large golgi area of an acinar cell after IPR. Sections of short, slightly curved tubules are seen adjacent to the rough endoplasmic reticulum. Very few randomly oriented golgi stacks and many vesicles are visible. TS: tubuler structures, V: vesicles GS: golgi stacks. x 25500.

discharge. In the first stage of the secretory cycle after IPR, there is no massive depletion of accumulated secretory material. Secretogoque effect is continuous but not so powerful. Some repopulation of granules can be seen after 2-3 hours. Acinar cells accumulate large numbers of granules between 6-13 hours and the process appears to be completed by 24 hours[2, 3, 6, 7] It has been shown that granule formation can be best demonstrated between 12-24 hours after IPR.[3, 4, 6]

The results of the present investigation are in accordance with the above mentioned effects of IPR. There were no significant alterations in the number of secretory granules and in the amount and distribution of

Figure 7

Typical arrangement of tubular structures around a maderately dense homogeneous material (arrow) after IPR. x 25500.

rough endoplasmic reticulum. This explains the slow secretogoque effect of IPR. After stimulation of secretion, discharge of secretory material became acclerated, but this increase was not an acute one. Newly formed secretory granules progressively accumulated in the cell as the old ones were being discharged. Synthesis of secretory macromolecules is a continuous process, and the given IPR did not alter the rate of synthesis of secretory proteins.

It has been known that mammalian submandibular glands produce neutral and acid glycoproteins. Acid glycoproteins can be either sialomucins or sulphomucins.

Figure 8

Portions of two adjacent acinar cells containing large golgi areas. From IPR treated group. In the golgi area on the right of the micrograph, tubules, vesicles and few golgi stacks are visible. In the left of the micrograph golgi area is seen to be composed of different profiles of tubular structures. Helical arrangement of tubular structures around a dense material is seen within the rough endoplasmic reticulum (Arrow).RER: Rough endoplasmic reticulum, TS: Tubular structures, GS: Golgi stacks, V: vesicles x 25500.

Submandibular acinar cells mainly produce sialomucins. But they stain poorly with alcian blue which generally stains sialic acid containing mucosubstances. Acinar cell granules also show a slight staining with PAS which is a selective stain for neutral mucopolysaccrahides. According to these moderate alcian blue and PAS staining, acinar cells are generally considered as seromucous cells.[4, 5, 8] Morphologically, seromucous secretory granules resemble classical mucous granules. Ultrastructurally, they are large, pale, membrane-bound structures containing

a filamentous secretory product. granule substructure has been shown to be highly fixation dependent. In tissues fixed with gluteraldehyde and osmium tetroxid, fine filaments and vesicles are prominent, and the filaments often aggregate into fibrils in fixatives containing phosphate buffer, whereas vesicles are prominent with fixatives containing colloidine buffer.[4, 5, 7, 8]

Our material was fixed with acrolein-gluteraldehyde (double aldehyde) and osmium tetroxide in phosphate buffer. In the control group, we identified three types of secretory granules according to their substructure. Large pale granules containing loosely arranged fine filaments and denser ones possessing condensed filaments were abundant. In very few granules intragranular secretory material was in the form of tubular structures. Granules with vesicular content were not seen in our specimens. These vesicles probably correspond to the cross sections of tubular substructures seen in our material. Therefore, it has been thought that differences described in the granule density and content might probably be related to the maturation of the secretory product within the cell rather than being the result of fixation. We observed that denser, early secretory granules were preferentially located in the vicinity of the golgi complex, while pale, mature ones were concentrated at the apical cytoplasm.

The effect of IPR is complex. It stimulates excretion of saliva as well as DNA synthetic and mitotic activity of acinar cells.[2] It has also been shown that IPR increases the synthesis of sialomucins and alters the composition of the granule matrix. Chronic IPR administration causes the condensation of secretory material within the submandibular acinar cell granules. These condensations often form some unusual structures such as lamellae, sprials, cap-like structures.[4-6]

These changes in the chemical composition of the secretory product reconstitued after IPR can be seen morphologically since the morphological substructure of fixed and stained granule material is assumed to reflect chemical composition and intra granule aggregation of secretory molecules.[5, 9]

In the present study, it was found that granules reconstituted after IPR were much more electron dense than those of the control group. Intragranular secretory product was mostly in the form of condensed fibrils and tubular aggregates (Granules containing tubular structures were significantly increased in number). Tubular structures and filaments undoubtly represented the different chemical constituents of a normal secretory product. Their significant increase in amount, however, indicates that synthesis of these secretory macromolecules became ac-

celerated after IPR. The exact chemical nature of these tubules and filaments has not been fully established yet.

Luzzatto et al have shown the presence of crystal-like structures in rat submandibular acinar secretory granules. These structures appear as a stack of parallel tubules sometimes arranged in helices, and are probably composed of glycoproteins.[10] Similar rods or tubules have also been described to be located in rat submandibular acinar cell granules in post osmicated tissue.[5] It has been proposed that carbohydrate containing components of both parotid and submandibular gland secretory granules may be bound to a lipodial component to form glycolipids or lipophilic glycoproteins.[7] This lipoidal material was seen as vesicles and tubules in post osmicated tissues.[5, 7]

The role of the golgi complex in the formation of secretory granules has long been known. Proteins synthesized in rough endoplasmic reticulum are carried to the golgi complex. These products are collected in the golgi complex and the carbohydrates are added to the protein core. Secretory granules are formed by the dilatations of golgi membranes. These dilatations are gradually pinched off from the golgi membranes to form secretory granules.

We have not observed such a membraneous relationship between golgi membranes and the early secretory granules. Such a system would not be true for the formation of seromucous secretory granules. Moreover, the structure of the golgi complex was significantly different from the usual structure of this organelle in both control and IPR treated groups. After the IPR the golgi complex was seen to be composed of tubules and vesicles with very few golgi stacks. These different structural appearances of the golgi complex after IPR would probably indicate a different function of the organelle under stimulated conditions. Tubular membraneous structures appear to be formed by the alteration of golgi membranes.

Another function of the Golgi complex is the formation of membrane for replacement of the plasma membrane. This organelle also plays an important role in membrane recyling during the secretory cycle.[11]

Tubuler (Golgi) structures might also be related to the two functions of the organelle mentioned above.

We have also observed a very specific arrangement of these tubular (probably golgi) mebraneous structures around a homogeneous material after IPR. We believe that such an arrangement has not been reported before. It remains to be elucidated whether these unique arrangements indicate the formation of secretory granules or not.

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Experimental Epiphyseal Distraction*

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Summary

The effects of two different rates of epiphyseal distraction on the morphology and the function of the epiphyseal plate were compared in immature white rabbits. Distal femoral epiphyseal distraction was performed using a unilateral external fixation frame at rates of 1 mm/day and 0.25 mm/12 hours. In all rabbits symetrical femoral lengthening could be obtained.

Although epiphyseal distraction at the rate of 1 mm/day altered the epiphyseal plate, and caused premature epiphyseal fusion, slow distraction resulted in bone lengthening without harmful effects on the epiphyseal plate. The length obtained by slow distraction was maintained at the end of the growing period.

Key Words: Bone lengthening, growth plate.

Introduction

Bone lengthening by epiphyseal distraction has been gaining wider acceptance because of the simplicity of the method.[1-5] Lengthening is obtained by gradual distraction of Salter-Harris type 1 epiphyseal separation which occurred under tension.[6-14] The authors agree that the surgery should be performed at the end of the growing period since this procedure may lead to premature epiphyseal fusion.[2, 5, 6, 9, 10] Recently, some authors have shown that bone lengthening could be obtained by small distraction forces without altering the function and the morphology of the epiphyseal plate.[3, 7, 14]

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This is a preliminary study for a universal external fixation device which we intend to develop and our purpose is to investigate:

1- Whether a unilateral external fixation frame be successfull in bone lengthening by epiphyseal distraction?

2- Are there any differences in the effects of the distraction rates of 1 mm/day and 0.25 mm/12 hours on the function and the cellular and the vascular morphology of the epiphyseal plate?

Materials and Methods

The prototype external fixation devices were mounted on the right distal femoral epiphyses and diaphyses of 20 immature New Zealand white rabbits aged 10.5 $\pm$ 0.5 weeks. Surgery was performed under ether anesthesia using image intensifier (Figure 1). Sodium cephalothin was administered intramuscularly to every animal for 3 days postoperatively. The animals were separated into two groups. The distraction rate was 1 mm/day for 15 days in Group A, and was 0.25 mm/12 hours for 30 days in Group B. Four weeks after the end of the distraction period, the external fixation devices were removed and the animals were allowed to move freely. Animals from both groups were sacrificed at the end of the distraction period (Stage I). Four weeks after the end of the distraction period the second group of animals were killed (Stage II). The third group of animals were sacrificed when they were 24 weeks old (Stage III). 24 weeks is the age that bone growth ends in rabbits.[7] A mixture of barium sulphate and Indian ink was injected through the abdominal aorta.[15, 16] Both femora of the animals were removed extraperiostally, and their lengths were measured. The radiograms of the femora of the 24 weeks old rabbits were taken. The femora were fixed in 10 percent buffered formalin solution, and were decalcified in hydrochloric acid. Later the bones were cleared by the Spalteholz method.[15, 17] 5 micron sections of bone tissue were stained by Hematoxylen-Eosin dye and were observed under light microscope. Cleared sections of 1 mm thickness of bone were also evaluated under dissection microscope.

Results

Four animals were excluded from the study because of two cases of deep infection, one femoral fracture, and one death.

At the end of the distraction period, symmetrical bone lengthening could be obtained in all animals. Net gains in lengths are shown in Table I. As seen in the Table, differences in net gains in bone lengths at Stage III were statistically significant (The Mann Whitney U test, U: 9, $p < 0.05$).[18]

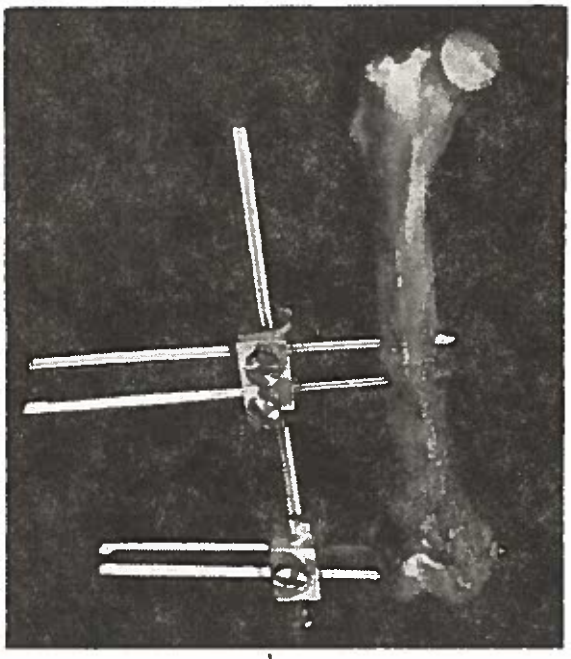

Figure 1
The prototype external fixator.

TABLE I
NET GAINS IN BONE LENGTHS IN GROUPS A AND B AT ALL STAGES

Group	Stage I	Stage II	Stage III
	10.5 mm	8.8 mm	0 (varus deformity)
A	11.6 mm	8.2 mm	2.3 mm
	11.1 mm		4.5 mm
	mean: 11.06 mm		mean: 2.3 mm
	10.2 mm	8.6 mm	7.5 mm
B	11.3 mm	9.1 mm	8.1 mm
	10.6 mm		7.8 mm
	mean: 10.7 mm		mean: 7.8 mm

Histologic and microangiographic findings in both Groups A and B at Stages I,II,III are summarized in Tables II, III, and are shown in Figures 2-7. As seen in Tables II and III, while the distraction rate of 1 mm/day caused epiphyseal separation, premature fusion and vascularisation of the growth plates, distraction rates of 0.25 mm/12 hours did not alter the growth plates significantly.

TABLE II
HISTOLOGIC FINDINGS IN EPIPHYSEAL PLATES IN GROUP A AND B AT ALL STAGES

Stages	Group A	Group B
Stage I	Epiphyseal separation distal to degeneration zone,	Increase in the thickness of the plate,
	Loss of columnar arrangement of the cells,	Columnar dissarrengement in some areas,
	Bone bridges in some areas.	Finger like expansion of the plate to metaphysis.
Stage II	Loss of normal appearance of the plate, Frequent bone bridges.	Nearly normal appearance of plate except loss of columnar architecture in some areas.
Stage III	Small islands of the plate in bone tissue.	The same findings as in Stage II

Figure 2
Group A Stage I histologic appearance. Bone bridges in some areas and loss of columnar arrengement of the cartilage cells are seen (Hematoxylen-Eosin, X 63).

Radiographic findings at Stage III: In Group A epiphyseal lines were irregular and were interrupted by bone bridges. A femoral varus deformity was seen in one animal due to asymmetrical epiphyseal fusion. In Group B normal epiphyseal lines were encountered.

TABLE III

MICROANGIOGRAPHIC FINDINGS IN GROUPS A AND B AT ALL STAGES

Stages	Group A	Group B
Stage I	Epiphyseal plate vascularisation in some areas	Avascular epiphyseal plate (normal appearance)
Stage II	The same findings as in Stage I	Normal
Stage III	Complete vascularisation of the the plate	Normal

Figure 3

Group B Stage I histologic appearance. Finger like expansion of the plate to metaphysis (Hematoxylen-Eosin, X 63).

Discussion

Usually bilateral of hemicircular external fixators are used for epiphyseal distraction.[1, 2, 4, 5, 11] By using unilateral external fixator and half pins, we were able to lengthen the bones by epiphyseal distraction as De Bastiani et.al, reported.[3, 7]

Although it has been shown by some authers that distractional epiphysiolysis is not harmful to epiphyseal plate in children, experimental results in animals are variable.[4, 6, 9, 10, 12, 13, 19] However, it sounds logical and safe to lengthen the bones without altering the continuity of

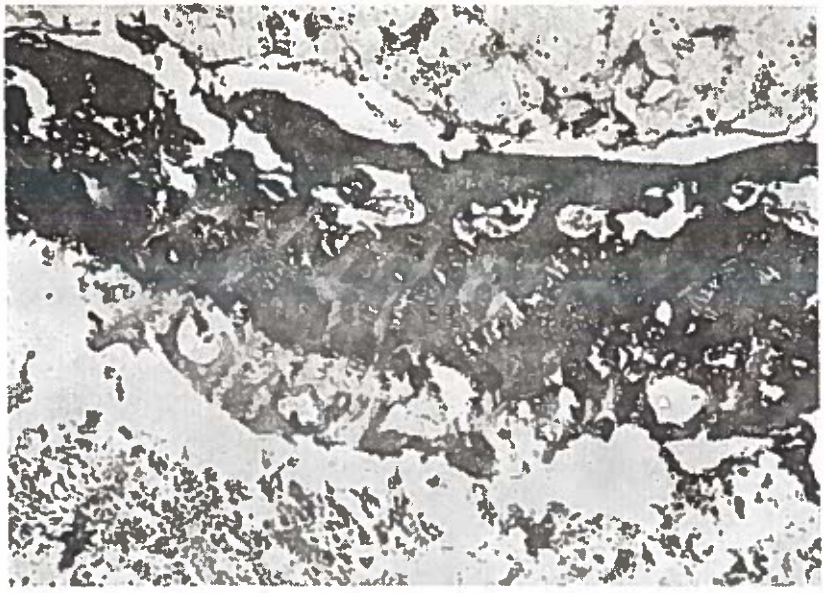

Figure 4
Group B Stage III histologic appearance. Nearly normal appearance of the plate (Hematoxylen-Eosin, X 63).

Figure 5
Group A Stage I microangiographic appearance. Epiphyseal plate vascularisation in the central region (X 6).

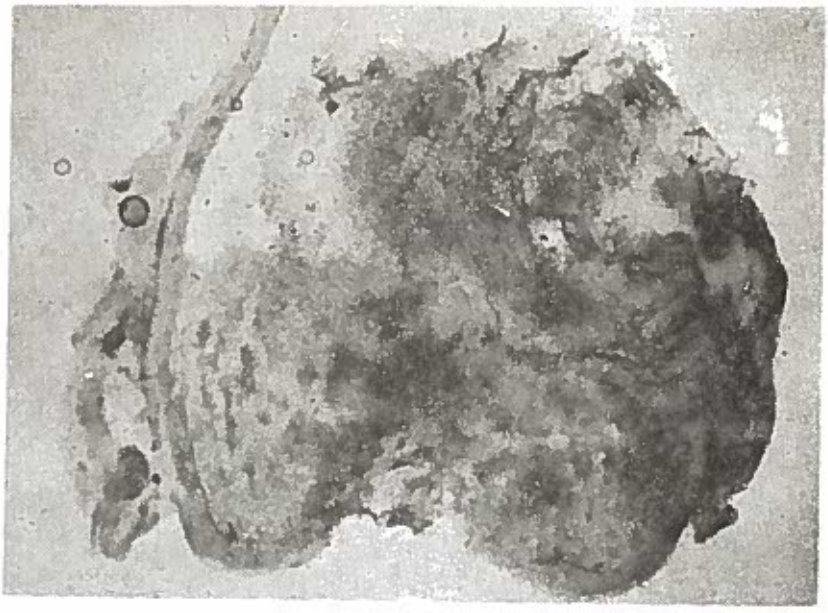

Figure 6
Group A Stage III microangiographic appearance. Complete vascularisation of the plate (X 6).

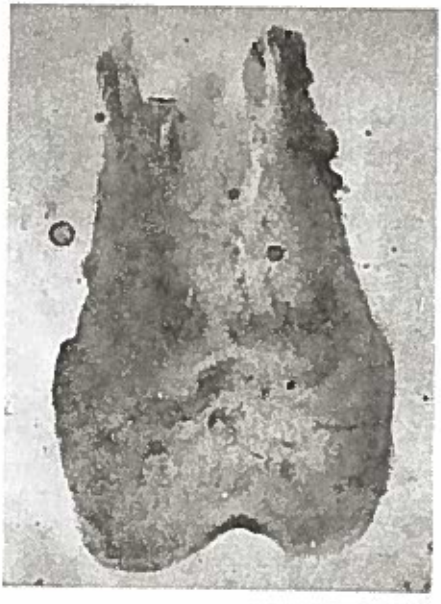

Figure 7
Group B Stage III microangiographic appearance. Normal appearance (X 6).

the plate. It has been reported that bone lengthening could be obtained by small distraction forces without altering the continuity, but slightly reducing the function of the plate.[3, 7, 14] The force for such a bone lengthening procedure was found to be 5-10 % of the breaking force of the physis.[20]

The epiphyseal plate is an avascular tissue.[16, 21] Trueta and Amato[22] have shown that epiphyseal ischemia resulted in epiphyseal plate necrosis, and metaphyseal ischemia resulted in thickening of the plate. It was also shown by the same authors that when metaphyseal circulation returned to normal, epiphyseal plate made a finger like expansion to the metaphyseal side. Additionally, the pins placed for epiphyseal distraction were found not to be harmful to the growth plate.[10, 11] Although we could not show any obvious loss of vascularization in epiphyseal distraction, we have thought that the changes which occurred in the plate during epiphyseal distraction were the result of ischemia due to stretching of the vessels. Frequent microangiographic and blood flow studies and tissue O_2 tension measurements may clarify this subject.

In conclusion, bone lengthening can be obtained by slow epiphyseal distraction (0.25 mm/12 hours) without harmful effects to the growth plate, using a unilateral external fixation frame.

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The Groin Flap in Reconstructive Hand Surgery

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Summary

Eight cases of hand injuries complicated with tissue loss were treated with the use of groin flap. Seven out of 8 flaps survived completely restoring a satisfactory hand function.

Key Words: Groin flap, superficial iliac circumflex vessels.

Introduction

Acute hand injuries with significant loss of skin and subcutaneous tissue exposing deep structures, such as tendons, nerves, bone and joints, cannot be treated by direct closure, skin grafts or by local flaps.[1] In such cases a distant flap may be utilized as a mean of primary closure.[2, 3] In another category of patients, resurfacing of the cicatricial hand wound with a flap may be performed as an elective secondary procedure.[4] In the management of soft-tissue defects in the hand requiring distant flap coverage the conventional groin flap based on the superficial iliac circumflex vessels was chosen. The groin flap was first described by Ian Mc Gregor[5] (Figure 1).

Material and Method

This study consists of 8 cases of groin flap performed at the Hacettepe University Medical School Department of Plastic and Reconstructive Surgery, between February 1985-September 1986.

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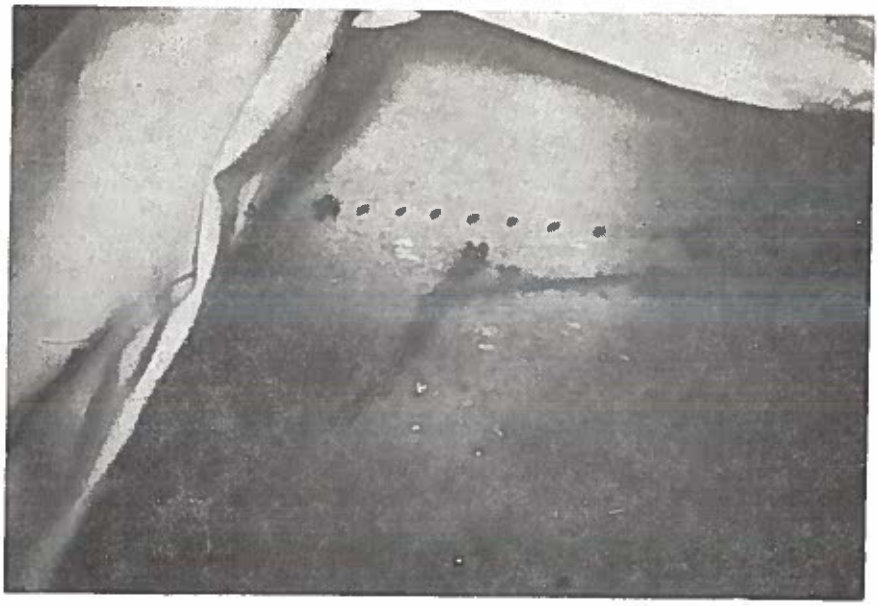

Figure 1
The design of groin flap according to Mc Gregor.

In 5 patients the flap was utilized to resurface defects exposing tendons, bones or nerves. In 3 patients the groin flap was used as an elective secondary resurfacing procedure to eliminate scar tissue and contracture.

Following the removal of all nonviable or scar tissue, a groin flap based on the superficial iliac circumflex vessels was prepared. The dimension of the groin flaps in this study ranged from 16 to 24 cm in length and 8 to 10 cm in width. Primary closure of the donor site defect in the groin was performed in all cases. The flaps were sutured to defects and the hand was fixed into desired position with bandages. 15 days after the first operation the base of the flaps were divided and completely transferred to cover the defect.

Results

The length of follow-up ranged from 6 months to 18 months. All of the 8 flaps in this study were viable at the time of initial elevation and application to the tissue defects. 7 flaps healed with no complications.

In one case a partial necrosis occurred after division of the base of the flap. After removal of the necrotic portion of the flap the defect was skin-grafted.

In 2 cases the groin flap was used to cover defects caused by crush injuries.

In 3 cases the groin flap was utilized to resurface skin defects following ring avulsions. In 3 cases the groin flap was used in elective secondary resurfacing procedures 1 to 2 years after the original injury (Table I).

TABLE I

Cause of injury	Patients	Complications
Crush Injury	2	–
Avulsion Injury	3	1
Secondary resurfacing	3	–

7 of the 8 flaps survived completely and covered hand defects successfully restoring a satisfactory hand function (Figure 2-7).

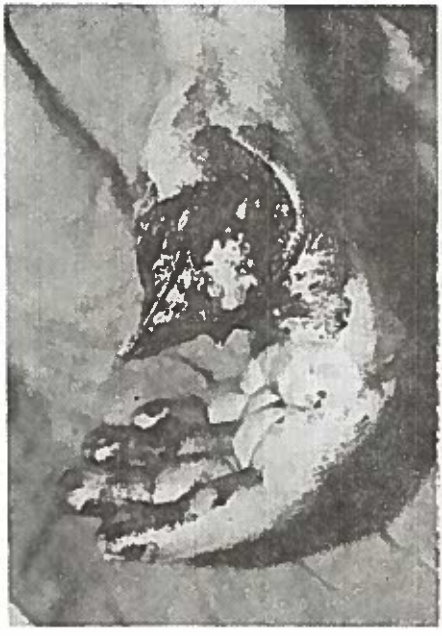

Figure 2
An acute hand injury complicated with amputation of the thumb.

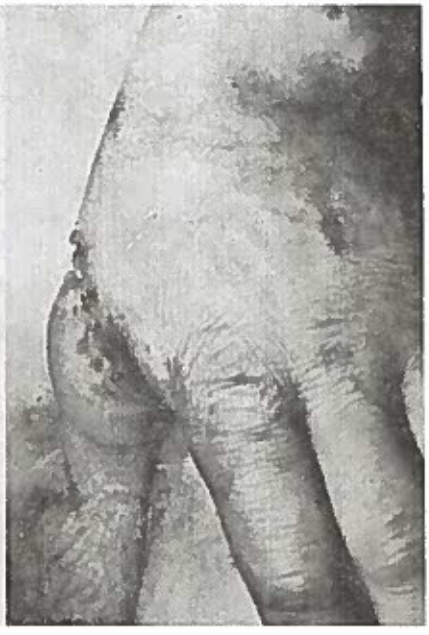

Figure 3
The groin flap was used to reconstruct the thumb in this case.

Figure 4
The Reconstructed thumb after division of the base of the flap.

Figure 5
A Ring avulsion with amputation of the distal phalanx.

Figure 6
The Groin flap was used to reconstruct the ring finger defect.

Figure 7
The reconstructed ring finger, after division of the base of the flap.

Discussion

The groin flap[2] is a single-pedicled flap with its base centered on the point of origin of the superficial circumflex iliac artery in the femoral triangle. The flap passes laterally and slightly upward along the line of the vessel, that is, parallel to the inguinal ligament.

It is an axial pattern flap[4] (arterial flap) contains the superficial circumflex iliac arterio-venous system, which runs along the axis of its length. The presence of this system allows it, although raised without prior delay, successfully to sustain a ratio of length to breadth which would not be possible were the system is absent. This advantage and this relative freedom from the shackles of the length breadth ratio it shares with the other axial pattern flaps, the deltopectoral and the hypogastric. In this respect this flap contrasts strikingly with flaps lacking an axial system, that is, flaps with a random pattern of blood vessels.

Soft tissue defects of the hand particularly when combined with exposed bone, tendon or nerve require distant flap coverage[2, 3] Although distant flap coverage is usually not performed complications such as infection, necrosis soon follows the injury.[6] Necrosis, when it does occur,

has a distinctive pattern of development. It contracts with the pattern of necrosis in a random pattern flap, in which necrosis develops quickly in a matter of hours when it occurs.

Distant flap coverage of the hand can be done in two ways. One of them is microvascular free flap transfer and the other is axial pattern flap.[5-7] The groin flap was used in our 8 cases. Groin flap has a little subcutaneous fatty tissue and it is thiner than the others. For this reason the conventional groin flap in preference to microvascular free flaps in situations where both techniques will deliver equally good final results was utilized in our cases.

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Comparison of Silicone and Latex Urethral Catheters in the Development of Urethral Stricture Following Open-Heart Surgery

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Summary

A prospective study was undertaken to assess the incidence of urethral stricture in 50 men undergoing open-heart surgery. A preoperative urological history was taken and peak urine flow rates were measured before surgery, at 1 and 10 weeks after operation. Twenty three of the patients were catheterised in the theatre with 14 F latex urethral catheter and details were recorded. In the remaining 27 patients, the same procedures were done and 14 F silicone urethral catheters were used. Urethral stricture was found in 2 (8.7 %) of latex catheter group and in none of the silicone catheter group. It is recommended that silicone catheters be used routinely in men undergoing open-heart surgery.

Key Words: Open-heart surgery, Urethral stricture.

Introduction

Temporary urethral catheters are used for monitoring purposes during and following open-heart surgery. A large number of urethral

strictures have been reported recently following catheterisation for cardiac surgery.[1-5] In all of these studies latex urethral catheters were used. On the other hand, some reports showed that silicone catheters had no tissue toxicity while latex catheters were toxic.[6-8] As a result, this prospective study to assess the problem in men undergoing open-heart surgery either by latex or silicone urethral catheters was planned.

Materials and Methods

All male patients over the age of 18 years undergoing elective cardiac surgery were considered for the study. This minimum age was chosen to avoid the problem of the smaller urethral calibre in younger patients. Any patient who had had previous instrumentation or surgery at the lower urinary tract, any history of urinary infection or peak urinary flow rate lower than 15 cc/min was excluded. The primary operative procedures are outlined in Table I. In the week before open-heart surgery the urinary flow rate was measured by a standard uroflowmeter (Wolf 2118.09). At the time of surgery the patients were randomised into one of two groups according to their file numbers; odd numbers to receive 14 F latex foley catheter (30 patients), even numbers 14 F silicone foley catheter (30 patients). Catheterisation were done by our junior medical staff using a polyvidon-iodine antiseptic solution to clean the genitalia and vaseline liquid as a lubricant. Catheters were left *in situ* for 48 hours in all cases. All patients were monitored at the 7th day and at the 10th week postoperatively by urologic history, examination, urinalysis, urine culture and uroflowmetry. The investigation for urethral strictures included retrograde urethrography and urethrocystoscopy, when indicated.

TABLE I

PRIMARY OPERATION

Procedure	No of Patients
Coronary artery bypass grafts	22
Aortic valve replacement (AVR)	7
Mitral valve replacement (MVR)	14
AVR + MVR	7

Results

Sixty patients were included in this study. Two of these patients died in the immediate postoperative period and eight did not come to 10th week control. Therefore 50 patients were eligible for this study; 23 in latex, and 27 in silicone urinary catheter group. Their ages ranged

between 18 and 62 years (mean 39.4) in latex and 18-65 years (mean 41.4) in silicone catheter group.

Two (8.7) of the 23 patients with latex urethral catheters postoperatively suffered strictures compared to none of the silicone urethral catheter group. The site of the stricture was in the membraneus urethra in both patients. One stricture was diagnosed in the 7th postoperative day and the other in the 10th week control. In those patients maximal flow rates were lower than 15 cc/min and urethral stricture was diagnosed in retrograte urethrography and urethroscopy. Both were initially treated with a combination of internal urethrotomy and dilatation.

Discussion

Recent reports and correspondence in the literature indicate that there have been a disturbingly high incidence of urethral strictures following catheterisation for open-heart surgery with latex catheters. Epidemic stricture formations were reported from Finland effecting 12 % of 478 men[2], and from Australia 48 % of 71 men[3] undergoing open-heart surgery. This rate was found to be 10 % in Ireland[4] and 36 % in Canada.[5] On the other hand, Ferrie *et. al.*, had shown an incidence of 2 % in the United Kingdom[9] and Nacey *et. al.*, 4 % in New Zealand.[10] Our study has shown an incidence of 8.7 % with a mean follow-up of 10 weeks. Strictures have been reported, however, up to 21 months after cardiac surgery.[11] Therefore, we can project that our urethral stricture rate will increase as the follow up period is prolonged.

Catheter material is being regarded as an important factor in stricture formation. Recent literature show that silicone catheters have no tissue toxicity while latex ones do, by *in vivo* and *in vitro* investigations.[6-8] Two prospective randomised studies have been reported from New Zealand and the United Kingdom comparing latex and silicone catheters in men undergoing open-heart surgery.[10, 11] In the former study, stricture rate of 4 % with latex catheters and none with silicone catheters were reported with a 6 month follow-up. In the latter one, stricture rate was 5.2 % in latex and none in silicone catheter groups with a 28 month follow-up. Our results are similar in silicone catheter group with 0 % urethral stricture rate.

The cause of urethral strictures in patients who have undergone open-heart surgery still remains unclear. Numerous causes have been suggested for urethral strictures following open-heart surgery including the role of the lubricant,[12, 13] chemical toxicity of latex material,[14] powder from surgical gloves,[15] alterations in the tissue perfusion during cardiopulmonary bypass[3] and urethral ischaemia.[10, 16-18]

On the basis of literature and our study, we suggest that silicone catheters be used routinely in men undergoing open-heart surgery.

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Pupillary Dysfunction in Myasthenia Gravis

Possible Involvement of Ciliary Ganglion

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Summary

A myasthenic patient with anisocoria had rapid improvement of pupillary dysfunction after an intravenous injection of edrophonium hydrochloride. A battery of pharmacological tests were performed to investigate the nature of this defect. 2 % homatropine, 1 and 4 % cocaine, 1 % physostigmine, 3 % ephedrine and 0.125 % pilocarpine were instilled with at least 48 hours time intervals. Our findings suggest that pupillary involvement and anisocoria may occur in myasthenia gravis and ciliary ganglion seems to be the site where the cholinergic transmission is defective.

Key Words: Myasthenia Gravis, pupil, ciliary ganglion.

Introduction

There have been a few reports on pupillary dysfunction in myasthenia gravis (MG)[1-6] although some other investigators have found no pupillary abnormalities.[7-9] Anisocoria and weakening of pupillary light response are indeed unexpected findings if one considers the pathophysiology of myasthenic muscular weakness and the innervation of the iris spincter and dilator muscles. The pupil like other smooth muscles with cholinergic innervation, has a muscarinic type of acetylcholine receptor (AChR) whereas skeletal muscle endplate is endowed with nicotinic AChRs. Nicotinic antagonists like curare lead to skeletal muscle pa-

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ralysis without affecting the pupil. On the other hand, muscarinic antagonists (e.g.atropine) cause mydriasis but they lack any effect on skeletal muscle. The similarity between curare poisoning and MG was recognised long ago and an autoimmune reaction to the nicotinic AChR of skeletal muscle has been well established as a cause of myasthenic weakness.[10] Therefore, pupillary abnormalities in MG evidently require further investigation to elucidate the seemingly contradictory data on hand.

Recently, we had a myasthenic patient with an apparent pupillary involvement and performed a battery of pharmacological tests to establish the nature of the pupillary dysfunction.

Patient and Method

A 49 year old man was admitted to the hospital on June 26, 1985 with chief complaint of drooping of the right eyelid, double vision and generalized weakness. Eye movements were limited on adduction, up and down gaze in the right eye which had ptosis. There was some limitation of abduction and adduction in the left eye. The right pupil was 2 mm larger than the left and the light response was sluggish. He had weakness in all four limbs, more marked in proxymal muscle groups. He responded well to edrophonium which opened the palpebral fissure, made the patient able to move his eyes in all directions and rendered the pupils isocoric. He was given pridostigmine (Mestinon) 60 mg five times a day and prednisolone was added to the treatment 60 mg on daily basis. A thymectomy was performed on July 7, 1985. He was discharged on August 1, 1985 in asymptomatic condition.

He was readmitted on Nov 8, 1985 with recurrence of ptosis, this time on the left eye in association with bilateral upgaze limitation and proxymal muscle weakness. His symptoms gradually disappeared with the regular administration of Mestinon (7 x 60 mg) and alternate day prednisolone treatment. Pupils were equal or sometimes the left pupil was 1/2 mm larger than the right; under evenly distributed light conditions. It was consistently demonstrated, however, that the left pupil could not maintain its contraction and returned to the starting diameter within three seconds when exposed to a constant source of light. A battery of pharmacological tests were performed to investigate the nature of the pupillary dysfunction. One drop of different drug solutions was instilled into each eye and the patient was observed every 15 minutes for the next hour. At least a 48 hour interval was allowed between the instillation of different drugs. The results of the tests are shown in Table I.

TABLE I

PUPILLARY DIAMETERS (right / left, mm) MEASURED BEFORE AND AFTER THE INSTILLATION OF VARIOUS AUTONOMIC DRUGS

A. Mydriatic Drugs

	Homatropine 2%	Cocaine 1%	Cocaine 4%	Ephedrine 3%
Dark	5 / 5	5 / 5	3 / 3.5	4.5 / 4.5
Room light	3 / 3	4 / 4	2 / 2.5	3.5 / 3.5
After drug	7 / 7	5 / 6	4 / 6	5 / 6
Difference*	+4 / +4	+1 / +2	+2 / +3.5	+1.5 / +2.5

B. Myotic Drugs

	Physostigmine 1%	Pilocarpine 0.125%
Dark	3 / 3.5	4.5 / 5
Room light	2 / 2.5	2.5 / 2.5
After drug	1 / 1 (1 hr) 1 / 1 (7 hrs) 1 / 3 (24 hrs)	2.5 / 2
Difference*	−1 / −1.5 (1 to 7 hrs) −1 / +0.5 (24 hrs)	0 / 0.5

* Refers to change from the value measured in room light.

Pupil sizes were measured in the dark and in room light at their horizontal diameter by using a pupil guge of a near vision card. Measurements were repeated by two different observers.

Results

Before the instillation of drugs the left pupil was 1/2 mm larger then the right in four out of six tests performed and in the other two tests, pupil sizes were equal (Table I). 2 % homatropine, an antimuscarinic agent, caused dilatation of both pupils to 7 mm. Two days later we conducted 4 % cocaine test which produced 2 mm dilation in the right pupil and 3½ mm dilation in the left 1/2 hour after the instillation. Another symphatomimetic agent ephedrine 3 % and a weaker solution of cocaine also had a more pronounced mydriatic effect on the left pupil, producing 1 mm difference between the pupils in 1/2 hour. 1 % physostigmine (eserine), a cholinesterase inhibitor, produced a strong miosis which lasted more than 72 hours on the right eye while the left pupil showed a faster recovery so that an anisocoria appeared 24 hours after the instillation, the left being 1½ mm larger than the right. Finally, we tried 0.125 % pilocarpine which caused constriction of the left pupil and no change on the right.

All the tests and pupillary examinations were conducted while the patient was receiving his regular anticholinesterase medication because he refused the cessation of the drugs. He was unwilling for any further tests which would prolong his hospital stay.

Discussion

Several group of investigators reported the involvement of pupils in MG; however, they did not study the responses to the autonomic drugs. Yamazaki and Ishikawa demonstrated in seven myasthenic patients that the amplitude, velocity and acceleration of pupillary constriction were diminished and these pupillary abnormalities could be corrected by edrophonium injection.[4] Lepore *et al.*, found that mean pupil cycle time was significantly increased in 25 patients with MG compared to the control group.[5] Subsequently, Dutton *et. al.*, showed that the pupillary light reaction could be fatigued in 11 out of 12 myasthenics following continous exposure to a narrow beam of light.[6] Moreover, there are two reported cases wih apparent involvement of the pupil (mydriasis) in the literature.[1,2]

In the present case, reversal of mydriasis by edrophonium injection in his first admission, subsequent remission and reappearance of the pupillary dysfunction, this time on the other eye, are strongly suggestive of a myasthenic involvement of the pupil.

Homatropine test clearly showed that the patient's iris sphincter had muscarinic type of AChRs. Tests with a nicotinic agonist or antagonist would have been very valuable to elucidate the possibility of a mixed type (nicotinic + muscarinic) AChRs like those in the central nervous system.[11,12] Since d-tubocurarine is not expected to penetrate the cornea and, to our knowledge,there is no known practice of applying nicotine to cornea, we could not perform tests with these substances. The test with dilute pilocarpine, however, demonstrated an increased cholinergic sensitivity rather than a reduced postsynaptic response due to loss of hypothetical nicotinic receptors.

Presynaptic parasymphathetic fibers utilize ACh as their transmitter acting on the postsynaptic nicotinic AChRs. In the ganglion cell the nicotinic receptors, however, have different features than the nicotinic AChRs at the motor endplate. For example, decamethonium acts as an agonist on muscle and an antagonist at ganglia[13] and alpha-bungarotoxin does not block transmission in symphathetic ganglia of mammals although it is an irreversible ligand of the endplate AChRs while d-tubocurarine has an equal potency in blocking transmission at the ganglia and neuromuscular junction.[14] It can then be assumed that a sub-

type of polyclonal antibodies[10] against endplate AChRs may be directed to a common reactant on ganglionic type of nicotinic AChRs in our case. Indeed, increased sensitivity to pilocarpine of the iris sphincter supports this view. This phenomenon is a well known feature of Adie's tonic pupil and is attributed to cholinergic denervation of the iris due to the involvement of the ciliary ganglion.[15-17] Moderate pupillary contraction elicited by pilocarpine on the left eye while the right pupil remained unresponsive, suggests a supersensitivity caused by partial denervation of the sphincter muscle of the left iris.

Another point of interest is whether the symphathetic superior cervical ganglion was also involved. The tests with symphathomimetic agents may be interpreted as demonstrating a less effecient symphathetic function on the right eye. However, the larger pupillary dilation observed with symphathomimetic drugs on the left can also be explained by a reduced parasymphathetic tonus as indicated by fatiguable light reaction, faster recovery from physostigmine effect and increased sensitivity to pilocarpine.

In conclusion, pupillary dysfunction in MG may be due to antibodies to nicotinic AChR in the cliary ganglion. This subtype of anti AChR antibodies may be sufficiently high to cause mydriasis in occasional patients.

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Fertility Following Abdominal Myomectomy

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Summary

The role of abdominal myomectomy in subsequent fertility, was evaluated. Fifty-six patients with infertility underwent abdominal myomectomy for correction of infertility. Twenty-seven (48.1 %) of these patients conceived. There was a total of 35 pregnancies. These conceptions have resulted in 25 (71.4 %) full term pregnancies. 62.9 % of the patients conceived in the first year after operation. The age of the patient, duration of infertility, number of the fibroids did not necessarily predict success.

Key Words: Abdominal myomectomy, Infertility.

Introduction

Indications for abdominal myomectomy have included correction of infertility, habitual abortion, relief of symptoms and discovery of a pelvic mass at the time of exploration. Theoretically, myomas can cause infertility by distorting the uterine cavity, by possible tubal occlusion or by causing vascular and inflammatory changes in the endometrium and underlying stroma which prevent implantation. Rubin claimed that 40 % of married women with multiple fibroid tumors have a history of childlessness.[1]

Despite the high prevalence of myomata in the female population, myomectomy is an operation infrequently performed solely for the correction of female infertility.[2,3] Many women with myomata readily

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conceive and carry to term. Nevertheless, after myomectomy for infertility, pregnancy rates of 35 to 50 % have been reported.[4] In two recent studies, 61.5 % and 65.2 % pregnancy rates were found in patients with infertility who underwent abdominal myomectomy.[5, 6]

In this study, the role of abdominal myomectomy in infertility, with regard to subsequent conception is evaluated.

Materials and Methods

Two hundred and ninty-eight patients who underwent myomectomy at Hacettepe University, School of Medicine, Department of Obstetrics and Gynecology from January 1970 to December 1984 were reviewed. In 94 patients, the indication for operation was infertility. Patients who had a fibroid tumor size more than 5 cm., multiple fibroids or fibroids localized near the cornua were included into the study. Patients who had vaginal myomectomies and those unavailable for a follow-up study or who were not actively trying to conceive were excluded from the study.

The hospital records were reviewed to evaluate any preoperative or postoperative infertility information and subsequent reproductive and gynecologic history, particular attention being paid to the operative notes and pathology reports. Follow-up data were also obtained from personal correspondence with those patients who had moved from the area.

Fifty-six patients who underwent myomectomy and desired pregnancy are the subject of this report. The patients ranged in ages from 25 to 41 years old (mean age 33.4 $\pm$ 6.2 years). The duration of infertility ranged from 2 to 12 years (mean 4.8 $\pm$ 2.1 years). 37 patients were primary, 19 patients were secondary infertile. Of the 19 patients with secondary infertility, 12 had a history of previous abortions and seven had prior term births.

Prior to surgery, all patients had undergone complete infertility investigation including laparoscopy.[4-6] No abnormalities other than the fibroids were noted. 19 patients had solitary, and 37 patients had multiple fibroids. The uterine cavity was entered in 21 patients and surgical dissection was described as being in the cornual area in 41 patients. There was no additional treatment during and after surgery to enhance fertility. A total of 213 myomas were removed, an average of 3.8 per patient. All patients were advised to try to conceive at least three months after surgery. All had at least one year exposure.

Student's t test was used for calculations the differences between values.

Results

The patients were observed for a median of 42.4 months for 16 to 130 months. Of the 56 patients trying to conceive, 27 (48.1 %) became pregnant (total of 35 pregnancies). These conceptions have resulted in 25 (71.4 %) full term deliveries, 5 (14.3 %) premature deliveries and 5 (14.3 %) spontaneus first trimester abortions. 23 of 56 patients have been delivered of, at least one viable infant. The pregnancy rates after myomectomy as a function of age of patient, the duration of infertility are listed Table I.

TABLE I

PREGNANCY RATE AS A FUNCTION OF AGE AND DURATION OF INFERTILITY AT THE TIME OF MYOMECTOMY

	No. of Patients	No. of Pregnancies	%
Age (years)			
< 30	11	8	72.7
30 - 34	21	10	47.6
35 - 39	16	7	43.7
> 40	8	2	25.0
Years infertile			
< 5	32	19	59.4
5 - 10	15	6	40.0
> 10	9	2	22.2

18 (48.6 %) of 37 patients with primary infertility, 9 (47.3 %) of the 19 patients with secondary infertility conceived. The difference between these groups was not statistically significant ($p > 0.005$). Pregnancy follow-up data are summarized in Table II.

TABLE II

PREGNANCY FOLLOW-UP DATA

	Primary Infertility		Secondary Infertility		Total	
	No	%	No	%	No.	%
Patients who conceived	18	48.6	9	47.3	27	48.1
Patients who did not conceive	19	51.4	10	52.7	29	51.9
Total pregnancies	21	60.0	14	40.0	35	100
Term pregnancies	16	76.2	9	64.3	25	100
Premature deliveries	2	9.56	3	21.4	5	100
Abortions	3	14.3	2	14.3	5	100

Nine of 21 patients in whom the uterine cavity had been entered during the operation became pregnant. 10 (71.0 %) of 14 patients on whom surgical dissection was performed near the cornua conceived. Surgical findings and conception rates are summarized in Table III.

TABLE III
SURGICAL FINDINGS AND CONTRACEPTION RATES IN 56 PATIENTS

	Total	Pregnant		Nonpregnant		
		No	%	No	%	
No. of patients	56	27		29		
Median no of myomas found	3.8	3.1		5.2		
Cavity entered	21	9	42.0	12	58.0	$p > 0.005$
Dissection near cornua	14	10	71.0	4	29.0	$p < 0.005$
Solitary myomas	19	9	47.3	10	56.7	$p > 0.005$
Multiple myomas	37	18	48.6	18	48.6	$p > 0.005$

The average length of time from operation to conception was 14.6 ± 3.7 months. 17 (62.9 %) of the patients conceived in the first year after the operation, eight (29.6 %) of the 27 patients conceived two years after the operation.

Discussion

Although decreased fertility has been reported by Rubin and Davis when myomas are present, it is obvious that not every patient with myomas has difficulty conceiving or maintaining a pregnancy[1, 7] Despite the frequency with which myomectomy is performed as a primary procedure for infertility, several reports, including the present series, demonstrated significant pregnancy rates after abdominal myomectomy in women with infertility.[2, 3, 5, 6, 8]

The way in which fibroids impair fertility is not fully understood. It has been speculated that fibroids interfere with sperm transport, impinge on tubal lumina, distort the course of Fallopian tube, compress cervical canal, or alter the position of cervix, thereby interfering with sperm capture in the posterior fornix.[9] Fibroids may also distort the endometrial cavity and distrupt uterine physiology, thereby interfering with implantation.[3, 9] Abnormalities of myometrial venous patterns because of compression and obstruction of venous plexi by fibroids have been demonstrated radiographically.[10] Histologic abnormalities of endometrium associated with fibroids are often seen.[11]

Infertility alone is a rare indication for myomectomy.[3, 12, 13] In our study, the incidence of myomectomies performed were solely because of the indication of infertility is 31.5 %.

Babaknia *et al.* previously reported no success after myomectomy in patients older than 35 years.[8] Results of this study indicate a higher pregnancy rate for younger women, although there may be other reasons for reproductive failure in addition to myomas in the older age group (Table I). Seven (43.7 %) of the 16 patients older than 35 years and two of the 8 patients older than 40 years conceived after myomectomy. Rosenfelt reported the same rates as 75 % and 50 % respectively.[6] As in the previous reports patients with a shorter duration of infertility were more likely to conceive after myomectomy (Table I).[4, 6, 8] Of patients with less than 5 years of infertility, 59.4 % conceived after myomectomy, whereas 40.0 % of the patients with more than 5 years of infertility conceived. Nevertheless, two of the 9 (22.2 %) patients with more than 10 years of infertility conceived after myomectomy.

No statistically significant difference was found between primary infertile and secondary infertile patients in terms of achieving pregnancy after myomectomy (Table II). The rate of premature delivery was significantly higher in the secondary infertile group of patients ($p < 0.005$).

Malone found that patients with multiple fibroid tumors were more likely to require future definitive surgical treatment and were less likely to conceive and carry to term than those patients with a single fibroid tumor.[12] Berkeley *et. al.*, reported that in patients older than 30 years of age, those with fewer, smaller fibroid tumors were more likely to conceive following the operation, and in those younger than 30 years of age the extent of disease had no effect upon the outcome.[4] In our study we found approximately the same conception rate after myomectomy in women with solitary and multiple fibroids (Table III). Entering into the uterine cavity was not a problem in term of achieving pregnancy after myomectomy. We also found that after the removal of myomas localized near the cornua, the chance of pregnancy was significantly improved (Table III). Similar results have been reported by Berkeley *et al.*[4]

In the present study, as in others, most of the patients (62.9 %) conceived within the first year after the operation[5, 6, 8]

In conclusion, despite the absence of specific criteria, myomectomy should be considered in patients with unexplained infertility of more than 2 years' of duration. The myoma need not necessarily be submucousal in location or solitary of multiple. The operation is not contraindicated in patients who are older (35 years) or who have a longer duration of infertility. The pregnancy rate may be significantly improved if myomas are localized near the cornua.

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Immune Response to Hepatitis B Vaccine in Hemodialysis Patients

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Summary

Hepatitis B vaccine was administered to 15 patients, whose ages ranged from 14-58 years, under a continuous hemodialysis program. Despite the supression of the immunity described in such patients, a positive antibody response was elicited in 53.53 % after the first dose, 69 % after the second, and 71 % after the third dose. The risk of Hepatitis B infection is expected to diminish with this program.

Key Words: Hemodialysis, Vaccination, Hepatitis B.

Introduction

Hepatitis B virus infection continues to be a serious problem in hemodialysis units. It is well known that hepatitis B virus infection risk is high among both the uremic patients undergoing treatment in Hemodialysis centers and the staff employed.[1]

Hepatitis B virus infection causes HB,Ag carrier at the rate of 60 % in uremic patients.[2] Determination of HB,Ag in the serum of 70-90 % of HB,Ag carriers indicates a high rate of infection risk in such persons.[3, 4]

In literature it has been reported that determination of HB,Ag carriership in about 20 % of hemodialysis patients and the presence of

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anti HB, antibodies in 25 % of the patients with negative HB,Ag is a strong indication that approximately 50 % of the patients contracted Hepatitis B virus infection either previously or during that period.[5]

These patients are continuous infection sources to their environment, particularly for dialysis personnel and other patients. Therefore, hemodialysis patients and doctors, nurses and other staff working in hemodialysis centers should be immunized by Hepatitis B virus vaccine. It is indicated that 56.5 - 89 % antibody response could be elicited in hemodialysis patients as against the immunity of 90 % in healthy persons by administering Hepatitis B virus Vaccine.[2, 6]

In this study, we attempted to vaccinate patients under the continuous hemodialysis program and to determine the development of immunity and complications.

Materials and Method

From September 1984 to April 1986, 15 uremic patients between the ages of 14-58 years were vaccinated in the Hemodialysis Unit of Hacettepe Hospital when they were on the continous hemodialysis program for on average period of 17.8 months (range 7-42 months). These patients were placed under dialysis treatment 2-3 times and 6-7 hours per week, and disposable dialysers were used for each dialysis.

Before administration of vaccine, patients' sera was checked for the presence of HB,Ag and anti HB,Ag antibody by use of Enzyme Immunoassay.

Their serum alanine aminotransferase, aspartate aminotransferase and bilirubin values were also assessed. One ml (20 µgr) hepatitis B virus vaccine (Merck) was administered intramuscularly in the deltoid muscle of patients whose liver function tests were normal, and in whom HB,Ag and antibody were negative.

Approximately 1 to 6 months after the administration of the first dose, second and third vaccines were administered at the same dose. All patients were checked for a period of 18 months for liver function tests, the presence of HB,Ag and anti HB,Ag antibodies.

Results

In 8 of the 15 patients administered the first dose of vaccine, anti HB, antibody was found to be positive (53.53 %). The second dose was administered to 9 patients and the percentage of patients with anti HB, antibody was increased to 60. The third dose could not be administered

to 8 patients due to various reasons. In the 5 patients out of 7, who were administered the third dose, anti HB, antibodies were observed before and after vaccination to be 71.4 %. It was determined that the rate of immunity of the patients 12 months after the vaccine administration did not change but this rate fell to 57 % after 18 months (Figure 1).

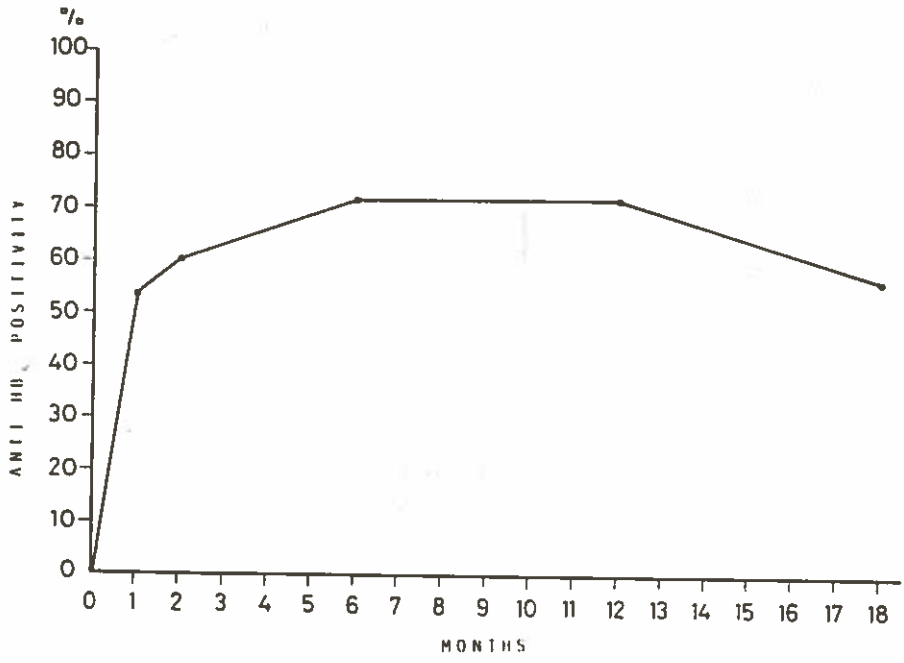

Figure 1
Antibody response elicited to Hepatitis B virus vaccine in Hemodialysis patients.

During the observation of our patiets, it has been noted that, the serum aspartate aminotransferase level was raised to 640 units and the alanine aminottransferase level to 325 units in only one patient and these values returned to normal levels in ten days. It was also noted in one patients serum that anti HB, appeared in 13 months after the first dose. This antibody response probably was not related to vaccine administration.

While the Hepatitis B surface antigen carrier rate in our unit was 34.88 % during the period of vaccine administration, this rate decreased to 27.27 % after the administration of the vaccine (Figure 2).

During our observation period of 18 months, there was no new patient with possitive HB,Ag.

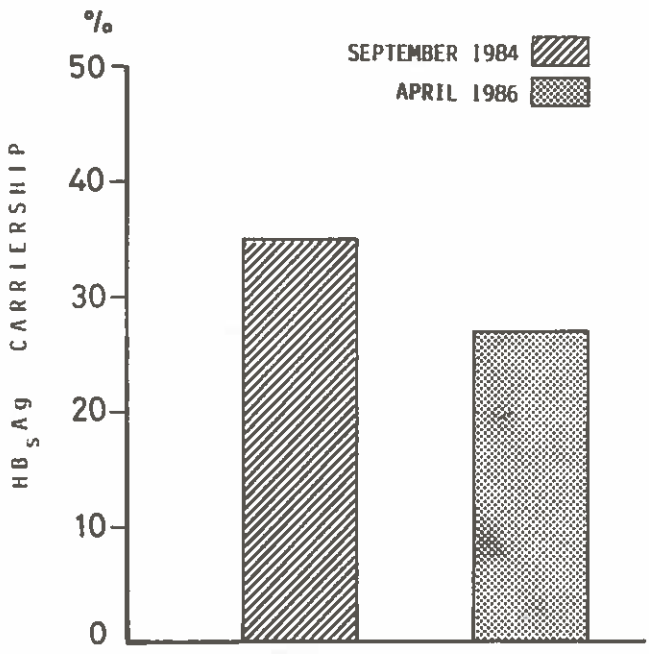

Figure 2
Rate of HB_sAg carriership in Hemodialysis Unit.

Discussion

The incidence of hepatitis B virus infections is high among dialysis patients. The European Dialysis and Transplantation Association has reported that about half of the European Dialysis units are contaminated by the Hepatitis B virus, and that more than 22 % of uremic patients treated by hemodialysis are carriers of HB_sAg.[7]

Hepatitis in hemodialysis patients generally is not severe, and because the patients are mostly unicteric, the acute phase may be overlooked. Lack of necessary measures creates the possibility of infection for all health personnel and patients nearby.

Several aspects of an abnormal immune function have been reported in uremic patients on chronic hemodialysis. These include impaired delayed hypersensitivity, lymphocytopenia involving both T and B cells, decreased monocyte phagocytic ability and impaired leukocyte migration capacity. A qualitative defect in lymphocyte function has also been reported.[8]

For this reason, the antibody response elicited in these patients against Hepatitis B virus vaccine is quite low as compared to healthy

persons. Previous studies indicate that immune response rate in patients under a continuous hemodialysis program is 11.6 % in the first month, 44-46 % in 6 months after the third dose, and 50-60 % in 12 months.[1, 2, 4]

It has been reported that, immune response of the children under continuous hemodialysis, has been elicited with the Hepatitis B virus vaccine at the rate of 72.2 % in 3 months, at the rate of 81.3 % in 6 months, and 87.5 % in 9 months and it has been stressed that the humoral immune response of these children may be better than those of adults.[1]

In another study, the antibody response after the second dose was-79.9 % and it reached 89 % after the third dose.[9] In our study, we determined 53.53 % anti HB_s (+) after the first dose, which increased to 60 % after the administration of the second dose. The antibody response elicited in the 6th month after the administration of the third dose was 71.4 %. Furthermore, we have observed that this response continued without change up to the 12th month and that it came down to 57 % in the 18th month.

In literature, it is recommended that chronic hemodialysis patients be administered 40 µgr Hepatitis B virus vaccine.[1, 3] It may be considered that the anti HB_s positivity at lower rates is probably a result of using 20 µgr. of vaccine.

It is reported that the highest anti HB_s antibody titration level has been observed in the 7th - 8th months, and that these levels are ten times less that the titration levels attained in healthy specimens.[9] Quantitative evaluation of anti HB_s antibodies has not been made in our patients.

It was reported that in patients who did not show anti HB_s antibodies after 12 months, a forth injection then led to the appearence of anti HB_s in 37 % of the patients and a fifth vaccination only led a seroconversion when a brief or borderline anti HB_s could already be demonstrated previously.[2] On the basis of this study, we also recommend booster vaccine doses at the 12th and 18th months in order to increase the diminished antibody response.

Such factors as age, nutrition, care, severity and duration of renal failure on entry into the dialysis program in hemodialysis patients may be important with respect to the immune response to Hepatitis B virus vaccine. Literature states that antibody response is better in patients with a dialysis period of less than 6 months.[1, 2, 6] A long dialysis period may be the cause of low antibody response in our patients.

Finally, the results of this study suggest that the Hepatitis B virus vaccine may diminish the Hepatitis B virus infection risk by providing considerable antibody despite the immune suppression in uremic patients under hemodialysis program.

We believe that an extensive program should be performed and the medical staff must also be included.

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Primary Pulmonary Neurilemoma

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Summary

In this paper we have reported a case of primary pulmonary neurilemoma who applied to the hospital with complains of cough, shortness of breath and chest pain. After the chest-X Ray had revealed a large opacity in the left lower thorax, several laboratory investigations, including radionuclid first pass study and thoracic computed tomography were performed. At the left posterolateral thoracotomy a large solid tumor which was diagnosed as primary pulmonary neurilemoma was found. It was totaly removed. Neither clinical nor radiological recurrence was detected 12 months after surgery.

Key Words: Primary Pulmonary Neurilemoma, Pulmonary neurilemoma, Neurogenic tumor.

Introduction

Primary neurogenic tumors of the lung are among the most uncommon tumors seen in the lung.[1,2] Since the first detailed description by Rubin in 1940, 54 cases of neurogenic pulmonary tumors have been reported in the literature.[1-5] These tumors arise from the cells of Schwann Sheat enwrapping the nerve bundles of the autonomic nervous system.[2,3,6] This report represents a case of primary neurogenic tumor of the left lung which was about 2750 grams and totally excised. The rarity of this tumor type has prompted us to describe another case of primary pulmonary neurilemoma.

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Case Report

H.K., a 37 year old man was admitted to our clinic with a two year history of non-productive cough, shortness of breath and left pleuritic pain. On examination, respiratory sounds were weak on the left basal area of the chest with dullness on percussion. Routine laboratory investigations were normal, whereas a chest-X Ray revealed a large opacity in the left lower chest (Figure 1). Bronchoscopy revealed that the left upper lobe orifice was oedematous and the lower lobe bronchus was partially narrowed by an extramural mass. Radionuclid first pass study which was performed with Tc^{99} (mRBC) revealed that the mass did not have any vascular component like aneurysm. Perfusion scintigraphy of the lungs (Tc^{99}-mMAA) showed a large perfusion defect in the left lower chest. Thoracic computed tomography demonstrated a solid tumor filling the lower and middle areas of the left chest and shifting the mediastinum to the right (Figure 2). On histopathologic examination of the sputum no malignant cells were detected.

The patient underwent a left posterolateral thoracotomy under general anesthesia. A large, lobulated mass, 20 x 25 x 15 cm in size which

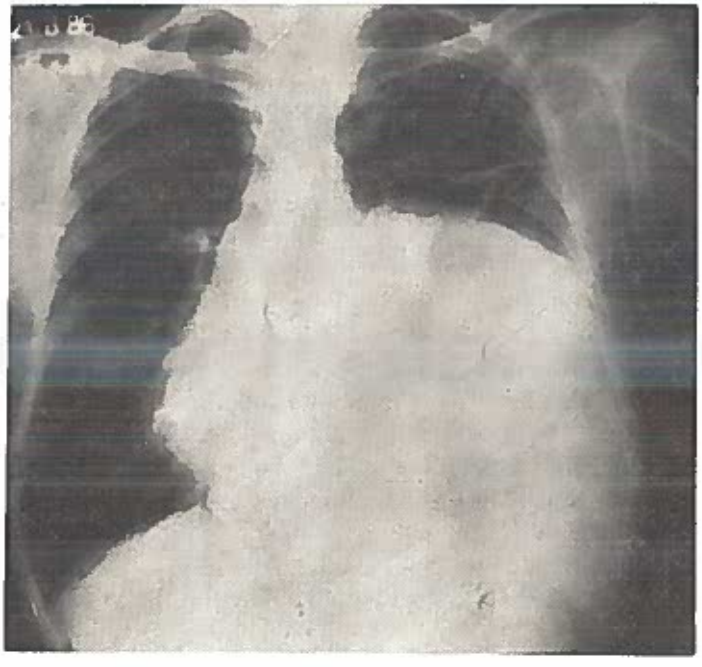

Figure 1
Preoperative posteroanterior chest X-ray.

was filling more than half the space of the left hemithorax was found. The mass was connected to the superior basal segment through a broad pedicle and the collapsed left lower lobe of the lung. This remarkably vascular tumoral mass had some adhesions to mediastinal pleura and pericardium. The pedicle was divided from its origin following ligation and the mass was totally excised.

The specimen weighing 2750 g was well encapsulated and had a lobulated smooth surface. On section, a firm yellowish tissue containing many blood vessels and some cystic structures were found (Figure 3). Histological sections revealed a benign, fibrous encapsulated neurilemoma with lines and palisades of spindle like cells forming organoids and ovoid cells forming loosened structures. The specimen was remarkably vascular with microcystic changes.

The patient had an excellent postoperative recovery period and was symptom-free without any radiological recurrence when he was seen 12 months later.

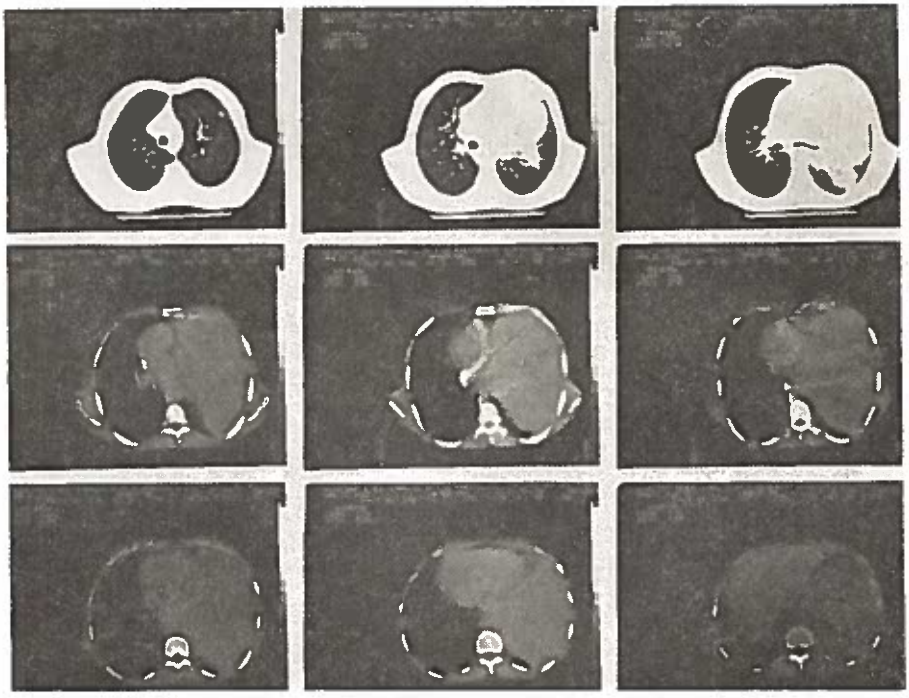

Figure 2
Preoperative computed tomography of the left lower chest showing the shifted mediastinum to the right by the large solid tumor.

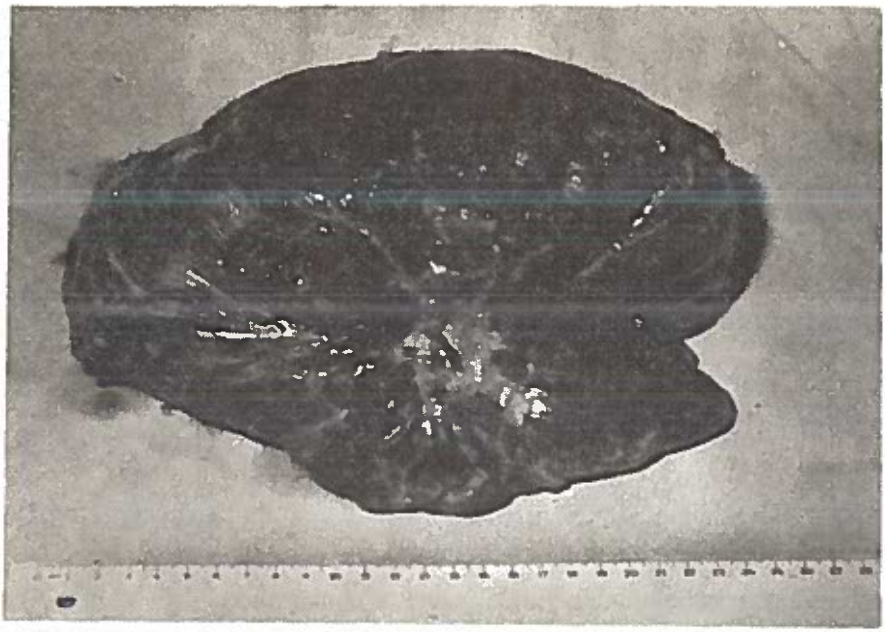

Figure 3
Anterior aspect of the excised mass.

Discussion

Neurogenic tumors are very rare neoplasms among pulmonary tumors.[1-4]

Since most of the patients with pulmonary neurilemoma are asymptomatic, these tumors are usually detected during routine X-Ray examination of the lungs.[4, 5] When symptoms do appear, they are usually nonspecific such as cough and pleuritic pain if the tumor is peripherally located.[1-5]

Primary intrathoracic neurogenic tumors can originate anywhere in the thorax but are most frequently found in the posterior mediastinum and the costovertebral angle.[2, 4, 6] Intrapulmonary neurogenic tumors deriving from nerve fibers associated with the bronchial tree are exceedingly rare.[6, 7] Neurilemomas seem to occur predominantly in women whereas all age groups may be affected.[1, 8]

The radiological features of these tumors are also nonspecific.[9] They usually appear as round, well defined ovoid or lobulated homogenous masses. If any compression to a large bronchus occurs the chest X-Ray may reveal massive collapsed area of involvel lung. In our case, since the mass was unexpectedly enlarged, left lower lobe was totally atelectatic.

Primary pulmonary neurilemomas frequently can be seen at bronchoscopy as a polipoid mass bulging into the bronchial lumen.[10, 11]

Some others may appear as an elevated, nodular mass covered with normal bronchial mucosa rarely showing oedema or superficial ulcerations. Preoperative bronchoscopic examination of our case showed nonspecific findings with an oedematous left lower lobe orifice and externally compressed left main bronchus.

Neurilemomas are usually small in size whereas in our case, the mass weighed 2750 g. Although this size may resemble malignant schwannoma that vary greatly in size, it is known that malignant transformation of pulmonary neurilemoma is an absolute rarity.[12, 13] Also malignant neurilemomas are not encapsulated and usually invade the surrounding tissues. To our knowledge, our case is one of the largest primary pulmonary benign neurilemoma case that was asymptomatic and has been totally removed with no recurrence at one year follow up.

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Bilateral Suprascapular Nerve Entrapment

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Summary

The authors describe the syndrome of suprascapular nerve entrapment neuropathy that occurs as this nerve passes beneath the transverse scapular ligament and discuss two case histories with bilateral suprascapular nerve entrapment remarkable on the left sides in two left handed sportsmen. The cardinal findings include weakness of shoulder abduction and external rotation, pain in shoulder and a positive electromyography. The patients refused surgical treatment. Physical therapy was applied and it was not effective on atrophy and weakness but shoulder pain disappeared. It was concluded that hyperprotraction of the shoulder overstretches this nerve with repeat activities and overuse of the joint. Both of the patients were popular volleyball players and interestingly, although the atrophy and weakness persisted they continued their professional life as volleyball players without any problem.

Key Words: Entrapment neuropathies, Suprascapular nerve, Shoulder pain.

Introduction

Suprascapular nerve entrapment may be more common than is generally believed, and may be an unrecognised cause of pain in the shoulder.[1] The suprascapular nerve is a mixed sensory and motor nerve that arises from the upper trunk of the brachial plexus.[2] It runs deep to the trapezius muscle and through a notch on the upper border of the scapula to supply the supraspinatus and infraspinatus muscles. The

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suprascapular notch has a varied shape and size and is bridged by the suprascapular ligament.[3, 4]

Injury to this nerve produces pain and weakness of shoulder abduction and external rotation, with wasting and weakness confined to the spinatus muscles. There may be tenderness on palpation of the suprascapular notch.[5, 6] This nerve is most frequently damaged by injuries in which the scapula is fractured or as a part of more extensive brachial plexus lesions.[2] True entrapment neuropathies within the suprascapular notch are rare.[7, 8] In some of these patients there is a clear history of prior shoulder injury[9, 10] whereas in others the entrapment occurs spontaneously.[1, 11]

Anatomically, the suprascapular nerve is tethered in the fibro-osseous tunnel of the suprascapular notch, especially in the extremes of the movement of the shoulder. Protraction or forward rotatory movement of the scapula seems to make the nerve very tight. Hyperprotraction of the shoulder overstretches the nerve with resultant swelling and creating a pain-swelling cycle similar to entrapment of the median nerve in the fibro-osseous tunnel of the wrist.[9]

The purpose of this paper is to report two cases with suprascapular nerve entrapment. A search of the literature disclosed only one case of neuropathy due to bilateral suprascapular nerve entrapment.[2]

Case Reports

Case 1: A 27 year old left handed sportsman, previously in good health was first noted on December 1984 by coincidence by a physiotherapist while he was practicing. His left supraspinatus and infraspinatus muscles were remarkably atrophic. When he was questioned, he reported that he was playing volleyball for 14 years as spiker and setter. He complained of left shoulder pain when he was asked if he had any problem and the pain was of a dull aching character. He also reported that he had been addmitted to another hospital and treated with the diagnosis of periarthritis of the shoulder. Later on September 1985 he was referred to our department because of pain in his other shoulder.

The only abnormalities on physical examination were related to the bilateral scapular regions. There was obvious decrease in muscle mass over the infraspinatus and supraspinatus muscles especially on the left side (Figure 1). No obvious fasciculations were noted, and there were no sensory changes. In muscle testing there was marked weakness of external rotation and abduction of the shoulder joints. Otherwise there were no abnormal neurological findings.

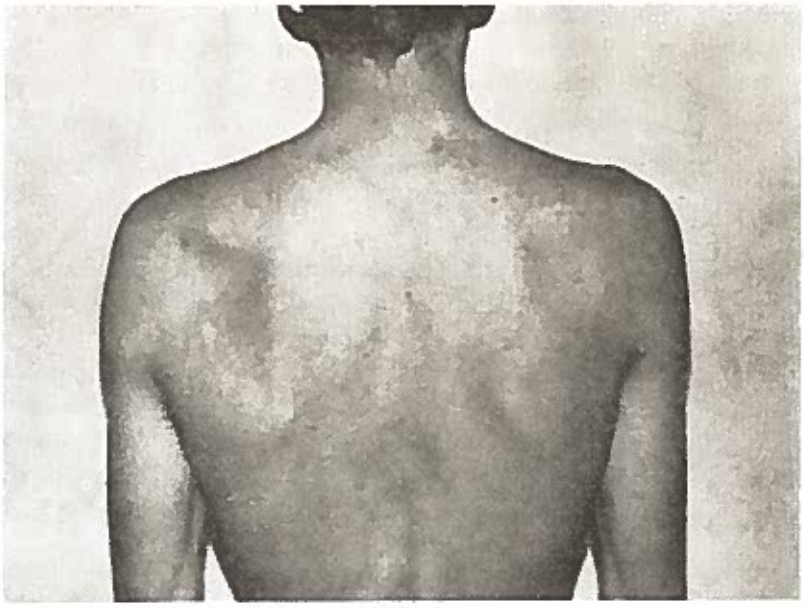

Figure 1
Bilateral decrease in muscle mass over the infraspinatus and supraspinatus muscles in case one, remarkable on the left side.

In radiological examination this cervical and thoracal spine and shoulder joints and special anteroposterior radiographies of the suprascapular notches were interpreted as normal.[5] Further shoulder artrography was negative. In the myelography and cervical CT there were no pathological findings. Infiltration of the suprascapular notch with local anesthetic provided temporary relief. In electromyographic examination denervation potentials and a decrease in motor unit action patentials (MUAPS) was observed in supraspinatus and infraspinatus muscles bilaterally.

Nerve conduction studies demonstrated delayed conduction in the right and left suprascapular nerves (Table I). All the other muscles and nerves of the upper extremities and paraspinal muscles were normal electromyographically. Laboratory investigations, including a complete blood count, serum chemistries, and urinalysis were normal. Serological tests for syphilis, tests for latex fixation, antinuclear antibodies, and lupus erythematosus preparation were negative.

The patient did not accept surgery for decompression of the right and left suprascapular nerve. Conservative measures were applied, including measures directed toward pain relief and shoulder girdle strengthening by means of exercises to elevate the shoulder. He still demonstrated

considerable wasting and weakness of these muscles but since the pain in his shoulders had subsided, he again refused surgery. The patient has remained well since and he has been playing volleyball without any problem for two years.

Case 2: A 20 year old left handed young man was first discovered while practicing also. He was playing volleyball for 3 years as a spiker and complaining of shoulder pain. There was remarkable atrophy of bilateral supraspinatus and infraspinatus muscles in the left side (Figure 2). Except the weak abduction and external rotation of the shoulders, no other abnormality in the musculoskeletal system was observed. Systemic physical examination, blood and urine analysis were within normal limits. The radiological examination of the cervical and thoracal spine and the shoulders were normal. No pathological change was observed in the myelography and computerized tomography (CT) of the cervical area.

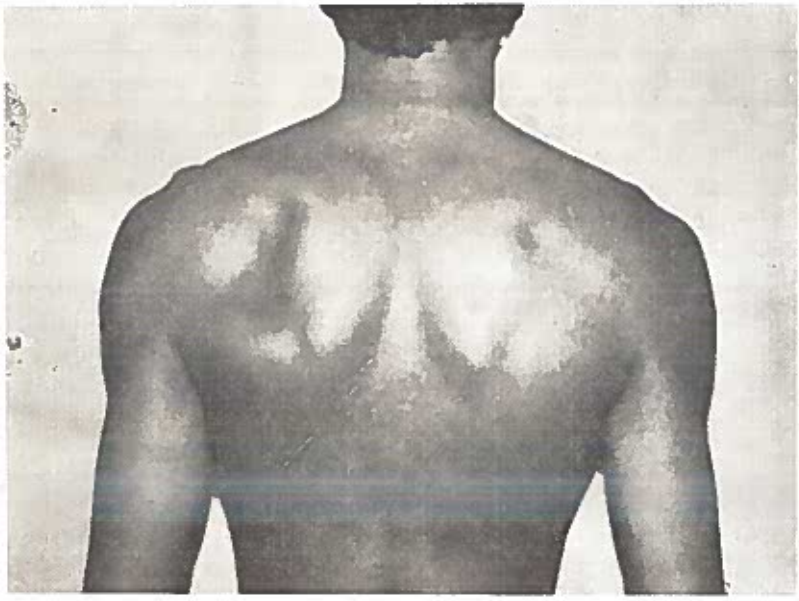

Figure 2
Atrophy of bilateral supraspinatus and infraspinatus muscles especially on the left side in case two.

In electromyographical examination denervation potentials and a decrease in MUAP's was observed in supraspinatus and infraspinatus muscles. Nerve conduction studies demonstrated delayed conduction in the right and left suprascapular nerve (Table I).

TABLE I
SUPRASCAPULAR NERVE LATENCIES
(IN MILISECONDS)*

	Case 1	Case 2
Left	4.1	4.2
Right	3.9	3.8

* Normal Values: 2.2 to 3.7 miliseconds.[2]

All other muscles and nerves in the upper extremities and paraspinal muscles were normal.

He also refused surgery. The same conservative therapy was given and was called back for a control. One month later it was observed that there was bilaterally suprascapular and infrascapular atrophy, but he did not have any pain or limitation in shoulder joint motion. Since then he has been playing volleyball without any problem.

Discussion

A lesion of the suprascapular nerve may be suspected clinically when there is a selective wasting of the supraspinatus and infraspinatus muscles associated with weakness of external rotation and of abduction of the shoulder.[1, 5, 6, 7]

Selective entrapment of the motor branches to the infraspinatus muscle would occur if the nerve underwent compression as it transversed and coursed around the edge of the lateral border of the spina of the scapula. Neurophysiological studies showing progressive delay of nerve conduction and denervation potentials also support the diagnosis.[7, 12] Theoretically, the compression could be due to either intrinsic or extrinsic pressure applied to the nerve. Prolonged entrapment can cause atrophy of the supraspinatus and infraspinatus muscles.

Isolated paralysis of the suprascapular nerve is rare, but the nerve can be damaged by traction along with the axillary nerve. There have been previous reports in the literature that certain arm motions might be related to injury of the suprascapular nerve.[4] Entrapment of the suprascapular nerve may be caused by traction or compression, since the serve is relatively fixed where it passes through the notch of scapula.[1, 7] The suprascapular notch, being a natural foramen, is the most frequent site of entrapment of the suprascapular nerve, which may be due to abnormal configuration of the notch,[10] a traction phenomenon,[4] or neoplasia.[3]

A relative fixation of the suprascapular nerve at the foramen, which is a constantly moving point because of the excursions of the scapula during movements of the arm can be commented. For this reason the nerve is subject to friction at this site and this could lead to inflammatory swelling and constriction of the nerve. While transmitted forces, direct injuries and traction all play a part in the cause, a traction injury may be the most significant cause, and this could also be postulated in the present cases. The shoulder is the most overused joint of volleyball players, and this is the reason why pain in this joint is very common. Injury to the suprascapular nerve produces pain and weakness of shoulder abduction and external rotation. Hyperprotraction of the shoulder overstretches the nerve with resultant swelling and creating a pain-swelling cycle while repeating the same activities and overusing the joint during volleyball games like our cases because both of them are left handed and the atrophy is remarkable on the left sides. In some cases, simple ablation of the fibrous component of the tunnel is not sufficient as the nerve remains taut and swollen and pain persists.

According to Clein, the treatment of the suprascapular entrapment neuropathy is by division of the transverse scapular ligament. He reported that two of his five cases returned to normal, but the remaining cases still had weakness and atrophy after the operation and none of them had pain anymore. Surgical decompression of the nerve by relasing it beneath the transverse suprascapular ligament has relieved all patients of pain almost at once. The improvement in function has been coexistent, but slower.[1]

Garcia and Mc Queen reported a bilateral suprascapular nerve entrapment syndrome. In their case the left side returned to normal although the patient was still complaining of mild atrophy in the right side after the bilateral operation.[2]

No return of function or bulk of the left infraspinatus muscle although on operation was performed for the dissection of a ganglion cyst was also mentioned by Ganzhorn *et. al.*,[3]

Rask reported two patients, having temporary relief from simple transverse scapular ligament release and later lasting relief of pain from scapular notch resection and stated that primary wide notch resection could be the preferable procedure.[9]

In literature it is proposed that this entrapment neuropathy had no effective conservative therapy method, but the results of the operative therapy were not satisfactory either. The major gain by the operative treatment is the relief of pain, but the muscle atrophy and function does not return to normal in most of the cases treated surgically. Although

remarkable atrophy in supraspinatus and infraspinatus muscles and shoulder pain was observed in both of our cases because of their refusal of surgery they were treated conservatively by physiotherapy. The physiotherapy was effective on pain but both of them still demonstrated considerable wasting and weakness of these muscles. Interestingly, although, both of their bilateral shoulder external rotation and abduction was weak, they could easily go on playing volleyball professionally. These young men are still under the control of our Department and both of them play volleyball without any problem since 1984.

As a conclusion we want to propose that hyperprotraction of the shoulder can overstrech the suprascapular nerve and cause shoulder pain and atrophy in supraspinatus and infraspinatus muscles in volleyball players.

Yet this remarkable atrophy does not prevent sportive activities if the pain subsides.

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Factors Affecting Post Invitro Fertilization (IVF) Implantation

Timur Gürgan, M.D.**

Post IVF Implantation

As the techniques and procedures that together constitute IVF/ET therapy have been refined to achieve improved success in pregnancy rates, one aspect in particular has still confounded most efforts to understand and control it. With the increasing diversity and flexibility of drug induction regimes, and also the improved consistency and standardization of egg and spermatozoal culture, the production and freezing of good quality embryos is no longer the haphazard business it once was, but the unpredictable fate of these seemingly viable embryos remains the least understood area of human IVF. The following commentary considers many of the varied factors that appear to affect post IVF implantation, and its chances of occuring. These general areas are:

1. Embryo replacement technique
2. Endometrial 'priming' and receptivity
3. Pregnancy related proteins
4. Utero-fetal immunoregulatory mechanisms
5. The seminal ejaculate
6. Platelet activating factor (Figure 1)

These disparate areas perhaps have more in common with each other and, more pertinently, have more immediate relevance to implantation than may at first appear to be the case. Certainly several areas overlap, some less cryptically than others, and the clinical nature of human IVF therapy has led to some emphasis on control over the first two areas.

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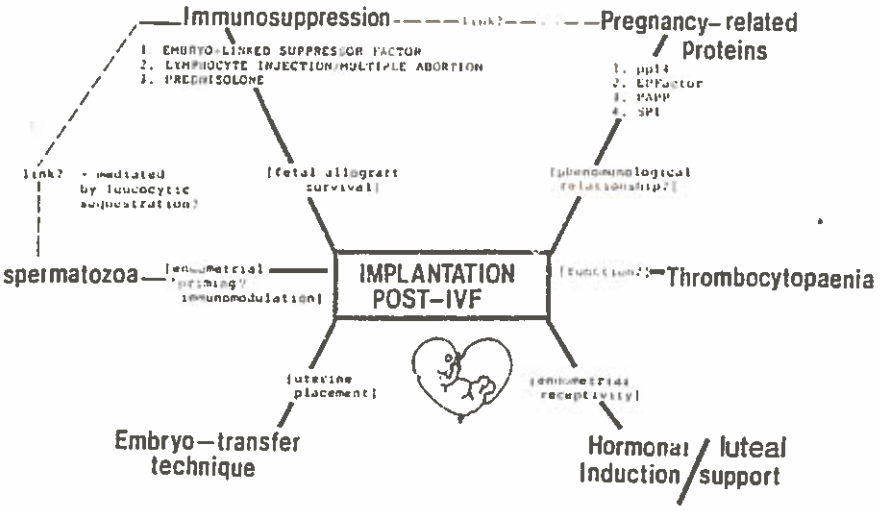

Figure 1

1. A wide variety of embryo transfer/replacement catheters exists, and transfer medium volumes and constituents also vary enormously. This is partly historic, in part economic, and more recently due to an effort to establish what is the optimal medium in which to replace the embryos, and in what manner. Placement of the embryos is thought to be critical; too high a transfer increasing chances of tubal ectopic implantation[1] too low giving rise to embryo loss through the cervix, or placenta previa.[2] Traumatization of the endometrium and cervical canal with the transfer catheter can be detrimental due to bleeding and possible inflammatory response with related uterine prostaglandin release,[3] and it can be said that clinicians favour the 'gentle touch'. Surgical transfer[4] and the use of ultrasound guidance[5] have enabled the observation of where the transfer of the embryos occurs within the uterine cavity. These physical considerations, though important however, have little to do directly with the subsequent implantation of the replaced embryos, as the embryos are then left to wait unphysiologically in the potentially hostile uterine environment for up to six days prior to implantation, depending on the cell stage at which the embryos are transferred. Meanwhile a concatenation of other events needs to occur before implantation may proceed successfully.

2. The fact that the hormonal status of the luteal phase is a direct consequence of any foregoing hormonal induction regime, has meant much research has been done into the need, if any, for luteal hormonal support to correct or 'save" the endometrium due to any deficiencies

resulting from insufficient or excessive follicular stimulation. Consequent inappropriate estrogen/progesterone ratios may arise, and human chorionic gonadotropin (hCG; e.g.,[6]) or progesterone (e.g.,[7]) have been used to attempt a redressing of the balance, through neither has proved of value except to prolong the luteal phase. However, it is possible that earlier and sequential treatment with these drugs may allow a more physiological, and hence successful support.[8] hCG has been used successfully in the treatment of habitual aborters where support was continued up to the sixteenth week of gestation.[9] This luteal support might be more eloquently explained in terms of endometrial receptivity, and more specifically the steroid hormone receptors therein. Although synchronism between the early embryo and endometrial evolution appears not to be as strict in the human as for other species, as indicated by pronucleate embryo transfer,[10] nonetheless, incorrect or incomplete modulation of the number of cytoplasmic receptors, and subsequent level of nuclear triggering of steroid target cells, will render the endometrium hostile to unimplanted embryos.[11] This is clearly seen in the relationship between estrogen binding sites and the biological response of uterine endometrial growth.

3. Moving away from the relatively more obvious factors when considering post IVF implantation, there are those areas when observable changes at, and features of implantation are used to allow a 'back bearing' to be taken in an attempt to predict the viability of any implantation. hCG has become the normal 'marker' for monitoring early implantation[12] though more recently a new generation of pregnancy related proteins has been described and provide potential prognostic markers of pregnancy, for example, PAPP-A and SP1,[13] alpha-1 and alpha-2 PEG.[14] Some confusion exists where some of the proteins have been characterized in different laboratories and have been given different names; roles for most of these proteins have not been worked out either, although there is some suggestion of an immunomodulatory function for both PP14/alpha-2 PEG,[15] and the extensively researched though poorly characterised Early Pregnancy Factor (EPF;[16]). To consider EPF more closely, it is said to be pregnancy-dependent, and seems to be released within hours of fertilization, and subsequently throughout much of any ensuing pregnancy. Its precise make-up remains elusive, but it is thought to be a suppressor-releasing hormone effecting an immunomodulation of the uterus to ensure that immune-rejection of the fetus does not occur. Its continued production is taken to indicate a maintenance of fetal viability. Pituitary prolactin production, an 'ovum factor' and a 'tubal factor' are all said to be contingent upon early production of EPF. With reference to IVF, unwitting mistreatment of oocytes

in culture *in vitro* may well currently jeopardize initiation and continued production of EPF, and consequently lessen chances of implantation; indeed, this may hold true for any one or more of the proteins/factors related to early pregnancy. The means by which immunomodulation of the uterine environment is worked has not been established for these proteins.

4. Following hard on the heels of the pregnancy-related protein studies is the elegant work of Daya and Clark,[17] who are approaching the question of utero-fetal immunoregulation from the cellular level, addressing the longstanding paradox: why is the fetal allograft not rejected? Their studies have led to the discovery of two immunosuppressor cells in the human uterus. The absence of such cells correlates with pregnancy failure, whilst the presence of these non-specific cells evidently confers protection on the fetal allograft, and pregnancies may succeed. It seems an immunosuppressive factor released from some human embryos calls into action these two cell propulations, and this has brought us closer to one definition of a viable embryo whereby it must secrete this factor to enable it to implant.

The converse of an embryo modifying its host's immunology has been explored by Mowbray *et. al.*,[18] who have considered active recognition of paternal antigens on the fetus to be central to the maintenance of any pregnancy. Though some controversy exists as to the real prognostic value of associated anti-paternal cytotoxic antibodes[19] nevertheless, the immunization of recurrent aborters with paternal lymphocytes has met with some success. Immunosuppressant drugs themselves have been used[20] as supplements in IVF therapy, althogh how this might relate to the subtle workings of an active materno-fetal immunorecognition is unclear.

5. It has been proposed[21] that a 'missing cog' in the 'clockwork' of IVF is the very physiological presence of semen. Currently in many IVF programs it is likely that many couples refrain from sexual intercourse throughout an entire IVF cycle, thus embryos are returned to an environment devoid of any 'spermatozal priming' (cf, the greater success of GIFT where spermatozoa are present), that putatively is mediated by the extensive cervical and uterine leucocytosis that would normally occur *post coitus*. This rather pleasingly innovative concept provides a nice explanation of the function of the excessive numbers of sperm in the ejaculate, and is based on a sporadic but reasonable amount of evidence from the literature that has highlighted the importance of the normal presence of spermatozoa (see:[22, 23]), and has even gained acceptance as a relevant factor in a few IVF programs (e.g.,[24]). The fate of such

large amounts of 'non-fertilizing' spermatozoa is not entirely clear, and the means by which they effect embryonic cleavage and implantation is also unclear.[25] Sperm significantly stimulate endometrial protein synthesis in the rabbit for example,[26] and such an impact on the female reproductive tract that is separate from fertilization itself does imply a greater role for spermatozoa in general, the relevance of which is not yet fully understood.

6. A somewhat suprising factor that has been observed and adopted as a monitor of embryonic viability is the drop in the platelet count (thrombocytopaenia) during the pre-implantation phase of pregnancy.[27] Viable embryos are said to produce platelet activating factor (PAF) and a drop in the platelet count is throught to be a good indication of possible successful implantation, where in contrast non-viable embryos lack the ability to produce PAF, and pregnancies are not established. No explanation of the basis of why this humoral response occurs has so far been forthcoming.

Concluding Remarks

No attempt is made here to draw together this series of seemingly unrelated factors that have some bearing on implantation into some theory of unification. It is more an attempt to direct thoughts to a new framework within which the natural embryonic wastage, and the even higher failure of post-IVF implantation may become more readily explicable.

However, in accepting that subtle and perhaps even important links may exist between the factors discussed here, then a certain general proposition may be made. To think of these factors as being discrete and unrelated would deny the interrelated continuum of events that occurs thoughout an ovulatory cycle. Central to implantation is the condition and 'receptivity' of the uterine endometrium, and indeed all of the previously considered factors have some relationship to the state of the endometrium. The absolute level and gradient of the estrogen rise, and the subsequent luteal phase progesterone/estrogen ratio directly influence proliferation and maintenance of the endometrium. The normal presence of semen and spermatozoa at the time of ovulation elicit a marked invasion of leucocytes into the endometrial tissues, and a related non-specific protein synthesis is noticed at this time. The presence of fertilized embryos interacts with the uterine environment to cause a systemic thrombocytopaenia throughout the pre-implantation stage of pregnancy, and at this same time the luteal-phase suppressor cells described by Daya and Clark[17] migrate into the endometrium. Gentle transfer of

IVF embryos to the uterine cavity is necessary not to disturb the endometrial milieu. Finally, the temporal array of pregnancy-related proteins comes into play prompted by some form of embryonic signal that stimulates endometrial production of this host of factors from earliest pregnancy to term.

Whatever the relative significance of these factors in the overall context of implantation, it can be seen that they all relate in some way to the development, control and maintenance of the endometrial tissues, be it the stroma, luminal or glandular epithelium. It would be wrong to try to propose theoretical links where none exist; but equally it would be a mistake to divorce all these factors from each other, when so many clues seem to indicate a universal involvement with endometrial implantation.

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