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Labelling and Evaluation of ^{99m}Tc-α-D-Glucose 1-Phosphate for Radionuclide Angiocardiography

Meral T. Ercan, Ph.D.* / Seydi Aksüt, M.D. / Tülin Aras, M.D.***

Summary

α-D-Glucose 1-phosphate (GP) was labelled with ^{99m}Tc and the labelling efficiency was determined by thin-layer chromatography (ITLC). Over a time period of 24 hours the amount of impurities stayed below 2 %. The radiochemical was administered to 2 rabbits and scintigrams were obtained at certain intervals up to 24 hours. It was also administered to 3 normal subjects. Blood samples were collected at 10 and 30 minutes, 1, 2, 6 and 24 hours. Whole blood and plasma samples were counted against a standard. Protein binding was determined in plasma samples by the TCA precipitation method. Urine was collected at 3 hour intervals and a sample from each was counted. Scintigrams of the heart were taken by the use of a gamma camera at intervals up to 24 hours. In another group of 5 normal subjects, the ejection fraction (EF) of the left ventricle was calculated by the use of a pre-prepared program (MUGA) with both ^{99m}Tc-GP and ^{99m}Tc labelled erythrocytes (RBC).

The results indicated a slow removal of radioactivity from the vascular space. Only 15 % was excreted in urine within the first 3 hours. Scintigrams of the heart were of high-contrast good-quality, deliniating the cardiac blood-pool. An ejection fraction of 53.3 ∓ 5.0 % was obtained with ^{99m}Tc-GP and 53.0 ∓ 3.3 % with ^{99m}Tc-RBC's in the same subjects.

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Key Words: ^{99m}Tc Labelling, ITLC, Protein-Binding, Radionuclide Angiocardiography, MUGA, Ejection Fraction (EF).

Introduction

Radionuclide angiocardiography has become an important tool in clinical cardiology. This technique provides noninvasive visualisation of cardiac motion and anatomy. The information obtained in such studies are useful in the diagnosis and treatment of cardiovascular disorders.[1] A number of radiopharmaceuticals have been developed for this purpose, such as ^{99m}Tc labelled human serum albumin (TcHSA),[2] ^{99m}Tc-dextran[1,3] or red blood cells (RBC) labelled in vivo[4] or in vitro[5,6] with ^{99m}Tc *In vivo* labelling method introduced by Pavel *et al*,[4] has found wider application and has been adopted for clinical routine use, because of the simplicity and ease of labelling. However, organs such as thyroid, stomach and intestines compete with RBC's for the uptake of $^{99m}TcO^-_4$ and as a result, the labelling of RBC's is low. An often encountered problem is the uptake by the stomach which prevents the calculation of the left ventricular ejection fraction (EF) by the use of a multigated acqusition (MUGA) program.[1]

In our search for a better radiopharmaceutical for cardiac blood-pool imaging, we labelled α-D-glucose 1-phosphate (GP) with ^{99m}Tc and evaluated it first in rabbits and then in normal volunteers.

Materials and Methods

Radiopharmaceutical Preparation and Quality Control: α-D-Glucose 1-phosphate (GP) was purchased from Sigma Chem. Co., U.S.A. Technetium (^{99m}Tc) was obtained from a generator (Amersham, England). The following procedure was used for labelling: 30 mg of GP was dissolved in 2 ml distilled water. The pH of the solution was adjusted to 5-6 with 1 N HCl. 0.5 ml $SnCl_2 2H_2O$ solution (1 mg/ml) was added and stirred for a few minutes. The mixture was passed through a 0.22 μ Millipore filter directly into a sterile glass vial. An appropriate amount of pertechnetate ion ($^{99m}TcO^-_4$) in saline was added to the vial. It was shaken for 30 seconds.

Impregnated thin-layer chromatography (ITLC) plates (ITLC-SG) were used to determine the labelling efficiency. Saline or acetone were used as solvents. Each batch was analyzed 30 minutes after preparation. ITLC was repeated at 3 and 24 hours to determine the *in vitro* stability of ^{99m}Tc-GP at room temperature.

Animal Experiments: Two rabbits were administered through the ear vein with 5 mCi of ^{99m}Tc-GP. Scintigrams were obtained at 5, 10, 30,

60 minutes, 2, 4, 6 and 24 hours post-injection by the use of a gamma camera (Scintiview II, Siemens).

Human Studies

1. Blood Clearance and Urinary Excretion: Three normal volunteers were administered 20 mCi ^{99m}Tc-GP in a volume of 0.5-1.0 ml. 10 ml blood samples were collected at 10, 30 minutes, 1, 2, 6 and 24 hours. 1 ml blood from each sample was saved and the rest was centrifuged at 3000 rpm for 10 minutes to seperate the plasma. Urine was collected at 3 hour intervals up to 24 hours. The volume of each urine sample was measured. 1 ml samples of whole blood, plasma and urine were placed in special counting tubes and the radioactivity was counted in a gamma counter (BF 5300, Berthold, F.R.G.) against a standard.

2. Affinity of ^{99m}Tc-GB to RBC's and Protein Binding: The five ml blood samples obtained above were centrifuged at 3000 rpm for 10 minutes. The plasma was separated. Both the RBC and the plasma fractions were counted in the gamma counter.

Protein binding of ^{99m}Tc-GP was determined by protein precipitation with trichloroacetic acid (TCA). Five ml of a 5 % TCA solution was added to 0.5 ml plasma. Ten minutes later the mixture was centrifuged at 3000 rpm for another 10 minutes. The supernatent was decanted into another tube. Five ml TCA solution was added to the precipitate and was stirred. Ten minutes later the mixture was centrifuged again and the wash solution was saved. The washing was repeated 5 times. Both the precipitate and the wash solutions were counted in the gamma counter. The fraction of radioactivity precipitated with the proteins was calculated.

3. Scintigraphic Studies: Three subjects were administered with 20 mCi ^{99m}Tc-GP while lying prone under the collimator of the gamma camera. Kinetic study of the heart was started immediately. 120 frames, each 0.5 sec were collected. Static scintigrams of the heart were obtained in the same position by collecting 500.000 counts for each image at 5, 30 minutes, 1, 2, 4, 6 and 24 hours.

4. Multigated Equilibrium Cardiac Blood-Pool Study (MUGA): 5 normal subjects were administered with a bolus of 0.5 ml ^{99m}Tc-GP (20 mCi). The high-resolution parallel-hole collimator of the gamma camera was placed 45° left anterior oblique position. Data were accumulated in synchronization with the electrocardiogram and stored. By the use of "auto edge cap" program the ejection fraction (EF) of the left ventricle was calculated automatically. A repeat MUGA study was

performed a week later on the same subjects, this time by the use of Pavel *et al's in vivo* method of RBC labelling.[4] The EF's obtained through both methods were compared.

Results

^{99m}Tc-GP was prepared with a high efficiency (> 99 %). It stayed at the point of application in acetone and moved with the solvent front in saline (R_f: 1.0). Two contaminants are expected in technetium labelling: $^{99m}TcO^-_4$ and $^{99m}TcO_2$. $^{99m}TcO^-_4$ moved with the solvent front (R_f: 1.0) in both saline and acetone. Its amount was determined in acetone. $^{99m}TcO_2$ stayed at the origin in both solvents. Its amount was determined in saline. Thirty minutes after preparation the amount of $^{99m}TcO^-_4$ was 0.80 ∓ 0.05 % and TcO_2 0.14 ∓ 0.10 %. The total amount of contaminants was negligible even after 24 hours (Table I).

TABLE I

STABILITY OF ^{99m}Tc-GP AT ROOM TEMPERATURE, DETERMINED BY ITLC USING ACETONE OR SALINE AS SOLVENTS

Elapsed time after preparation	No. of observations	% Impurities, Mean ∓ SD	
		$^{99m}TcO^-_4$	$99mTcO_2$
30 min	8	0.80 ∓ 0.05	0.14 ∓ 0.10
3 h	5	0.77 ∓ 0.27	0.94 ∓ 0.65
24 h	5	1.00 ∓ 0.12	1.21 ∓ 0.45

Scintigraphic studies with rabbits indicated that ^{99m}Tc-GP stayed in the vascular space up to 24 hours. The cardiac blood-pool could be visualized during this time (Figure 1).

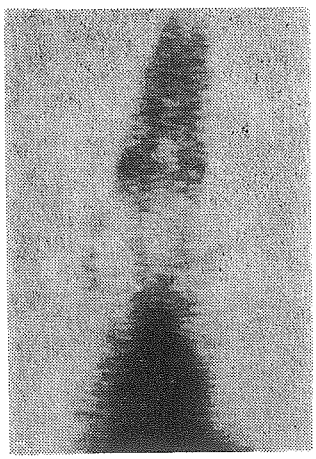

Figure 1
Scintigraphic image in a rabbit at 30 min post-injection of ^{99m}Tc-GP.

The whole blood and plasma clearance curves of ^{99m}Tc-GP are shown in Figure 2. Blood radioactivity levels decreased at a slow rate. Urinary excretion was 14.6 % within the first 3 hours and reached a plateau after 24 hours with 48.7 % excretion (Figure 3).

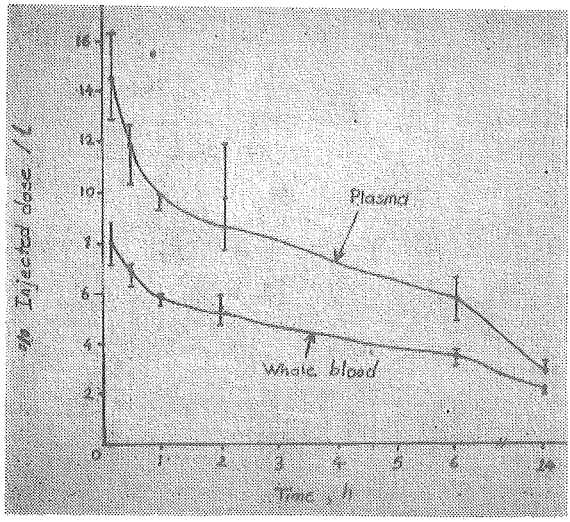

Figure 2
The whole blood and plasma clearance curves of ^{99m}Tc-GP in man.

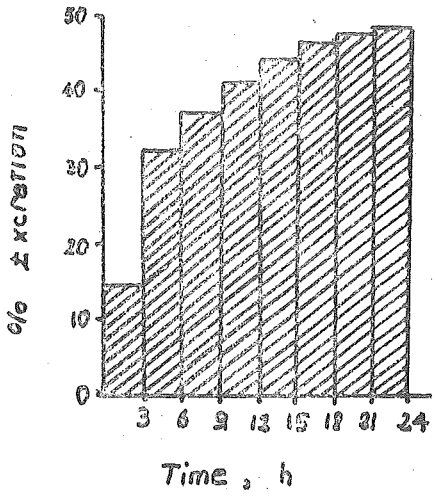

Figure 3
Cumulative urinary excretion of ^{99m}Tc-GP in man.

Centrifugation of the blood and the counting of RBC's and plasma showed that 4.0∓0.8 % was in the RBC fraction and the rest was in the plasma. These findings indicate that little, if any ^{99m}Tc becomes attached to RBC's. Even after centrifugation the packed red cells still contained 2-4 % plasma.[1] Protein binding was 79.05 ∓ 7.67 % of the radioactivity present in the plasma fraction.

Scintigraphic kinetic images of the heart obtained in a normal subject is shown in Figure 4. The static images obtained at times indicated are shown in Figures 5 and 6. The heart was well visualized up to 24 hours, indicating that the radioactivity was still in the blood-pool. The kidneys, and to a lesser extent the liver and the spleen were also visualized. There was no accumulation of radioactivity in the thyroid or stomach indicating the absence of $^{99m}TcO^-_4$ ion *in vivo*.

The results of a MUGA study is shown in Figure 7. The mean EF of the left ventricle obtained in 5 subjects was 53.3 ∓ 5.0 % with ^{99m}Tc-GP. When ^{99m}Tc-pyrophosphate was used to label the RBC's in the same subjects a mean EF of 53.0 ∓ 3.3 % was obtained. EF's within the range of 50-75 % were considered normal.

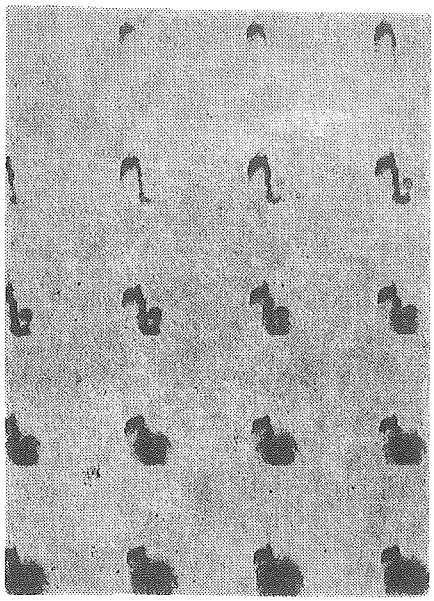

Figure 4

Kinetic (first-pass) images of the heart in a normal subject.

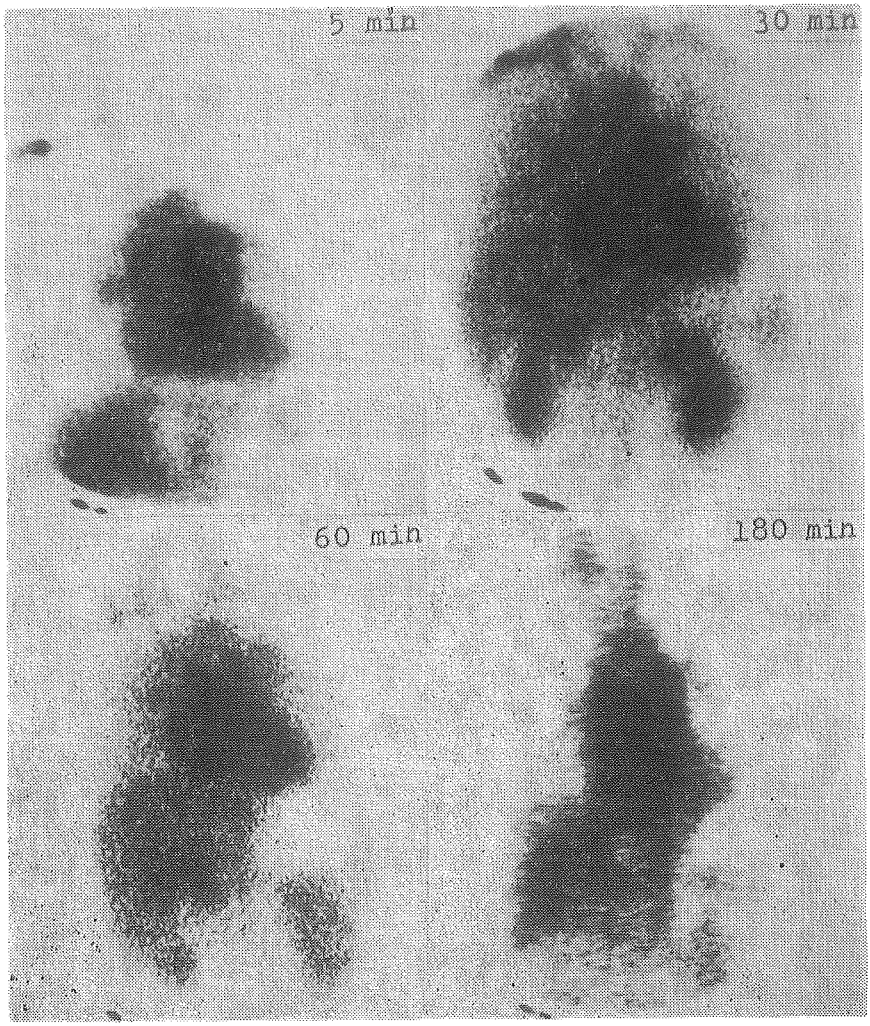

Figure 5
The static images of the heart at times indicated in a normal subject, taken in anterior position.

Discussion

None of the currently used radiopharmaceuticals for blood-pool studies is satisfactory. ^{99m}Tc-HSA leaks out of the vascular space, the labelling efficiency is variable and the accumulation in the liver is quite high.[4, 5, 7] ^{99m}Tc-dextran is removed very fast from the vascular space and accumulates in the liver where the dextran molecule is oxidized and is broken down to smaller fragments that are eventually excreted by the kidneys.[3] The cardiac image deteriorates starting at 5 minutes post-injection in favor of liver image. RBC labelling *in vivo* with ^{99m}Tc re-

quires separate injections of (Sn) pyrophosphate and $^{99m}TcO^-_4$ 30 minutes apart and does not always provide adequate labelling of RBC's[8] The EF cannot be accurately determined because of high stomach activity in some subjects.[1] Semi *in vitro* labelling procedures were introduced to improve the labelling yield.[8, 9] In *in vitro* RBC labelling techniques almost 100 % labelling in possible.[5, 6] However, the labelling procedure is tedious and time-consuming. There is also some risk of contaminating the patient's blood since too many steps are involved. A single injection *in vivo* labelling method is to be preferred in blood-pool studies.

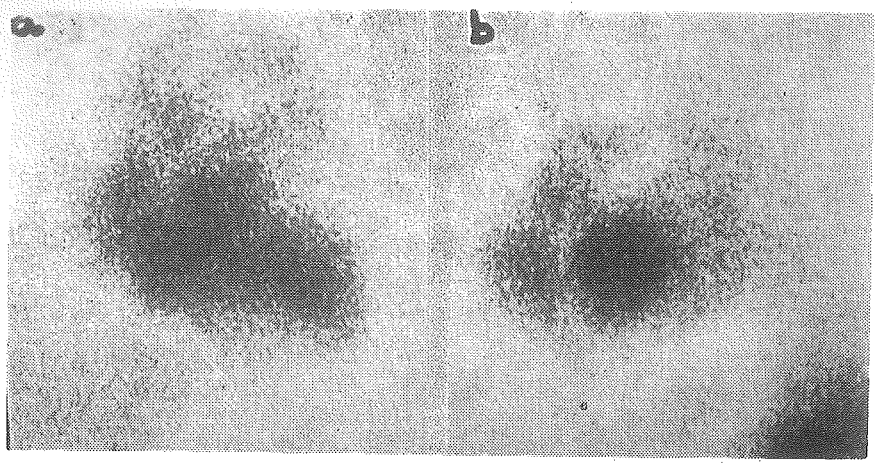

Figure 6
Close-up view of heart from a) anterior and b) 45° left anterior obligue position.

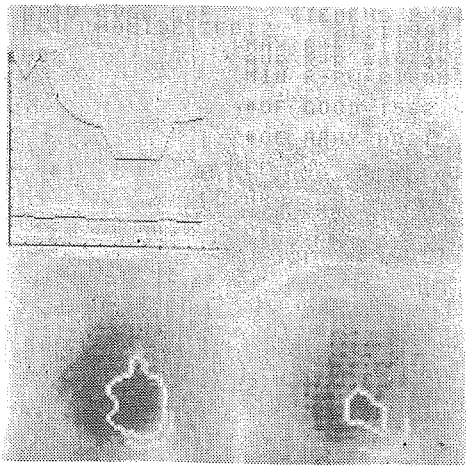

Figure 7
The results of a MUGA study in a normal subject administered with 20 mCi ^{99m}Tc-GP, indicating an ejection fraction (EF) of 54 %.

Our results with ^{99m}Tc-GP indicate that it is an adequate radiopharmaceutical for cardiac blood-pool studies. It is prepared with a high specific activity, so a bolus injection of 20 mCi in 0.5 ml is possible for first-pass studies. The radiopharmaceutical is stable up to 24 hour after preparation (Table I). It does not necessiate two injections as in the case of *in vivo* RBC labelling. There is no risk of contaminating the patient's blood compared to *in vitro* methods. Visualization of the heart persists up to 24 hours. The study is usually completed within the first hour. During this time decrease in blood radioactivity levels and urinary excretion is low. The absence of $^{99m}TcO^{-}_{4}$ *in vivo* is a great advantage and allows the calculation of EF in each study. The absence of radioactivity from the neighboring organs such as the liver, the stomach and the lungs is clearly an advantage. The only disadvantage is the excretion via the kidneys which are also visualized but do not interfere with the cardiac studies.

In this study we have shown that ^{99m}Tc-GP is a better radiopharmaceutical than the ones currently available for radionuclide angiocardiography. However, the ideal radiopharmaceutical would be the one that would label RBC's *in vivo* to almost 100 % efficiency with a single injection. This agent is yet to be found.

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High Dose Gamma Globulin Therapy in Childhood Acute and Chronic Immune Thrombocytopenic Purpura (ITP)*

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Summary

Twelve children with ITP (six chronic and six acute) were given intravenous gamma globulin 400 mg/m²/day for five days. All six of the new cases responded to therapy and five of these patients remain disease free. One patient in this group later returned to thrombocytopenic state and now has chronic ITP.

The six cases of chronic ITP showed variable responses, albeit transient.

Routine use of intravenous gamma globulin in acute and chronic cases of ITP is not justified and the only reason for using this mode of therapy may be in steroid resistant cases in preperation for splenectomy or any other surgical procedure.

Key Words: Idiopathic thrombocytopenic purpura, Immune thrombocytopenic purpura, Gamma-globulin therapy.

Introduction

High dose intravenous gamma globulin (HDIgG) therapy was first used by Imbach[1] *et al* in 1981 for childhood acute and chronic ITP. Since

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that time, there have been several cases reported on children and adults with acute and chronic ITP, with variable results.[2-6]

This study was initiated to assess: 1. whether HDIgG will prevent any of the patients from advancing into chronic state when administered at the time of diagnosis, 2. whether any of the chronic cases could be induced to remain in permanent remission, and, 3. whether there is any difference in Saudi children in terms of response to HDIgG therapy.

Material and Methods

Six children (3 girls and 3 boys) who were known chronic ITP patients and had been on intermittent prednisone therapy were admitted for HDIgG therapy. None of these patients had been treated by any other medication and two had splenectomy, 6 and 20 months prior to HDIgG therapy. All six patients had platelet counts below 20,000/mm^3 immediately before the HDIgG therapy and they were not on any medication at that time. Chronic ITP was defined as a platelet count less than 60,000/mm^3 for at least six months and as increased number of megakaryocytes in the bone marrow with no other existing condition to account for the trombocytopenia.

Six other children (2 girls and 4 boys) who were consecutively diagnosed as having acute ITP and did not receive any prior therapy were also admitted for HDIgG.

Routine diagnostic evaluation included physical examination, complete blood counts by Coulter-S before therapy, daily during therapy and once a week thereafter for 8 weeks. Platelet counts below 50,000/mm^3 were repeated manually. Bone marrow aspiration was done on every patient before therapy. Serum electrolytes, renal and liver function tests were monitored before and after therapy at regular intervals. Serum IgG and IgM levels were measured by immunodiffusion (Meloy Laboratories, VA) before therapy and at weekly intervals for four weeks.

The intravenous gammaglobulin (Swiss Serum Vaccine Institute, Berne) was given at a dose of 400 mg/m^2/day for 5 consecutive days over 1.5 - 3 hours infusion.

Duration of response was defined as the number of days during which platelet counts remained more than 20,000/mm^3 following the HDIgG therapy. Remission was defined as the maintenance of a platelet count above 150,000/mm^3 without any form of therapy.

An informed consent was obtained from the parents of each patient prior to treatment.

Results

Clinical information and the results of HDIgG treatment on acute and chronic patients are shown on Tables I and II.

TABLE I
PATIENT CHARACTERISTICS AND RESPONSE TO HDIgG IN CHILDREN WITH NEWLY DIAGNOSED ITP

Patient No	Sex	AGE Years	Pre Tteatment Platelets/mm^3	Post Treatment Platelets/mm^3
1	M	8.5	5,000	113,000
2	M	5	13,000	434,000
3	M	10/12	20,000	166,000
4	F	8	9,000	454,000
5	F	4	15,000	240,000
6	M	5	12,000	320,000

TABLE II
PATIENT CHARACTERISTICS AND RESPONSE TO HDIgG IN CHILDREN WITH CHRONIC ITP

Patient No	Sex	AGE Years	Pre Treatment Platelets/mm^3	Post Treatment Platelets/mm^3
1*	F	9	5000	38,000
2	M	2.5	12,000	29,000
3	F	7	20,000	219,000
4	M	2.5	5,000	484,000
5*	F	4	2,000	110,000
6	M	7	9,000	71,000

(* Splenectomized)

All six newly diagnosed patients responded with a significant rise in platelet counts on day 4 or 5 of the HDIgG. Average pretreatment platelet count was 12.300/mm^3 and the average peak platelet count was 288,000/mm.^{3} Five of these six patients are still in remission with a follow up period of five to nine months and require no further therapy. One patient (patient number 1), had a peak platelet count of 113,000/mm^3 on day 5 of HDIgG infusion, nevertheless, it dropped below 20,000/mm^3 within ten days following the treatment. During the follow up period of nine months, his platelet count has been fluctuating between 8,000/mm^3 and 40,000/mm.^{3} This patient, who incidently has Down syndrome, now has a chronic disease and proposed splenectomy was refused by the parents.

Six patients with chronic ITP showed variable responses to HDIgG therapy. While platelet counts were elevated significantly in three patients, the other three showed a minimal response (Table II). Nevertheless, all dropped below 40,000/mm^3 within two weeks following the HDIgG infusion. Previous splenectomy on two patients did not seem to have any effect on their response to HDIgG.

During the follow up period (ranging from 6 to 14 months), these six patients retained their chronic disease state, similar to their condition prior to HDIgG therapy with platelet counts fluctuating between 7,000/mm^3 and 50,000/mm^3.

Following the HDIgG infusion, serum IgG and IgM levels were elevated in both acute and chronic cases. There was no statistical difference between these groups ($p > 0.05$). Pretreatment IgG level was 858 $\mp$ 420 mg/dl and increased to 1850 $\mp$ 422 mg/dl, while pretreatment IgM was 84 $\mp$ 46 mg/dl and was elevated to 210 mg/dl $\mp$ 33 mg/dl after the treatment.

There were no significant toxicities that resulted with the cessation of therapy. Three patients experienced moderate headaches and one of them also complained of pain over the sternum. These side effects seemed to be related to the rapidty of the infusion. The infusion rates had been adjusted to run in 1.5 hours. After these complaints, infusions were given over 3-4 hours; this appeared to eliminate any further side effects.

Discussion

ITP in children is generally a benign, self limited condition with spontaneous recovery occuring in 85-90 % of the cases within a few weeks.[8,9] However, controversy over the management of the acute ITP has not yet been resolved.[10-12] While the discussions on the use of steroids in acute ITP continued, successful use of intravenous IgG by Imbach[1] offered a new scope for the discussions on the management of the acute and the chronic forms of the disease. Major questions to be answered were: 1. What is the mechanism of action of intravenous gamma globulin? 2. Can the chronic form of disease be prevented by using HDIgG at the time of diagnosis? 3. Can chronic patients be cured or saved from splenectomy?

Fehr *et.al.*[5] and Bussel *et.al.*[2] demonstrated that inravenous gammaglobulin inhibits the Fc-receptor mediated reticuloendothelial clearence. Therefore, the major mode of action of HDIgG in ITP seems to be the Fc-receptor blockade.

However, persistence of elevated platelets beyond the priod of receptor blockade in some patients, and the decrease, in spite of continuing blockade in others, suggest that other mechanisms may also be involved.[2] Inhibition of antiplatelet antibody synthesis has been speculated,[1] and was demonstrated in some patients.[2, 3]

In this study, one of the newly diagnosed patients developed a chronic disease in spite of his initial response. This behavior suggests that it is not entirely possible to abort chronicity by administering HDIgG at the time of diagnosis. However, whether the overall 10-15 % chronicity could be reduced by this mode of therapy needs to be shown in a larger group of patients. It may be worth noting that the peak platelet count on this patient was the lowest (113,000/mm^3) among the newly diagnosed cases, and as was observed by others,[13] this may have a prognostic significance.

There was no correlation between pre and post treatment levels of serum IgG and IgM levels and the ultimate outcome. Similar observations were also noted by others.[2, 3]

None of our chronic patients showed a persistent response. Although the number of patients are small, this finding is different from some of the other studies where some patients were reported to have recovered or maintained a safe level of platelet count, and splenectomy had been deferred with or without maintenance therapy.[2, 3, 13] Whether this observation reflects a different behaviour of the diasease on our patients needs to be shown on larger groups of patients. In a recent collaborative study comparing HDIgG with oral corticosteroids, there was no differences in the number of patients having platelet counts less than 30,000/mm^3 after 180 days of follow up period.[14]

Routine use of HDIgG does not seem to be justified. The lag period of 4-5 days before a rise in platelet count rules out the possibility of its nsefulness in an emergency situation. Nevertheless, according to one report,[4] combination of HDIgG with methylprednisolone may shorten this lag period. The current price of intravenous gammaglobulin is another disadvantage for its routine use. In our study, the average price of the drug alone, for five days of treatment has exceeded $ 4,500 (U.S.)

At the present time the only justification for using HDIgG in ITP appears to be in a steroid resistent patient in preperation for an elective surgery.

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The Use of Ender Nails in Tibial Fractures

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Summary

The results of 50 tibial shaft fractures treated with closed Ender nailing and early weight-bearing are reported. The average healing time was 14 weeks. One deep infection was seen in a grade II open fracture, nailed 12 hours after injury. As a major complication malalignment less than 10 degree was seen in 30 % of the cases. Only one case has had a 14 degree malalignment. It has been our experience that closed Ender nailing provides excellent rotational stability, allows early full weight - bearing and decreases hospitalization.

Key Words: Tibia, Fracture, Internal Fixation.

Introduction

The treatment of tibial shaft fractures is a well established method in cases of minimally displaced or non-displaced fractures which are a result of low energy trauma. However, the treatment of open deplaced and comminuted fractures due to high energy traumas are controversial. Closed reduction and cast immobilization, open reduction and internal fixation, external fixation, PTB cast, closed or open intrameduallary nailing with or without reamering are the most common methods of treatment. Closed reduction and cast immobilization often result in joint stiffness, circulatory disturbances and muscular atrophy.

The best method of treatment of tibial shaft fractures should provide good alignment of the fracture with the fragments, allow for some motion at the fracture site in order to stimulate external callus formation, use implants that are easily applied without exposure of the fracture site, allow early weight-bearing and cause few complications. Closed flexible intramedullary nailing of tibial shaft fractures appear to fulfill these criteria and compares favorably with other methods.

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Materials and Methods

Between January 1983 and March 1986 we treated 45 patients with 50 tibial shaft fractures by Ender's closed intramedullary nailing. This method was used for all patients with any fracture of the shaft of the tibia admitted to the SSK Göztepe and Trabzon hospitals during this period. The patients ranged in age from 18 to 68 (average 35 years). There were 35 men and 10 women. Twentythree patients fractured the right tibia, 17 the left and five had bilateral tibial fractures.

The fracture types were as follows: Transvers (eight), short oblique (fifteen), long oblique (ten), long oblique spiral (seven), segmented (three). Using Gustilo's[1] classifications of open fractures, there were 10 grade I and grade II open fractures (Table I).

TABLE I
CLASSIFICATION OF FRACTURES

Fracture Type	Proksimal third	Middle third	Distal third	Closed	Open	Total
Transvers	2	6	–	6	2	8
Short Oblique	–	6	9	11	4	15
Long Oblique	–	5	5	8	2	10
Oblique-spiral	–	2	5	7	–	7
Unicortical Comminuted	1	6	–	5	2	7
Segmented	3	–	–	3	–	3
Total	6	25	19	40	10	50

Hardware used in this operation are Ender's original flexible nails made of stainless steel. The appropriate legth of the nail was estimated by measuring the distance between a point 2 cm. proximal to the tibial tuberosity and the medial malleolus on the injured leg. Under general anesthesia the patient was positioned on the fracture table with the injured extremity placed over a right angle support. After preoperative skin cleaning and wrapping, two vertical skin incisions, 2,5 cm. long, were made on each side of the tibial tuberosity. Subcutenous tissue and muscle were dissected bluntly. After reaching the bone, the anterolateral and anteromedial cortex of the tibia was perforated with an awl, and two nails were inserted under direct visualization of image intensifier. All dressing and instrument were changed in the open fractures of the tibia, after careful surgical debridement of the wound and copious irrigation with saline. Nail insertion was performed in direct vision.

Postoperatively, full weight-bearing was allowed as tolerated, usually during the first week. Thereafter, the treated fractures were examined at monthly intervals on an outpatient basis.

Results

The patients were followed for a period ranging from 6 to 38 months postoperatively (average two years).

The average operative time for closed nailing was 30 minutes. The time spent on debridement of open fractures and wounds was not calculated in nailing time. The average hospital stay for 45 patients with tibial fractures was 16 days. Only five of these patients stayed in the hospital more than 20 days.

We considered the fracture united when the callus had matured and the fracture line was obliterated in at least three-quarters of the bone circumference. Forty fractures united between eight and twelve weeks, ten fractures united with delayed union between four and nine months (Figure 1-3). The average healing time was 14 weeks, the average time for

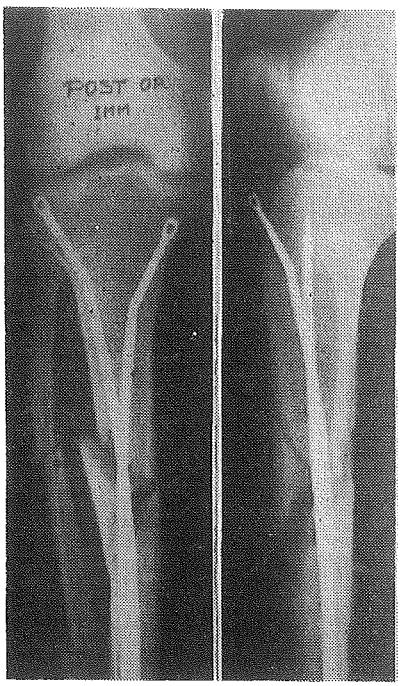

Figure 1
Tibial fracture with butterfly fragment. Roentgenograms show distraction at the fracture line immidiately after surgery.

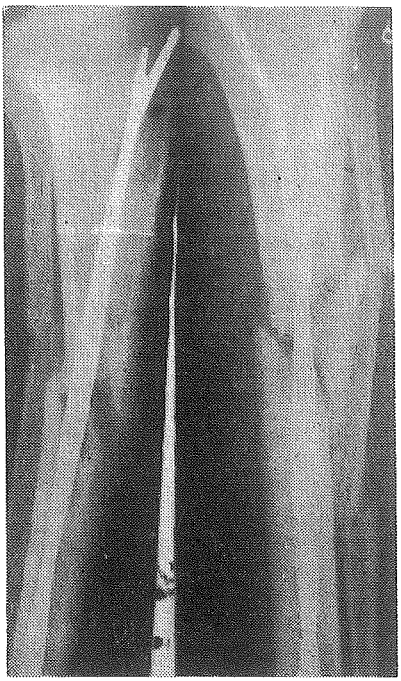

Figure 2
3 weeks later impaction occurred at the fracture line.

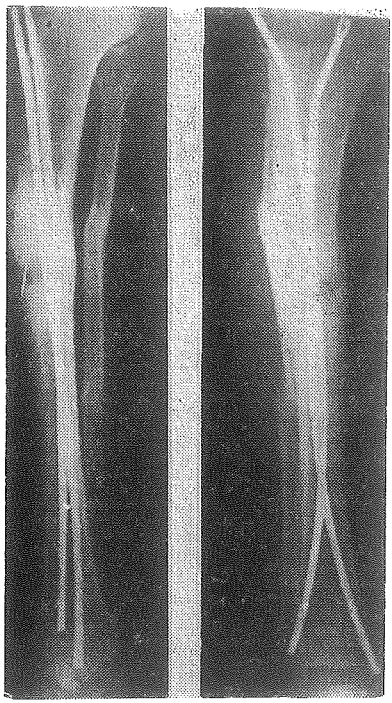

Figure 3
Views taken 9 months after nailing show the fracture healed with external callus formation.

bridging osseous callus was 8 weeks for closed fractures and 14 weeks for open fractures (Table II).

TABLE II
TIME FOR UNION

Time in weeks	Number	%
8 - 12	40	80
12 - 18	2	4
18 - 24	4	8
24 - 40	4	8

There were 6 delayed unions in 40 closed fractures while 4 delayed unions in 10 open fractures (Table III).

Complications: Two varus and two valgus deformities exceeding 7 degrees were seen in four patients, while 5 to 10 degrees recurvatum were seen in ten patients. There was only one deformity more than 10 degrees. All angulations were in the distal third of the tibia.

TABLE III
UNION RATES

	Union	Dilayed union	Non-union
Closed fractures	34	6	--
Open fractures	6	4	--
Total	40	10	--

Osteomyelitis developed in one case who had a grade II open fracture with unicortical comminution, and was debrided and nailed 12 hours after injury. This fracture united in nine months. In one case the distal tip of the nail penetrated the distal articular surface of the tibia and had to be replaced by a shorter one.

Fifty percent of the patients had minor discomfort in the knee joint especially with flexion more than 90 degrees.

Discussion

Fractures of the tibia is the most common fracture of long bones. The aim of the treatment is good anatomic reduction, rapid solid union and lack of complications. Flexible intramedullary nailing is a very suitable method for the treatment of tibial shaft fractures.[2, 3, 4]

A more serious problem lies in the variety of criteria and definitions used by different authors to judge their final results. Healing or union has been variously defined as the presence of bridging callus seen roentgenographically,[5, 6] the absence of pain or deformation at the fracture site,[7] the ability of the patient to bear full weight without an external support,[8] or a combination of these factors.[5, 9-15]

We have considered the roentgenographic bridging callus without limitations of motion on the ankle and knee joints as the most objective criterion for union.

Callus formation in the radiograms was about 18 weeks with conservative treatment, 22 weeks in open reduction and internal fixation and 18 weeks rigid intramedullary nailing[2, 7, 16] In our cases with flexible intramedullary nailing, callus formation started by 8 weeks. The mean healing time was 14 weeks. Forty of the fifty fractures healed in three months.

One of the best advantages of this method to rigid intramedullary nailing is that reamering of the medulla is not necessary. The so-called fracture disease is the main complication of conservative treatment of

tibial fractures. Muscles and joints rehabilitation were not a problem because of additional external immobilization, such as cast or brace, were not necessary.

It is well known that fracture hematoma is very important in the callus formation process. In the open reduction and internal fixation fracture hematoma is removed. This is the main reason for prolonged union in this method. It is obvious an advantage of our method that original fracture hematoma is preserved and there is no additional trauma to the any of the tissues in the fracture site.

Non-union, malposition and malunion, posttraumatic and postoperative osteomyelitis were major and serious complications in the treatment of tibial shaft fractures. All of the bilaterial tibial fractures were healed with delayed union but one. We thought that delayed weight bearing was the reason for this delayed union.

0-10 % non-union has been reported by various authors.[11-13] There was no non-union in our series. In our opinion, the reason for this zero incidence on non-union was to see an impaction between fracture fragments, on the fracture line in the x-rays was taken after 3 weeks post operatively in spite of obvious distraction at the fracture line seen immediately postoperative x-ray in 5 cases.

The most common complication in our series was malalignment. Many authors consider that less than 10 degree of malalignment is functionally insignificant.[2, 3, 10, 14, 15] There was only one patient more than 10 degrees of malalignmet (Table IV). All malalignments occured on the fractures at the lower third of the tibial shaft and none of them required renailing. There were no rotational deformities.

TABLE IV

MALALIGNMENT

Deformity	Varus	Valgus	Recurvatum
0 - 5°	1 (2%)	–	5 (10%)
5 - 10°	1 (2%)	2 (4%)	5 (10%)
10° up	--	--	1 (2%)

Some authors reported the incidence of osteomyelitis after surgical treatment of tibial fractures at the ratio of 2,5 - 15 %. Osteomyelitis of the tibia developed in one patient in a grade II open high-energy fracture which was debrided and nailed 12 hours after injury. The other nine open fractures were nailed immediately after debridement and healed without any problems.

In conclusion, we recommend Ender nails for all types of diaphysial fractures of the tibia. The advantages of this method are as follows: It is simple to use, does not require medullary reamering, does not inferfere with the fracture haematoma, eliminates the need of external immobilization, allows early weight-bearing through the bone, and decreases postoperative complications.

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Mucinous Adenocarcinoma of the Colon and Rectum in Children

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Summary

Between 1976-1985, seven children with mucinous adenocarcinoma of the colon and rectum were treated in the Hacettepe University Department of Pediatric Surgery. Abdominal pain, rectal bleeding, nausea and vomiting, changes in bowel habits were common symptoms; while two patients who were admitted with signs of acute intestinal obstruction. Rectum was the primary site of the tumor in five patients and rectal examination was diagnostic for all of them. The surgical treatment in our series consisted of permanent colostomies or wide excisions, other than resection of the tumor mass, with a low survival rate. These cases are presented with a review of the literature.

Key Words: Mucinous Adenocarcinoma.

Introduction

Mucinous adenocarcinoma is a rare histological type of colonic carcinoma in adults, while it is a common type in children.[1-14] Despite the tremendous progress in cancer treatment in recent years, this type of colonic carcinoma is characterized by a rapid growth and invasion and has a very poor prognosis.[2,3]

In this report, seven children with mucinous adenocarcinoma of the colon and rectum are presented and some aspects of the disorder are discussed with support from findings in the literature.

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Materials and Methods

Between 1976-1985, seven pediatric patients were diagnosed as having mucinous type of colorectal adenocarcinoma at the Department of Pediatric Surgery of the Hacettepe University Medical Faculty. We did not see any non-mucinous carcinoma of the colon and rectum during this period.

The records of the patients were reviewed and age, sex distribution, presenting symptoms and signs, staging, surgical procedures with other therapeutic approaches were noted.

Staging was made according to modified Dukes classification, were in Stage A only the mucosa and submucosa are affected, in Stage B, the disease is limited to the bowel, in Stage C, the disease is limited to the lymph nodes, and in Stage D, there are distant metastases or peritoneal implants or direct invasion of other viscera.[6, 14]

Results

The important clinical features of the patients are summarized in Table I.

Four patients were male and three were female. The age range was from 11 to 16 years with a mean of 13.7 years.

There was no history of ulcerative colitis or familial polyposis or another precancerous lesion in any of the patients. However, the father and two uncles of one of our patients had previously died of colonic cancer.

Abdominal pain was present in all patients. Rectal bleeding, nausea, vomiting, difficulty in defecation and bloody-mucous diarrhea were the other common symptoms. Two patients were admitted with signs of acute intestinal obstruction. The duration of symptoms in patients with intestinal obstruction was two days while in other patients it ranged from 15 days to 6 months.

The primary site of the tumor was the rectum in 5 patients, caecum and hepatic flexura in the other two. A 10x10 cm abdominal mass was found in the right lower quadrant in the patient with caecal tumor and barium enema revealed the diagnosis. The patient who suffered from hepatic flexura tumor was admitted with signs of intestinal obstruction and the diagnosis was made by exploratory laparotomy. These two patients were in Stage C and had radical tumor resections with 5 cm proximal and 7 cm distal bowel and primary anastomosis. Rectal examination was diagnostic in all rectal tumors. In this group, the patients were

TABLE I

CLINICAL FEATURES OF THE CASES

Patient	Age/ Sex	Presenting Symptoms and Signs	Duration of Symptoms	Primary Site	Stage	Surgery	Chemotherapy	Radiotherapy	Outcome
1 MB	13M	Abdominal pain, distention, vomiting, mass	15 days	Caecum	C	Radical Resection	5FU+MCCNU	–	Alive 4 years
2 AA	16M	Acute intestinal obstruction	2 days	Hepatic flexura	C	Radical Resection	5FU+MCCNU	–	Died in 1 year
3 HB	13M	Abdominal pain, bloody mucous diarrhea	6 months	Rectum	D	Miles	5FU	+	Died in 9 months
4 MT	13M	Acute intestinal obstruction	2 days	Rectum	D	Miles	5FU	+	Died in 6 months
5 GE	14F	Abdominal pain, rectal bleeding	1 month	Rectum	D	Colostomy	5FU+MCCNU	–	Unknown
6 İK	15F	Abdominal pain, difficulty in defecation	1 month	Rectum	D	Colostomy	Refused further therapy		Died in 6 months
7 ZK	11F	Abdominal pain, distention, difficulty in defecation	1 month	Rectum	D	Resection	5FU+MCCNU	–	Died in 8 months

found to be in Stage D. Two patients underwent Miles operation, two patients had palliative permanent colostomies and one patient had tumor resection and Swenson type anastomosis.

Histopathological examination of the biopsy materials revealed the diagnosis of mucinous type of adenocarcinoma in all patients (Figure 1).

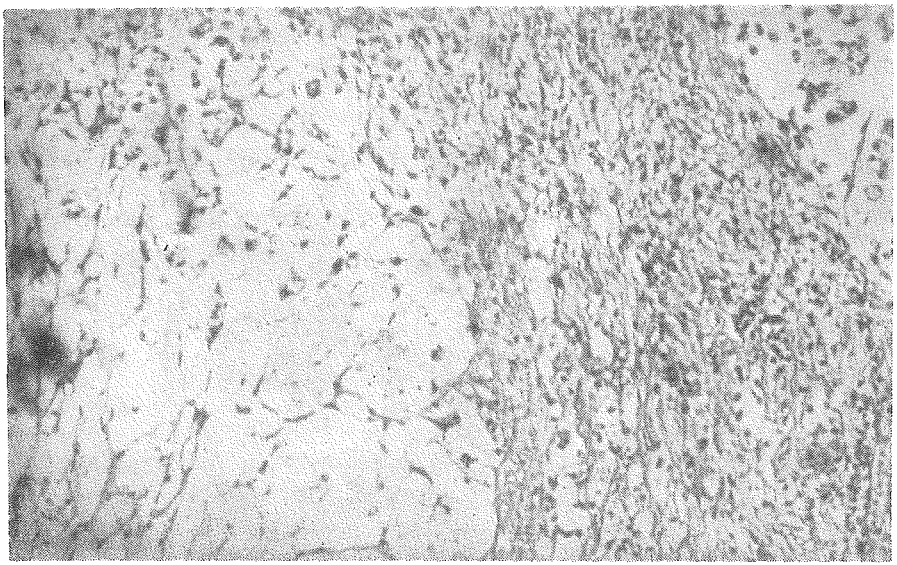

Figure 1
Histological specimen showing the typical appearance of mucinous adenocarcinoma with signet-ringformation. (HE x 100)

Discussion

Colonic cancer is a common neoplasm in adults while it is rare in childhood.[1] Not more than 350 pediatric cases have been reported.[1-14] These present series seem to be one of the largest series of colonic cancer in children.

Although mucinous or colloidal adenocarcinoma with signet cell formation is a histological subtype of the colorectal cancer, it has important distinctive clinical features when compared to non-mucinous carcinoma.[2,3] This type of adenocarcinoma is a rare from of adult colorectal carcinoma and is seen in about 5 % of the cases.[1] The incidence of mucinous carcinoma in children has been reported as more than 50 % colonic cancer, and was 100 % in this series.[3] Additionally, it grows so rapidly that regional lymph node involvement and diffuse peritoneal

spread is a common finding when the diagnosis is made and this leads to a fulminating course and a poor prognosis of the disease.[13, 14] In this series, five patients were in Stage D and two patients were in Stage C.

The incidence of the reported cases increases with age and especially after 10 years of age.[1] However, the case of a 9 months old baby was reported at postmortem examination.[4] All of our patients were older than 10 years of age. The reason for this increase is unknown.

In the reported series,[5] there is a 2:1 male: female ration in young patients, but we found only 4:3 male preponderance.

The exact etiological factors of the colorectal carcinomas are unknown, However, familial polyposis, ulcerative colitis and certain types of polyps are known precancerous lesions.[6] None of our patients had confirmed diagnosis of these disorders, but one patient had a family history of colonic cancer without a previous gastrointestinal complaint.

Abdominal pain, vomiting, weight loss, rectal bleeding, gastrointestinal motility disorders are the most common presenting symptoms.[3, 5, 7] These nonspesific symptoms are suggestive of more common pediatric disorders such as appendicitis and gastroenteritis, therefore the presence of a colonic tumor can be overlooked very easily. Sometimes, after a period of vague gastrointestinal complaints, typical signs and symptoms of acute intestinal obstruction may ensue as happened in our two patients.

On physical examination, an abdominal mass can be detected in some patients.[7, 8] Since rectal bleeding was the major symptom in a group of cases, gross or occult blood per rectum should be searched carefully.[9] Rectal examination was diagnostic in all of our patients with rectal tumor. This finding supports the importance of this examination in patients with any type of gastrointestinal symptoms. Barium enema, rectosigmoidoscopy and colonoscopy are all valuable tests for investigation and they may confirm the diagnosis of colonic cancer.

The primary site of colorectal carcinomas was the rectum in 33 % and sigmoid colon in 27 % of the adult patients according to Symonds and Vickery.[2] However, transverse colon was the most common site (39 %) in Anderson and Bergdahl's review of 79 children.[7] In our series, the carcinoma was located in the rectum in five of the seven patients. There was no reported correlation between the site of the tumor and the presenting symptoms.

Treatment principles of mucinous colorectal carcinoma in children are the same as in adults.[2, 10] Radical resection of the tumoral mass with the lymphatics and primary anastomosis is the procedure of choice. If

the patient is operated for acute intestinal obstruction without bowel preparation, temporary ileostomy or colostomy following resection may be added to the procedure. Palliative procedures should be done in the presence of peritoneal spread or distant metastasis or prevention of bleeding and obstruction. As our approach to solid tumors of childhood had become more aggressive in an attempt to remove more tumor tissue, we performed wide excisions like Miles operation even though the patients in Dukes D Stage. Unfortunately, we lost all of those patients 6 or 9 months later. In contrast to most of the pediatric tumors, it seems unlogical to perform radical operations when the tumor is at an advanced stage.

Radiotherapy is proposed for palliation, but it was not beneficial in our first two cases.[5, 11] 5-FU seems to be the most effective agent for these kind of tumors and it has been combined with many agents like MCCNU and Vincristine.[11, 12] Among these MCCNU was used in this series.

Long term survivors have been reported but the overall survival rate for 5 years is not more than 10 % in mucinous colorectal cancer.[1, 2, 7, 13] Because most of the survivors were free of regional lymph node involvement at the time of surgery, early diagnosis seems to be very important for a better prognosis.

Although it is a rare disorder in childhood, it must be kept in mind for adolescents with abdominal pain, rectal bleeding and defecation problems.

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Histologic Type of the Primary Vulvar Neoplasia and the Mortality Rates

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Summary

This survey reports 33 cases of primary malignancy of the vulva seen and treated at the Division of Gynecologic Oncology of Hacettepe University Medical School during the past 22 years. Of these 33 patients, 27 had squamous cell carcinoma, 2 had melanoma, 2 had basal cell carcinoma and 2 had sarcoma. The mortality rates were 25.9 % for squamous cell carcinoma, 50.0 % for melanoma, 0.0 % for sarcoma. The most common complication was wound infection and breakdown.

Key Words: Primary vulvar neoplasia, Radical vulvectomy, Malign melanoma, Mortality rate, Lymphadenectomy.

Introduction

The vulva is a highly specialized organ comprising one percent of the total body surface. It harbors a diverse group of neoplasms with different malignant properties (Table I).[1,2] Epidermoid cancer is the most common type accounting for 81 % of all vulvar malignancies and 3.5 % of all gynecologic malignancies.[2-4] The others range from highly aggressive malignant melanoma to sarcoma, adenocarcinoma and basal cell carcinoma.[1,5]

TABLE I
THE INCIDENCE OF VULVAR MALIGNANCIES ACCORDING TO HISTOLOGIC TYPES

Histologic Types	Literature (%)[1, 2, 4]	Hacettepe (%)
Squamous cell carcinoma	86.2	82.0
Melanoma	4.8	6.0
Sarcoma	2.2	6.0
Basal cell carcinoma	1.4	6.0
Bartholin gland		
squamous cell	0.4	–
adenocarcinoma	0.6	–
Adenocarcinoma	0.6	–
Undifferentiated	3.9	–

Vulvar malignancy includes lesions of the mons, labia majora-minora, clitoris, fourchette, vestibule, urethral meatus, Bartholin glands and subepithelial vulvar structures.[4, 6]

The standard surgical procedure is radical vulvectomy and inguinal node dissection and additional pelvic lymph node dissection in selected patients who have positive Clocquet and central lesions.[7] Additional radiation is given to patients who have positive lymph nodes and grade two or more tumors.[2, 4]

Prognosis for the primary vulvar malignancy is related to the histologic type of the tumor, stage, size, grade, depth of invasion, the area of the tumor and then treatment is undertaken.[8] A five-year survival rate should approach 90 % in the early stages.[9]

The purpose of this study was to evaluate the histologic type of primary vulvar malignancy and to review the related literature.

Materials and Methods

33 patients with primary malignancy of the vulva who were treated at the Division of Gynecologic Oncology of Hacettepe Medical School from 1964 to 1986, were reviewed. Data were collected from patients' charts, follow-up records and pathology reports. The mean age of these patients was 56 years (ranged from 32 to 83). Of these 33 patients, 13 had ulcus, 10 had ulcus and mass, 8 had only mass and two had discoloration and mass. Tumors in 7 of the patients were on the area of the clitoris.

The final diagnosis was made by histologic examination of specimens obtained from every patient. Of these patients, 21 were subjected to radical vulvectomy, bilateral inguinal lymphadenectomy,[9] had ad-

ditional pelvic lymphadenectomy, (12) 2 had simple vulvectomy and 10 had biopsy, hemivulvectomy, and excision of the mass (Table II). After initial surgery, chemotherapy was given to the two patients with sarcoma (VAD) and to three patients with squamous cell carcinoma (bleomycin). The two patients with sarcoma were subjected to inguinal lymph node dissection for recurrence after initial therapy. Radiation was delivered to one patients with melanoma and to those patients with squamous cell carcinoma who had a positive Clocquet-pelvic node and who were advanced cases. We reviewed the slides from the original biopsies and the operative specimens, taking particular note of the depth of stromal invasion, grade, lymphatic and vascular channel involvement and lymph node metastasis. The follow-up time varied from 2 to 17 years.

TABLE II
SURGICAL PROCEDURES AND HISTOLOGIC TYPES

Surgical Procedures	Histologic Types	No. of patients
Radical vulvectomy, inguinal lymph node diss.	Squamous cell Ca	9
Radical vulvectomy, ing. and pelvic node diss.	Squamous cell Ca	12
Simple vulvectomy	Squamous cell Ca	2
Only vulvar biopsy	Squamous cell Ca	4
Local excision and hemivulvectomy	Basal cell Ca	2
Local excision, ibsilateral lymph node diss.	Melanoma	2
Extripation of the tumor	Sarcoma	2

Results

Of these 33 patients, 27 had squamous cell carcinoma, 2 had basal cell carcinoma, 2 had melanoma and 2 had sarcoma (Table I). One of the 27 patients with squamous cell carcinoma had the microinvasive form. Four different patients in this group had additional lesions such as VaIN III(1), vulvar Paget (1), cervical invasive cancer (1) and lichen sclerosis (1).

Although the ratio of positive groin node in 21 patients with squamous cell carcinoma who were subjected to radical vulvectomy and bilateral lymph node dissection was 43.0 %, the figure in 12 patients who had pelvic node dissection was 8.3 % for positive pelvic node. One patient with tumor of the clitoris had both positive inguinal and positive pelvic nodes.

The stage (FIGO-TNM) of 27 patients with squamous cell carcinoma and in 2 patients who had basal cell carcinoma is shown in Table III.

TABLE III
STAGES IN PATIENTS WITH SQUAMOUS CELL CARCINOMA AND BASAL CELL CARCINOMA

Stage (FIGO-TNM)	Squamous Cell Carcinoma	Basal Cell Carcinoma	Total
I	11	1	12
II	12	–	12
III	2	–	2
IV	2	1	3
Total	27	2	29

One of two patients with melanoma had level II disease according to Clarc's classification and the remainder had level IV.

TABLE IV
MORTALITY ACCORDING TO HISTOLOGIC TYPES

Histologic Types	Deaths	/ Total	%
Squamous cell carcinoma	7	27	25.9
Basal cell carcinoma	1	2	50.0
Melanoma	1	2	50.0
Sarcoma	–	2	0.0
Total	9	33	27.2

Of the patients with sarcoma, one had angiosarcoma and the other one had rhabdomyosarcoma.

The overall mortality rate was found to be 27.2 % and its distribution according to histologic type is given in Table IV. There are many complications such as wound infection-breakdown, emboli, lymphatic edema-cyst which was published previously.[10]

Discussion

Primary malignancy can appear anywhere on the vulva with approximately 70 % arising on the labia.[1] In this study, 21 of the 33 patients had a labial tumor, 7 had tumor of the clitoris and 5 had a tumor outside the labia and clitoris. The most common tumor of the vulva is squamous cell carcinoma.[1, 2, 4] The incidence of primary vulvar malignancies is listed in Table I. As shown, 82 % of all patients had squamous cell carcinoma in the present study.

The prognosis is related to the histologic type of tumor, stage, grade, depth of invasion, lymph node involvement and treatment.[8] The mor-

tality rate according to the histologic type of tumor, in this study, is given in Table IV. In stage I and II diseases, a five-year survival rate should approach 90 %.[9] If the lymph nodes are negative, irrespective of the stage, over 90 % of the patients will survive five-years, whereas only one third will survive if the lymph nodes are positive.[1, 2, 4, 7]

The mortality rate, in this study, was 25 % in patients with positive lymph nodes and 9 % in patients without positive lymph nodes.[10]

One of two patients with basal cell carcinoma refused therapy after biopsy, and the other one survived for two years, after local excision (hemivulvectomy) without recurrence. Complete local excision is curative, although the recurrence rate is high and probably exceeds 20 %[11, 12] Careful follow-up is advised.

Two patients with sarcoma, in this study, survived for two years after surgery and chemotherapy. Therapy generally would be radical vulvectomy and bilateral inguinal lymphadenoctomy, except in low-grade lesions where nodal involvement is rare and wide local excision should be considered. Although the cure rate was low, longevity was increased by subsequent chemotherapy.[13]

The mortality rate in patients with melanoma was 50 % in this study. Ten-year survival rates associated with Clark's level II, III, IV and V tumors were 100, 85, 65 and 23, respectively.[14,15] Although it has been suggested that all patients with melanoma of the vulva should be treated with a radical vulvectomy and inguinal and pelvic lymphadenectomy, recently there has been a tendency to be more conservative. If the disease is intraepithelial, prognosis should be very close to 100 %. Even with level I or II melanoma, a wide local excision may be adequate treatment. As the melanoma extends deeper, the chance of lymph node metastasis increases and the prognosis decreases considerably The role of the lymph adenectomy in this disease is probably more prognostic than therapeutic.[2, 12-16]

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Gonadoblastoma Associated with XX-and XY-Gonadal Dysgenesis

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Summary

The clinical features and histopatological findings in 3 cases of gonadoblastoma are presented. This rare tumour and its association with gonadal dysgenesis is discussed.

Key Words: Gonadoblastoma, XX-Gonadal Dysgenesis, Primary Gonadal Failure.

Introduction

The term "Gonadoblastoma" was introduced in 1953 by Scully to designate a gonadal tumour which recapitulates the gonadal development more completely than any other type of tumour. In our medical center we have found 3 cases of gonadoblastoma between 1978-1983.

Case Reports

Case 1: (L. E.) An 18-year-old virgin, Caucasian female was admitted to the hospital for primary amenorrhea and lack of sexual development. Her past medical history was unrevealing.

She weighed 50 kg. and was 158 cm tall. Sexual characteristics showed Tanner 2 breast development and pubic-axillary hair growth. The clitoris was somewhat enlarged, the external genitalia and habitus were otherwise those of a normally developed adult female. Rectal

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examination revealed a hypoplastic uterus and slightly enlarged semisolid gonads on both sides. A routine laboratory work-up had been normal. Her hormonal evaluation showed a hypergonadotropic state; FSH and LH levels in blood samples being 90 mIU/ml and 120 mIU/ml respectively. The karyotype on peripheral blood lymphocytes was 46, XX.

Laparoscopic ovarian biopsy under general anesthesia was performed on an outpatient basis. Ovaries were ovoid in shape and were enlarged. The uterus and the fallopian tubes were infantile in appearance. The microscopic diagnosis of the ovarian tissue samples was gonadoblastoma. The patient underwent laparotomy on August 15, 1978. The uterus was 4x2x1 cm in size. The fallopian tubes were present bilaterally. The right gonad was 3.3x3x5 cm and the left gonad was 3x4x5 cm in dimension. Both were covered by an intact, gray-white dense capsule. The exploration of the abdominal organs and peritoneal washing cytologic studies revealed negative findings. Bilateral gonadectomy was performed. The patient has been well and disease free. She is currently receiving estrogen and progesterone replacement therapy.

Macroscopic Examination: The specimen consisted of the right and left gonad tissue measuring approximately 3.3x1.5x1 cm in dimension having cystic structures, and white capsule.

Microscopic Examination: The microscopic examination of the gonad revealed many cystic follicle structures like call-Exner bodies and leydig cells around them. These were compatible with gonadoblastoma.

Case 2: (R.A.) A 25-year-old married, Caucasian female presented with primary infertility. She had her menstrual bleeding at the age of 23 following hormonal treatment. Since then she had had no menstrual periods unless she took hormone pills. She had laparotomy for an adnexal mass when she was 13. No records could be obtained about the laparotomy (e.g what was performed and what the pathology was).

On admission to the university clinic, she weighed 52 kg. and was 155 cm tall. She appeared to have a female habitus with Tanner 3 breast development and scarce axillary and pubic hair. The external genitalia looked normal. Speculum examination revealed a functional vagina and cervix. On bimanual examination the uterus was felt to be hypoplastic and both ovaries were palpated to be enlarged and semisolid. The results of the routine labarotory studies were negative. The patient's FSH was 65 mIU/ml and LH was 50 mIU/ml in blood samples, both elevated compatible with the diagnosis of primary gonadal failure. The karyotype was 46 XY.

She underwent laparotomy on January, 1979. The uterus was 4.5x 2x1 cm. in size. Both ovaries were enlarged with cauliflower-like tumoral involvement. The right gonad was 9x8x7 cm and the left one was 8x4x3 cm in dimension both being enclosed in a thick intact capsule. There was no evidence of gross disease in the abdominal organs. Bilateral oophorectomy was performed. Currently she is under hormonal replacement therapy and responding well.

Macroscopic Examination: The specimen consisted of both the right and the left ovarian tissue. The right ovarian tissue was about 6x4.5x4 cm in dimension weighing 40 gm and revealed a neoplastic tissue diagnosed as Dysgerminoma and cystic teratoma. The left ovarian tissue consisted of 2 pieces; one of them was about 2.5x2x1.5 cm in dimension, the other being about 0.8x0.5x0.3 cm.

Microscopic Examinazion: Biopsy Number-255/79. Sections revealed two neoplastic patterns as Dysgerminoma and Gonadoblastoma.

Case 3: (Z.K.) A 16-year-old virgin, Caucasian female presented at the department of Obstetrics and Gynecology in September 1983, because of primary amenorrhea and lack of sexual development. Her past medical history was non-contributory. She was one of the 5 children of the family. Her two brothers and one sister were all in good health with normal reproductive function. Her younger brother who was 6 years old, had had several surgeries for the correction of undescended testes, inguinal hernia and hypospadias. The mother mentioned that she had taken several hormonal injections and pills to be able to abort her unwanted pregnancies without success and eventually delivered our patient and the one with the hypospadias, uneventfully.

She weighed 40 kg. and her height was 148 cm. She had no breast development and scarce pubic and axillary hair growth. X-ray of the left wrist showed a bone age of 13 years. The external genitalia were those of an undeveloped female. The length of the vagina was sounded through the hymenal opening as 7 cm. Rectal examination revealed a fibrous band-like uterus in the midline with no adnexal palpable masses.

The routine laboratory studies were negative. Hormonal studies included FSH: 97 mIU/ml., LH: 39 mIU/ml., estradiol 10.8 pg/ml. (N: 30-120 pg/ml.) and DHEA-S: 1665 ng/ml. (N: 1100-1900 ng/ml.). The karyotype determined on peripheral blood lymphocytes was 46, XY. On September 9, 1983 the patient underwent laparoscopic ovarian biopsy. The uterus was like a fibrotic band in the pelvis accompanied with infantile but normally developed fallopian tubes. Streak gonads were detected on both sides. On the surface of the right gonad a fragile,

Cauliflower-like growth of 2-3 mm. in size was noticed. Laparoscopic ovarian biopsy which was performed on both sides revealed Gonadoblastoma on dysgenetic gonads in microscopic examination. On October 10, 1983 the patient underwent laparotomy. No tumoural involvement was found in the abdominal organs. Bilateral gonadectomy was performed. She has been doing well under replacement hormonal therapy.

Macroscopic Examination: The specimen consisted of the right white ovarian tissue about 0.3 cm. in diameter and showed two cystic stuructures. The left ovarian tissue was also about 0.3 cm in diameter.

Microscopic Examination: Biopsy Number-6481/83. The specimen of the left ovary revealed Gonadoblastoma showing some Call-Exner bodies, and focal calcifications.

Discussion

The term Gonadoblastoma was introduced by Scully to describe "tumours" that could be seperated from the Dysgerminoma, Seminoma, and Arrhenoblastoma. This name was chosen because: 1. The neoplasms described appeared to recapitulate gonadal development more completely than any other type of tumour, and 2. Although patients were phenotypic females, their sexual development had been abnormal and the nature of the gonads in which their neoplasms arose could not be determined. Although Gonadoblastomas recapitulates the whole embryonic gonad since it contains germ cells, Sertoli-granulosa cells and Leydig-Theca cells, it seems more convenient to place Gonadoblastoma with the germ cell tumours.

Teter notes various androgenic and estrogenic combinations with these primary germ cell tumours, but utilizes the term Gonadoblastoma and designates several types according to the endocrine effect produced by the elements contained in the cell.[6]

Grossly the tumour is similar to a Dysgerminoma, may vary in size from millimeters to a large solid, soft, firm or gritty mass. Often there is disseminated calcification and may be recognized in abdominal X-rays prior to surgery.

Gonadoblastoma often occurs in the dysgenetic gonad or in the hermaphrodite, usually the patients are enuchoidal, have complaints of primary amenorrhea and lack of sexual development. Twenty-two percent arise in a streak gonad, 18 % in a criptorchid, dysgenetic testis and the majority in an abnormal gonad of indetermined type.[1] Over 80 % of patients are phenotypic females who are usually virilized and 90 % of all patients have a Y-chromosome, although it is not essential to

the diagnosis.[7] However if a Y-chromosome is absent the risk of gonadoblastoma is extremely small. An abnormal ring type Y-chromosome has been reported.[8] Gonadoblastoma has been very rarely reported with normal 46,XX karyotype.[1, 9, 10] All patients with a peripheral karyotype of 46,XX should be studied for HY antigen. In our first case we found normal 46,XX karyotype. Gonadoblastoma is rare in infertile women and very rare in true hermaphrodietes.[3, 4, 10-13] These patients may be mosaics as gonadal mosaicism can be difficult to establish.

In 20-30 % of the patients with pure XY-gonadal dysgenesis, gonadal malignancy would be present at the time of first admission. Prophylactic gonadectomy shoud be done before puberty.

Turner's syndrome may be present and the patients are masculinized. Patel and Pentice indicate that there seems to be no one consistent karyotype pattern with these neoplasms.[2]

The clinical course and histological pattern were generally benign, although various other malignant germ cell tumours such as dysgerminoma, teratoma, endodermal sinus tumor, embrional carcinoma or choriocarcinoma may be superimposed. In case 2, cystic teratoma and dysgerminoma were superimposed. Pure gonadoblastoma does not metastasise. Metastasis of dysgerminoma in gonadoblastoma is uncommon even where the tumour is bilateral and large. In contrast, other malignant combinations have been fatal within 18 months.[4]

In a recent article Tierman et al reported a case of a true hermaphroditism with a 46, XX karyotype having gonadoblastoma and dysgerminoma.[4] Van Nierkerk reviewed 367 cases of true hermaprodites and found one case associated with gonadoblastoma.[5]

Two further cases of true hermaphroditism associated with gonadoblastoma have been described.[12, 13]

The new concept of treatment in gonadoblastoma is bilateral salphingo-oopherectomy, this is because of the high risk of being superimposed by malignant tumours. Further treatment is determined by the histologic nature of any other mixed germ cell element. Irradiation must be considered where dysgerminoma has supervened.

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Peripheral Nerve Involvement in Sarcoidosis

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Summary

Peripheral nerve involvement in sarcoidosis is uncommon and there are some controversies on its pathogenetic mechanisms. A 27-year-old man with right ulnar and peroneal nerve paralysis, and hard nodules in his extremities is presented. Biopsy from one of these hard lumps showed sarcoid granulomas. The case was considered to be interesting since such sarcoidosis cases with peripheral nerve involvement are seen rarely.

Key Words: Sarcoidosis, Peripheral nerve, Compressive Neuropathy.

Introduction

Sarcoidosis is a systemic disease where the central nervous system is not commonly involved.[1] Peripheral nerve involvement is also rare.[2] We recently observed a case with ulnar and peroneal nerve involvement due to compression of sarcoid granulomas.

Case Report

A 27-year-old male patient who complained about swellings in his arms and legs, thinning and weakness of the right hand, and weakness in the right foot, was admitted to the Hacettepe University Hospital in January, 1984.

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He was hospitalized with the diagnosis of tuberculosis for 6 months at the age of 12. Later in 1972, he was readmitted with pain in his extremities, and a search for collagen diseases was negative. However, muscle-skin biopsy demonstrated nonspecific myositis. Following a course of oral prednisolone (1 mg/kg) he completely recovered. Symptoms similar at this admission had occured in 1979. At that time the patient refused a biopsy.

On physical examination, the right forearm was found to be diffusely swollen from elbow to wrist and immobile hard lumps of 2x5 and 2.5x6 cm size were palpated. Similar, but more localized hard swellings were also seen on anterior aspect of the left forearm, lateral aspect of the right knee, and medial side of the left leg (Figure 1). There were also signs of ulnar nerve involvement in the right hand, and foot-drop in the right (Figure 2). No other physical and neurological abnormalities were detected.

Routine blood biochemistry, routine blood and urine analysis were found to be normal including the erythrocyte sedimentation rate. Two successive serum calcium levels were 9.1 and 9.6 mg/100 ml. and phos-

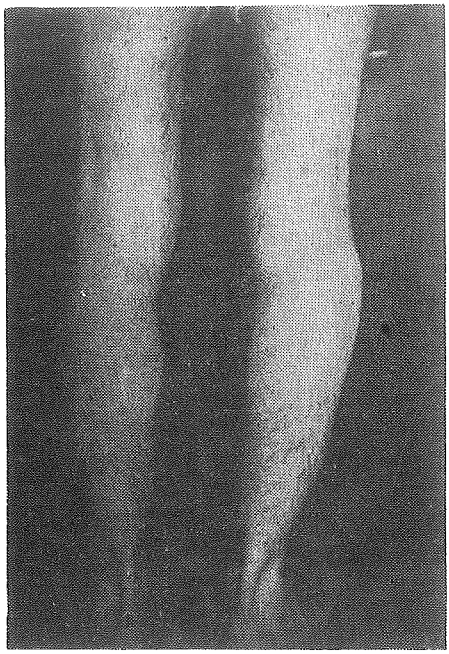

Figure 1
Fusiform swellings are seen in the extremities.

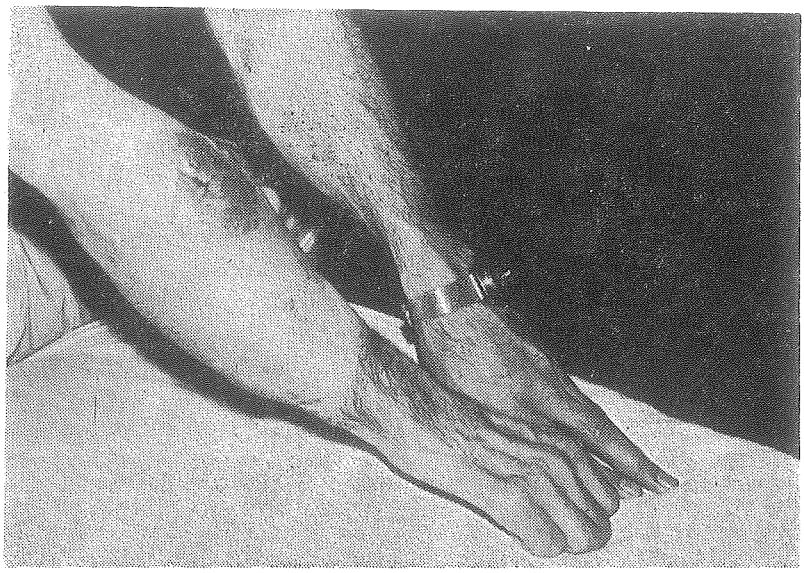

Figure 2
Marked atrophy is seen in the right dorsal interosseal muscles due to right ulnar nerve paralysis.

phorus levels were 6.1 and 4.5 International Unites. The X-ray was normal. The mantoux test showed 10 mm of induration. A lumbar puncture revealed no abnormalities. No leprosy bacilli were detected in a nasal smear.

EMG showed denervation potentials in digiti minimi and in the right extensor digitorum brevis muscles and no MUAP's were elicited with an attempt of voluntary contraction. No muscle action potential could be elicited from the abductor digiti minimi, and the extensor digitorum brevis muscles by stimulation of ulnar and peroneal nerves, suggesting neurotmesis. The remaining ENMG study showed no denervation potentials and nerve conduction velocities were within normal limits.

Right sural nerve biopsy revealed no abnormality. The biopsy obtained from the swollen area in the right forearm showed noncaseous granulomas around the peripheral nerve branches, hyalin fibrous tissue, and Langhans type giant cells which contained asteroid bodies (Figure 3a, b, c) There was no muscle tissue aruond the granulomas.

The patient was given prednisolone, 60 mg per day, for two weeks, subsequently tapered to 20 mg per day. The swellings rapidly diminished in size, but there were no changes in neuropathy signs. After two months, the swellings disappeared almost completely (Figure 4).

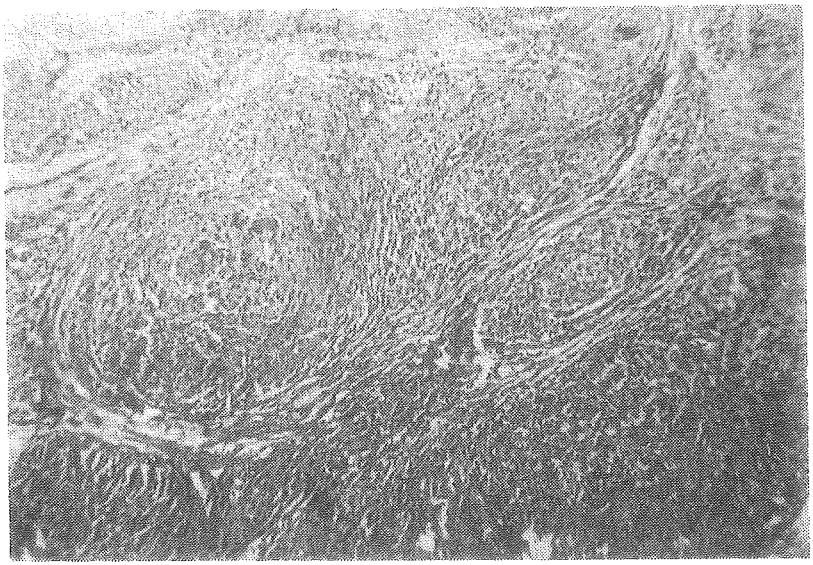

Figure 3a

Granulomas which are intersected by the fibrous collegenous bundles (H.E. x 37.5).

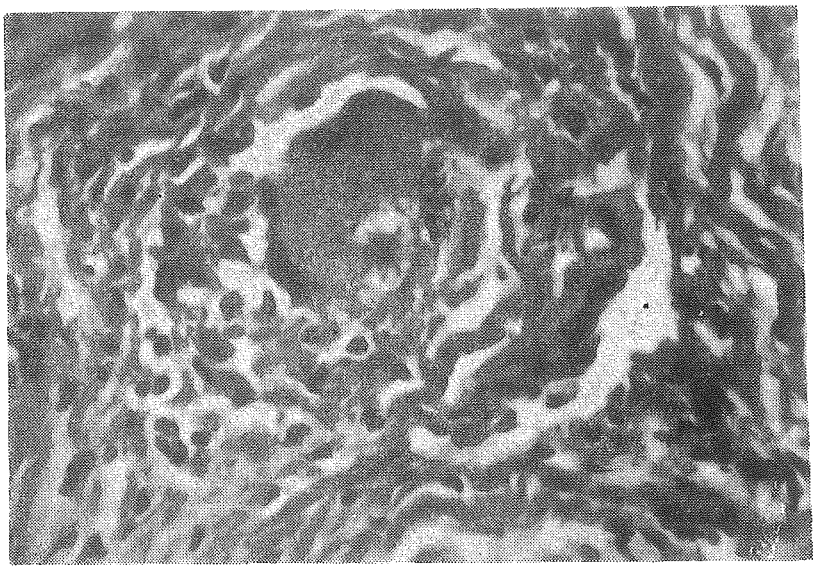

Figure 3b

Granulomas, one of them including a giant cell, around peripheral nerve branch (H.E. x 240).

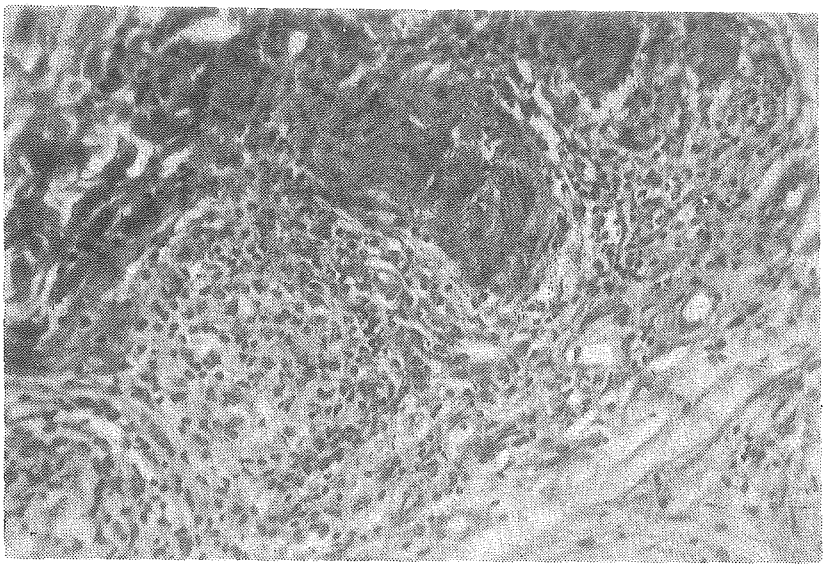

Figure 3c
Astreoid body in the cytoplasm of the giant cell in the granuloma (H.E. x 600).

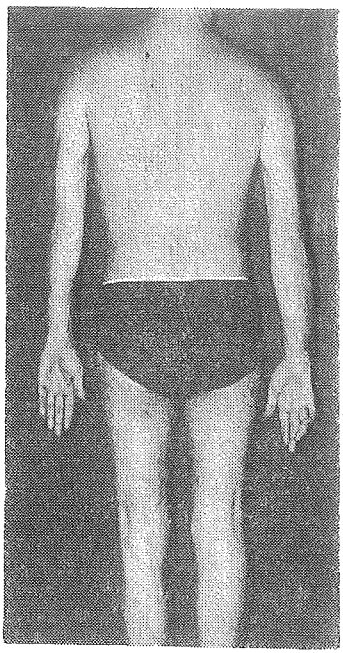

Figure 4
Extremities appear to be nearly normal after two months' prednisolone therapy.

Discussion

The nervous system involvement in sarcoidosis occurs in only 2-5 per cent of the cases.[3-5] The symptoms may vary depending on the site of the granulomas.[6] But there is no precise information on the peripheral nerve involvement. Since histopathologic diagnosis of peripheral and cranial nerve infiltration by sarcoid granuloma is reported quite rarely[7, 8, 9] it has been postulated that the peripheral and cranial nerve symptoms are toxic in origin.[10] Some authors claimed that the granulomatous infiltrations were the cause of peripheral involvement in sarcoidosis.[11, 12] Granulomatous vasculitis was also suggested as another explanation for peripheral nerve involvement.[13-16]

Boeck described a sarcoidosis case with palpable thickened ulnar nerve,[14] and Mazza reported a case with progressive nerve lesions and at autopsy he found irregular thickening of the involved nerves by sarcoid infiltration.[17] Our patient had hard lumps in his extremities. They might be considered as granulomatous nerve hypertrophies. It would indeed be interesting if we had confirmed this pathologicaly. However, it would be reasonable to assume that the hard lumps might be due to the granulomas within the connective tissue, rather than thickening of the ulnar and peroneal nerves, since the peripheral nerves in other two extremities have no clinical or ENMG abnormalities, despite the presence of similar hard lumps.

The patient was initially thought to be a case of neurofibromatosis, but it was proven to be sarcoid myopathy after histopathologic examination. Muscle involvement is not uncommon in sarcoidosis.[18] Ozer *et al.* reported a case with palpable nodules in his muscles.[19] However, in our case, histopathologic examinations with silver impregnation and trichrome mason stain did not show any muscle tissue, normal or atrophic, around the granulomas.

It was concluded that our case was unusual since sarcoidosis cases with such peripheral nerve compression are reported rarely.

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Acral Lentiginous Melanoma

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Summary

Acral Lentiginous Melanoma is an uncommon form of malignant melanoma. Its biologic behavior is similar to that of nodular melanoma. In this paper clinical and histopathological findings of a case which has characteristic features is presented.

Key Words: Malignant melanoma, Acral Lentiginous Melanoma.

Introduction

Acral Lentiginous Melanoma (ALM), described recently, is the fourth major type of primary cutaneous melanomas.[1-5] It occurs in the acral or peripheral portions of the limb, on the plantar or palmar surfaces of the hands and feet or the subungual areas of the fingers or toes. ALM is histologically and clinically distinct from nodular melanoma (NM), superficial spreading melanoma (SSM) and lentigo malignant melanoma (LMM).

ALM is an unusual type of melanoma which is characterized by acanthosis, elongation of rete ridges, and lentiginous proliferation of atypical melanocytes in the epidermis.[1, 3, 6] Although this form was thought to be a variation of LMM, it represents biologically and clinically a more agressive tumor, and shows a poorer prognosis.[1, 2, 6, 7]

In this report a case of ALM is presented.

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Case Report

A 53-year-old man with a tumoral lesion on his left heel persisting for two years was admitted to the Department of Dermatology in March 1986.

The lesion presented asymptomatic, verrucous, hyperkeratotic central nodule and adjacent dark brown pigmented macules (Figure 1) with an inguinal lymphadenopathy on the left side 8 x 8 cm in diameter. On physical examination an erysipelas-like picture on the left leg without local heat and tenderness was detected. Other findings were unremarkable.

Figure 1

ALM on the left heel. Patch radial growth surrounds hyperkeratotic central thickened area of tumor.

Routine laboratory tests showed no abnormality except slightly elevated ESR (32 mm/h).

Microscopically, both the primary lesion and inguinal lymph node was composed largely of epitheloid types of cells. The cells had bizarre pleomorphic nuclei with prominent nucleoli and abundant eosinophilic cytoplasm with well defined borders. Many of the atypical cells were multinucleated and many contained pseudonuclear inclusions. Mitotic figures including abnormal forms were small in number and some of these cells contained varying amounts of melanin (Figures 2,3).

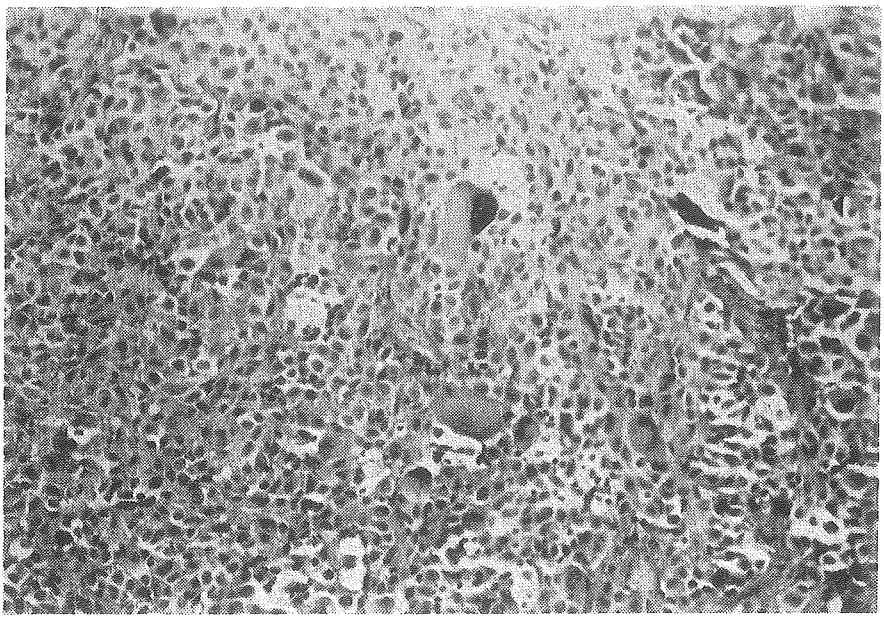

Figure 2
Photomicrograph of primary lesion (HE x 200). Melanocytic dysplasia with numerous melanophages.

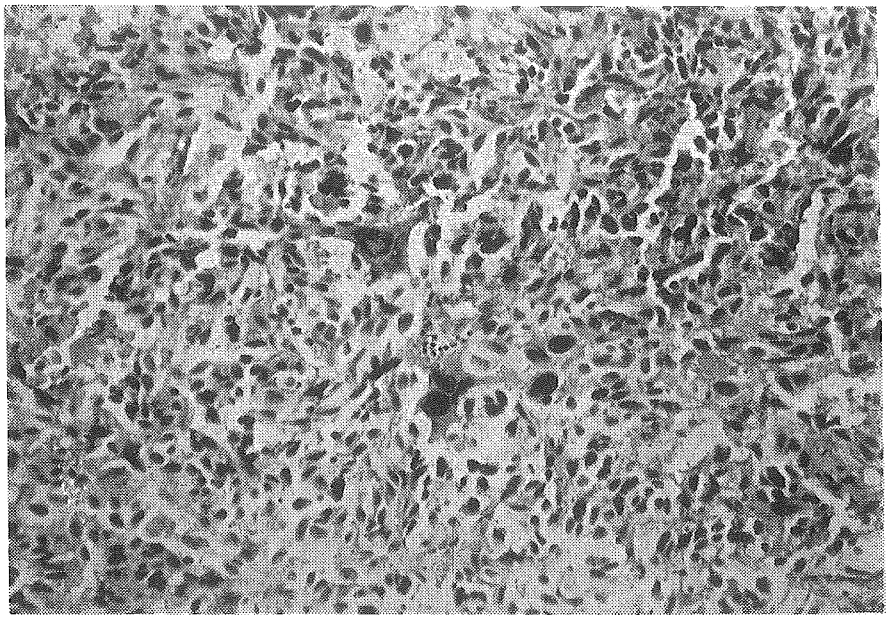

Figure 3
Photomicrograph of inguinal lymph node (HE X 200). Metastasis of ALM.

The patient was later transferred to the Oncology Hospital for further treatment.

Discussion

There is general acceptance of ALM as a distinct entity, the term being based on certain histologic similarities to LMM.[1, 3, 4] It occurs predominantly in the sixth and seventh decades of life.[3]

The incidence of ALM was reported ranging from 0.64-7.2 % of all melanomas.[1, 2, 5] Approximately 35-40 percent of patients with ALM were female.[1-3, 4] That the prevalence of ALM in Turkey is unknown may be due to the lack of reported cases.

The localization of ALM may suggest a trauma in the etiology.[5] Similary, in the case reported above there was also a trauma the story of a caused by a shoe-nail.

Delay in diagnosis has been a major problem with ALM. During the radial growth phase, lesions are macular and pigmentation may be very mild. Lesions on the sole are often mistaken for warts and pyogenic granuloma. Periungual and subungual lesions are frequently confused with subungual nevi, hematomas, paronychia and onychomycosis[1-4, 7]

There are five general histologic features used to characterize ALM.[1] They are: (1) The preliminary radial growth of atypical lentiginous cells at dermal epidermal interface, (2) expansive growth in the reticular dermis, (3) Cytology of the malignant cell (epitheloid or spindle cell), (4) response of the epidermis to the abnormal clone of melanocytes, (5) host immune response (lymphocytic infiltrates and patterns of regression).

In our case, the second and third histopathologic criteria and lymph metastasis were present.

Wide local excision with regional lymph node dissection is used for most of the patients with palmar-plantar lesions.[6, 7] All of the patients with subungual melanomas undergo amputation followed by lymph node dissection. Various forms of combination chemotherapy consisting of cytoxan, vincristine, BCNU(1,3-bis (2-chloroethyl)-1-nitrosurea), Alkeran, hydroxyurea, and DTIC (Dimethyltriazeno imidazole carboxamide)[6, 7] and immunotherapy with BCG or transfer factor were performed.[3] Recently, improved survival rates of patients with ALM were reported with hyperthermic isolation perfusion, wide excision and regional lymphadenectomy.[6] Patients with metastasis to lymph nodes at the time of diagnosis, as in our case, usually have shorter survival times than those without lymph node metastasis.

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Different Amount of Calorie Intake in the Incidence and Growth of Methylnitrosourea Induced Mammary Carcinoma in Rats with Some Biochemical Parameters

Meral Aksoy* / Martin R. Berger / Dietrich Schmähl****

Summary

In this study in which three groups of female Sprague-Dawley rats with MNU-induced mammary tumour were given diets containing 30, 40 or 50 Kcal/day, the effect of these calories on the tumour incidence and growth, plasma and liver lipids, vitamins A and E, were investigated. Feeding different amounts of energy did not effect on tumour incidence. The levels of plasma lipids and the vitamins were not altered. Nevertheless, the liver vitamin E was decreased during aging, while the vitamin A in the liver increased.

Key Words: Mammary carcinoma, calorie, nutrition, lipid.

Introduction

Animal models have been developed which clearly shown that breast cancer risk in animal systems is related to genetic as well as environmental factors including nutrition.[1,2] The influence of long term colorie restriction in reducing the incidence of tumours is well documented for rats, and mice.[3] In general, over nutrition favors and undernutrition inhibits tumour growth.[4] In most of these experiments, however, calorie intake was either excessive, or one of the nutrients in the diets was increased without altering other nutrients.

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It is well known that there is a close relationship between host and tumour. This relationship may alter the metabolism of lipids as well as some vitamins[5, 6, 7] In cancer, lipid stores are used to meet increased cellular energy requirement,[8] and, therefore, mobilization of lipids may cause increased plasma lipids.[9] On the other hand, plasma vitamin A and E levels of cancer patients is reportedly decreased.[10]

The following study, is therefore to be planned to show the role of energy intake in the incidence and development of methylnitrosourea (MNU) in duced mammary tumours in Sprague Dawley (SD) rats, including determination of the concentration of liver and plasma lipids and plasma vitamin A and E.

Materials and Methods

105 Female SD rats were divided into three groups according to matching body weight. They were housed, two rats per cage, in an air-conditioned room maintained at 21±2°C with a 12 hr.light-dark cycle. At 50 days, of age they were given (i.v.) a single dose of 25 mg/Kg MNU to induce primary tumours. Starting the day after induction, they were fed 30 Kcal/day 40 Kcal/day and 50 Kcal/day in groups 1,2,3 respectively. Tap water was given ad libitum. Food consumption was checked every two days.

The diets were obtained from Altromin International (F.R. Germany) (Table I) and kept frozen at –20°C. The diets were brought to room temperature immediatly before feeding.

TABLE I

DIET COMPOSITION OF GIVING DIET TO THE EXPERIMENTAL ANIMALS

Nutrients	%
Protein	27.8
Carbohydrate	41.0
Fat (total)	21.4
[Saturated*]	[76.4]
[Unsaturated*]	[23.8]
Mineral mixture	2.5
Vitamin mixture	0.5
[Vitamin A*]	[1500 I.U.]
[Vitamin E*]	[7.5 mg]
Cellulose	6.5

* The percent of total amount.

Every two months for six month (3 times), five animals were randomly chosen from each group. Blood samples were taken from the vena

cava of each animal after on overnight fast. Livers were also collected. Plasma was separated immediatly and, together with the livers, were stored in a deep-freeze (–80°C) until analysis.

The methods of Stähler *et.al.* and Trinder's[11] were utilized for the determination of plasma cholesterol while the method of Wahlefeld[12] was employed to measure plasma triglycerides. The levels of plasma vitamin A and E were determined of Hansen and Warwick's method.[13] The method of Thompson *et.al.*[14] was used in the liver vitamin extraction.

All the chemicals were of analytical grade and were obtained from Bohringer and Sigma Companies.

The significance of difference between groups was tested using multifactorial experiments for biochemical results and the chi-squared test for tumour incidence.

Results

Figure 1 represents the body and liver weight of the groups for the three time periods.

The tumour incidence of MNU-induced rats during the experimental period are shown in Table II. There was no significance differences in the number of tumour bearing animals between the groups consuming different amounts of energy. Hovever, the volume of tumours increased over the 6 months, and this increase was significant in group 3.

TABLE II

EFFECT OF GIVING DIFFERENT AMOUNTS OF CALORIE DAILY ON THE TUMOUR INCIDENCE AND VOLUME IN SPRAGUE-DAWLEY RATS TREATED WITH METHYLNITROSOUREA

Experimental groups	Animals with tumour Periods (Months)			The number of tumour Periods (Months)			The volume of tumour* Periods (Months)		
	2	4	6	2	4	6	2	4	6
1	5	20	25	5	34	56	0	0	0.75
2	4	18	26	4	29	60	0	1.2	4.8
3	1	5	18	1	8	30	0	1.3	8.0

* cm^3, median (95 %) confidence limits)

The total plasma lipid was found to be decreased in group 2 at the second time period and then increased at the third time period (Table III). There were no differences in the plasma cholesterol between the

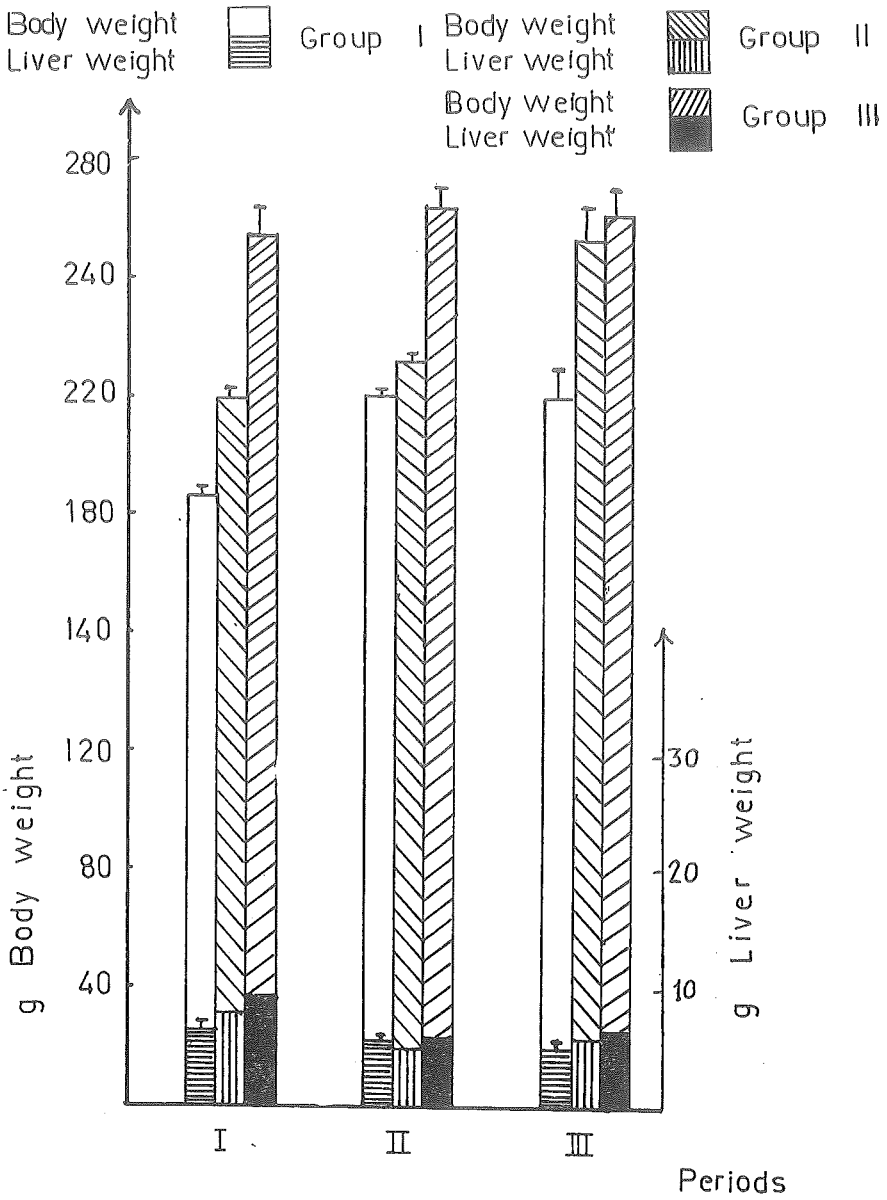

Figure 1

Body and liver weights of the experimental groups for three periods.

groups. The plasma triglycerides were incresad in group 2 at the first period, and then at the third period they were decreased in all of the groups.

TABLE III

EFFECT OF GIVING DIFFERENT AMOUNTS OF COLORIE ON THE LEVELS OF PLASMA TOTAL LIPID, CHOLESTEROL AND TRIGLYCERIDES IN SPRAGUE-DAWLEY RATS TREATED WITH METHYLNITROSOUREA (VALUES ARE THE MEAN OF FIVE ANIMALS ∓ SD*)

Experimental group	Periods (month) 2	4	6
Plasma total lipid (mg/dl)			
1	309 ∓ 16	388 ∓ 109	360 ∓ 71
2	302 ∓ 15	265 ∓ 59**	329 ∓ 43**
3	322 ∓ 36	389 ∓ 94	371 ∓ 214
Plasma Cholesterol (mg/dl)			
1	92 ∓ 11	99 ∓ 8	104 ∓ 11
2	78 ∓ 8	88 ∓ 15	106 ∓ 16
3	97 ∓ 16	105 ∓ 19	97 ∓ 56
Plasma triglycerides (mg/dl)			
1	56 ∓ 8	54 ∓ 9	39 ∓ 3
2	70 ∓ 17**	47 ∓ 4	*** 41 ∓ 5
3	57 ∓ 6	61 ∓ 6	46 ∓ 28

* P Value is 0.05

** Significance of difference between the groups at the same period.

*** Significance of difference between the general mean and the values of groups at the same period.

TABLE IV

EFFECT OF GIVING DIFFERENT AMOUNT OF CALORIE ON THE LEVELS OF PLASMA AND LIVER VITAMINS A AND E IN SPRAGUE-DAWLEY RATS TREATED WITH METHYL-NITROSOUREA
(VALUES ARE THE MEAN OF FIVE ANIMALS ∓ SD*)

Experimental group	Periods (month) 2	4	6
Plasma vitamin A (μg/100 ml)			
1	18.6 ∓ 0.9	17.8 ∓ 1.8	17.9 ∓ 7.0
2	19.2 ∓ 0.8	16.1 ∓ 2.0	24.4 ∓ 2.8
3	18.8 ∓ 1.5	15.0 ∓ 7.2	22.4 ∓ 14.4
Plasma vitamin E (μg/ml)			
1	5.8 ∓ 3.5	9.2 ∓ 0.6	10.8 ∓ 0.8
2 ***	7.0 ∓ 2.2	8.7 ∓ 1.2	16.1 ∓ 6.7**
3	9.5 ∓ 3.5	11.7 ∓ 2.2	10.9 ∓ 6.4
Liver vitamin A (μg/g tissue)			
1	189 ∓ 25**	405 ∓ 60**	805 ∓ 70**
2 ***	178 ∓ 15**	609 ∓ 60**	*** 779 ∓ 76**
3	180 ∓ 12**	512 ∓ 14	752 ∓ 90**
Liver vitamin E (μg/g tissue)			
1	22 ∓ 6	37 ∓ 13	14 ∓ 9****
2	30 ∓ 6	*** 52 ∓ 8	*** 26 ∓ 6
3	27 ∓ 4	54 ∓ 9	36 ∓ 17

* P value is 0.05
** Significance of difference between the groups at the same period
*** Significance of difference between the general mean and the values of groups at the same period
**** Significance of difference between the period values of the same group.

Total plasma A levels were constant in all the groups during the experimental periods (Table IV). At the first period, however, plasma vitamin E level was found to be lower than all the other periods in all the groups, and it was increased only at the third period in group 2. In contrast, plasma vitamin A level, liver vitamin A were increased with aging in all the groups. The vitamin E level of the liver was significantly decreased at the third period in group 1. All of the values, at the second and third periods were significantly different from three of the general mean.

Discussion

Consumption of different quantities of calories for six months, following tumour induction, has shown a parallel pattern which was expected, to that of excess energy, and body and liver weight gain (Figure1).

There are many reports about the amount of energy intake and the incidence of cancer.[15] It has been observed that an increased energy intake could also increase the incidence of specific tumours. The most notable of these are the hormone dependent mammary gland tumours.[16] In the present experiment, however, feeding the groups 30,40 and 50 Kcal/daily did not significantly change tumour incidence (Table I). 50 Kcal/day is accepted as normal energy for the rats.[17] Feeding 10 and 20 Kcal/daily less than the normal amount did not effect the incicidence and development of mammary carcinoma. Therefore, it could be said that the proportion of nutrients in diets, rather than the amount of calorie (except excessive calories) may play an important role in the incidence of tumours.

The plasma total lipid level of group 2 was decreased at period two and increased at period three. This could be due to individual differences seen in the group. Although there was no difference of the cholesterol level of plasma, plasma triglycerides were decreased with aging in all the groups. This finding confirms the work by Porta *et.al.* which showed a decrease in serum triglycerides from age 3 to 18 months in rats.[18]

The total vitamin A level of plasma was found to be constant in all of the groups at each period, while the liver vitamin A level increased with advanced age. It is reported that serum retinol concentrations are lower in patients with gastrointestinal tract and lung cancer than in controls,[10, 19] but the vitamin A stores of these patients were not measured. In the present study, however, the vitamin stores of liver was apparently enough to keep the vitamin A level of plasma in the

normal range. It is well known that the liver is a strong regulator of the fat-soluble vitamin concentrations in plasma. There was also no correlation between the plasma and the liver vitamin A levels and tumour volumes in either group.

The vitamin E level of the plasma, nevertheless, was increased in all of the groups during aging, and decreased in the liver. Further, there was no correlation between the plasma and liver vitamin E levels and tumour growth. The concentration of vitamin E in the liver tissue is related to the plasma concentration[20] and it is also well known that this vitamin is an antioxidant and is used to prevent lipid and vitamin A peroxidation. Therefore, this vitamin could have been used for the above purposes by the organism in the present experiment. A significant decrease in the vitamin E level of liver in group 1 could also be due to a direct relationship between adiposity and deposition of the vitamin,[21] because this group consumed the least amount of energy.

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Congenital Liver Damage After Treatment of Mother With Sodium Valproat in Mice

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Summary

Sodium valproate was given to pregnant mice in a dose of 100 mg/kg body weight per day orally in drinking water at different intervals of their 20-21 day pregnancies. After delivery, the body and liver weights of the siblings were lowest in the group whose mothers received sodium valproate during their last 13-16 days of pregnancy compared to the groups which received sodium valproate during their last 7-11th days or 2-4th days of their pregnancy. Although there was no correlation between liver weight and hepatocellular degeneration, degeneration was found to be much more severe in the siblings which had low liver weights.

Key Words: Intrauterin drugs effects, Hepatotoxicity, Mice, Sodium valproate.

Introduction

Although valproic acid (VPA) has an expanded role in the treatment of epilepsy, it also has serious side effects.[1] VPA has been respon-

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sible for grave disease or death in some patients when used by itself or with other anticonvulsant drugs. Most fatalities have been associated with hepatic failure,[2] and some with pancreatitis.[3]

Since antiepileptic therapy usually must be continued throughout pregnancy, there is a risk for the developing embryo and fetus. Anticonvulsant therapy during pregnancy may be associated with higher incidence of nonspecific fetal abnormalities as well as with significant and characteristic fetal syndromes. Very little is known about the effects of VPA on pregnant women and/or fetuses. VPA is a teratogenic agent in animals and it may cause congenital neural tube defects and some dismorphogenic facial defects.[4-7] But no report about toxic effects of VPA in fetal liver has been published, except one suspected case.[8] Therefore, we carried out a study on congenital liver damage in the offsprings of mice whose mothers were treated with VPA during their pregnancy at different interwals.

Materials and Methods

Pregnant albino mice were supplied by the Laboratory Animals Breeding Unit of Selçuk University for use in this study. The mice were divided into four equal groups of three mice each: groups A,B,C,D. Group D was separated as control and did not receive the drug. The other mice received sodium valproate (Depakin^R) dailly in a dose of 100 mg per kg body weight orally in drinking water at different interwals of their 20-21 day pregnancies. The mice were classified in three different groups according to their duration of sodium valproate intake.

The mothers and their siblings were killed by cervical dislocation after delivery. The livers of the mice were removed for examination under light microscopy. The body and liver weights were recorded.

Results were expressed as the mean $\pm$ SEM. Student's t test were used to compare the results.

Results

The results are summarized in Table I. Mother's body and liver weights were almost the same in all four groups. But, hepatotoxic effects of VPA was seen in the siblings who received the drug in early fetal development. The body and liver weights of the siblings were the lowest in group A who received sodium valproat during the last 13-16 days of their 20-21-day pregnancies.

The histological examination showed normal liver tissues in mothers, but there was hepatocellular degeneration in various degrees in the offsprings, except in controls. Although there was no correlation

TABLE I

THE BODY AND LIVER WEIGHTS OF MOTHERS AND THEIR SIBLINGS

Group	Beginning time of the drug in pregnancy	Number of mice	Mothers Body weight (g)	Mothers Liver weight (g)	Number of siblings	Siblings Body weight (g)	Siblings Body weight sibling/mother (%)	Siblings Liver weight (g)	Siblings Body weight/liver weight (g)
A	5-8. days	3	41.66 ∓ 4.92	2.89 ∓ 0.61	14	1.38 ∓ 0.03	3.25 ∓ 0.09	0.059 ∓ 0.003	4.29 ∓ 0.19
B	10-13. days	3	40.75 ∓ 1.05	3.33 ∓ 0.30	22	1.62 ∓ 0.04	3.99 ∓ 0.10	0.080 ∓ 0.004	4.92 ∓ 0.26
C	17-19. days	3	42.25 ∓ 0.85	3.22 ∓ 0.12	17	1.53 ∓ 0.01	3.63 ∓ 0.06	0.088 ∓ 0.004	5.73 ∓ 0.20
D	none (control)	3	40.20 ∓ 0.79	2.99 ∓ 0.17	16	1.95 ∓ 0.16	5.23 ∓ 0.43	0.094 ∓ 0.006	5.71 ∓ 0.13
				$p>0.05$ in all groups	A-B	$p<0.01$	$p<0.01$	$p<0.01$	$p>0.05$
					A-C	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$
					A-D	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$
					B-C	$p<0.05$	$p<0.01$	$p>0.05$	$p<0.02$
					B-D	$p<0.02$	$p<0.01$	$p>0.05$	$p<0.01$
					C-D	$p<0.05$	$p<0.01$	$p>0.05$	$p>0.05$

between the liver weight and hepatocellular degeneration, it was much more severe in the siblings who had lower liver weights.

Discussion

The Committee on Drugs of the American Academy of Pediatrics cautioned that, in view of increasing reports of toxic effects, VPA is not the first drug of choice for most patients with seizures.[9-11] The most important toxic effect is fatal hepatic dysfunction which occurs within six months of the initiation of VPA therapy. It tends to occur independent of serum concentration and is not dose related; it may progress even after drug withdrawal.[2] Postulated mechanisms for hepatic toxicidy include hypersensitivity and/or a direct toxic effect.[12-14] There may be two general forms of VPA-induced hepatic disease.[2] One is fulminant at the onset and rapidly progresses into hepatic necrosis and cholestasis; and the other is insidious at the onset and gradually progresses into temporary liver damage. Although cases of hepatic failure are rare, hepatic dysfunction indicated by reversible elevation of serum aminotransferases occur in 5 to 30 % of patients treated with VPA.[2]

VPA crosses the placenta and has been shown to achieve significant concentrations in the serum of a newborn.[15, 16] But there is only one report, according to our knowledge, concerning the fatal hepatotoxic effects of VPA treatment during pregnancy. The liver dysfunction and morphological damage of the liver observed in a female neonate whose mother had been treated throughout pregnancy with VPA and phenytoin, showed evident signs of liver damage confirmed by laboratory investigations and biopsy. Light microscopic findings showed pronounced changes with broad portal zones infiltrated predominantly by lympnocytes. Further, granulocytes were also present and there was cloudy swelling of the liver cells.[8] The histopathological findings in the liver showing diffuse inflamatory reaction and focal necrosis are consistent with previous reports of suspected hepatotoxicity of VPA.[1, 2] Although the maternal concentrations of VPA and liver function tests were normal when checked during pregnancy, it may be that the fetal liver is more sensitive to VPA than the adult liver. In this study neonatal liver morphologies were abnormal in contrast to their mothers liver morphology when VPA treatment was given during their pregnancies. Fetal plasma VPA concentrations have been reported to be higher than maternal levels, probably as a result of the difference in protein-binding capacity of VPA on each side of the placenta.[17] There is also a possibility that the metabolites of VPA are hepatotoxic.[13]

The timing of VPA introduction to pregnant animals is important. The effects of VPA on liver weights and histopathological signs of hepatic toxicity is much more severe in neonatal mice whose mothers received VPA from their early days of pregnancy as it is shown in the present study.

In summary, since antiepileptic therapy usually must be continued throughout pregnancy, there is a risk for the developing embryo and fetus. Taking the teratogenic and hepatotoxic side effects of VPA into consideration, we suggest that it should not be used in pregnant women and it is not the first drug of choice for most patients with seizures.[1,2]

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Thyroid Hormone Alterations in Surgical Patients

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Summary

Alterations in circulating thyroid hormone concentrations occur in a variety of non-thyroid disease states. In this study, two groups of patients were obserwed. In Group 1, there were 15 patients, (mean age 27 ± 4 years) undergoing emergency abdominal surgery for major abdominal trauma) and in Group 2, there were 10 patients (mean age 39.3 ± 2.9 years) undergoing elective abdominal surgery for intra-abdominal malignant tumor. Thyroid hormone levels were measured preoperatively (six hours before the operations) and postoperatively (twelve hours, one day and one week after surgery). Compared with preoperative values, the mean serum T_4,T_3 and TSH concentrations decreased significantly ($p < 0.01$), immediately following surgery in the trauma group. However, in the second group, thyroid hormone changes were not significant after surgical intervention. There was no direct correlation between the changes in T_3 - T_4 and TSH concentrations in the latter group.

These results suggest that in acutely traumatized patients, T_3 declines rapidly after surgery and remains depressed throughout the illness and remits with convalescence.

Key Words: Major trauma, Malignant tumor, Thyroid hormone alterations.

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Introduction

Changes in circulating thyroid hormone levels have been noted in a variety of acute and chronic medical and surgical illnesses.[1-5] While depressed serum total triiodothyronine (T_3) and elevated reverse T_3 (rT_3) levels occur consistently, variable total thyroxine (T_4) content have been reported.[6-8] Additionally, hypothyroxinemia may be a severe pathophysiologic event in certain clinical situations and result in a high mortaliyt rate in critically ill patients.[2, 3, 9]

The aim of this study was to determine the changes in thyroid hormone concentrations after surgery in patients with major trauma or who suffered from malignant disease.

Materials and Methods

Two groups of patients were studied. In Group 1, fifteen (13 men and 2 women, with a mean age of 27 $\pm$ 4 years) were admitted to Erciyes University, Medical Faculty Hospital, because of major trauma and were evaluated consecutively. There were more than two organ traumas in this group of patients. In Group 2, there were ten patients (7 men and 3 women, with a mean age of 39.3 $\pm$ 2.9 years) undergoing elective abdominal surgery. They all suffered from malignant diseases.

Venous blood samples were obtained from each patient in six hour intervals before surgery (Stage I) and during postoperative periods (twelve hour (Stage II), 1 day (Stage III) and one week (Stage IV) after surgery). All samples were processed immediately and stored at –20°C for later analysis. Each sample was assayed for total T_4,T_3 and Thyroid Stimulating Hormone (TSH) using specific radioimmunoassays (Amerlex-M T_3,T_4 and TSH RIA).

Serum total protein and albumin levels were measured twenty-four hours after surgery in both groups of patients.

Results are expressed as the mean $\pm$ SEM. Data were analyzed by students t-tests.

Results

All patients were submitted to major emergency surgical procedures after multiple trauma (twelve blunt and three penetran abdominal trauma). Types of trauma were: hepatic laceration, retroperitoneal hematoma, intestinal laceration, pancreatic hematoma and splenic rupture.

The preoperative mean value of T_3 was 0.91 ng/ml in the trauma group. Total T_3 values fell progressively following surgery, reaching their nadir at 24 hours (0.39 ng/ml). T_3 increased to 1.15 ng/ml on day 7. These changes are shown in the Table I.

The preoperative mean value of serum T_4 was 8.78 µg/dl in the trauma group. Total T_4 values decreased in early periods of surgery. But, T_4 increased again to 10.91 µg/dl on day 7 (Table II). Serum TSH concentrations were reduced at 24 hours after surgery and icreased on day 7 (Table III).

Total protein and albumin levels were in the normal ranges during the 24 hours following surgery.

TABLE I

SERUM T_3 LEVELS CHANGES IN THE TRAUMA GROUP

Stage	n	T_3 levels (ng/ml)*	Comparison of two stages		
			Stages	t	p
I	15	0.91 ∓ 0.09	I - II	7.32	< 0.01
II	15	0.57 ∓ 0.07	II - III	5.81	< 0.01
III	15	0.39 ∓ 0.05	III - IV	8.07	< 0.01
IV	15	1.15 ∓ 0.08	IV - I	2.58	< 0.05

* Values given are Mean ∓ SEM

TABLE II

SERUM T_4 LEVELS CHANGES IN THE TRAUMA GROUP

Stage	n	T_4 levels(µg/dl)*	Comparison of two stages		
			Stages	t	p
I	15	8.78 ∓ 0.74	I - II	0.03	> 0.05
II	15	8.74 ∓ 0.66	II - III	1.70	> 0.05
III	15	7.86 ∓ 0.65	III - IV	3.65	< 0.01
IV	15	10.91 ∓ 0.71	IV - I	2.20	< 0.05

* Values given are Mean ∓ SEM

TABLE III

SERUM TSH LEVELS CHANGES IN THE TRAUMA GROUP

Stage	n	TSH levels(µ IU/ml)*	Comparison of two stages		
			Stages	t	p
I	15	1.28 ∓ 0.21	I - II	0.30	> 0.05
II	15	1.53 ∓ 0.23	II - III	0.59	> 0.05
III	15	1.00 ∓ 0.08	III - IV	2.20	< 0.05
IV	15	1.74 ∓ 0.34	IV - I	1.43	> 0.05

* Values given are Mean ∓ SEM

In malignant cases, the tumor types were; colorectel carcinoma (five patients), gastric carcinoma (two patients), retroperitoneal malignant tumor (two patients) and portahepatic adenocarcinoma (one patient).

In malignant patients, total T_3 decreased to the lowest level 12 hours after surgery (0.55 ng/ml), then increased progressively and reached normal levels on day 7. Serum T_4 level changes were as T3, but there was no significant decrease.

Serum TSH concentrations changed during the course of the study, but only at the 12 hours interval was the statistically significant.

In malignant cases, postoperative total serum protein and albumin levels were lower than the trauma group ($p < 0.01$).

In the trauma group, serum T_3, T_4 and TSH values were compared with the malignant cases group. T_3 and TSH levels were found to be lower ($p < 0.05$) in the trauma group, than in malignant group cases during the 24 hours following surgery.

Discussion

Perturbations in thyroid hormone metabolism occur following elective surgery, thermal injuries, acute and chronic illnesses and bacterial sepsis.[1, 8, 10, 12] In general, these conditions cause a significant reduction in serum concentration of T_3, an increase in the level of biologically inactive rT_3, and little or no change in T_4. Our studies demonstrate that surgery after major trauma induce profound alterations in thyroid metabolism.

Moreover, the changes in patients with major trauma are characterized by a more rapid onset and longer duration than those observed following elective surgery for malignancy. Hormone levels had returned to baseline one week following surgery in both groups.

Triiodothyronine (T_3) is several times more active metabolically than T_4, while rT_3 seems to be biologically inactive.[7]

It has been known that plasma concentrations of T_3 fall in patients with severe systemic diseases, with a correlation between the severity of the disease and the reduction of total plasma T_3 levels.[2, 3, 9]

Several clinical situations have been reported in which T3 concentrations are decreased due to diminished extrathyroidal conversion of T_4 to T_3.[10, 13] Recently Chopra *et al* found a significant increase of plasma rT_3 concentrations in patients with severe systemic disease, total T_3 concentrations clearly below normal levels, and normal or slightly dec-

reased T_4 concentrations.[13] The reduction of T_3 levels was also reported in patients during the postoperative period following elective surgery.[4, 5, 14] Burr *et al.* showed a rapid decrease in T_3 levels after surgery, with simultaneous increase of rT_3 concentrations and slight fall of T_4 levels.[14] In the trauma patients we studied, there was a significant decrease of plasma T_3 levels, while the concentrations of T_4 generally remained in the normal range.

Thus, a reduction of the active thyroid hormones may be cosidered as an adaptive mechanism to catabolism. In the case of fasting, it occur by reducing the basal metabolic requirements, and in the case of severe catabolic illnesses or surgery it occurs by limiting the hypermetabolic state.[10]

The preferential generation of rT_3 from T_4, at the expense of decreased T_3 production, can thus be considered as a form of T_4 inactivation. The glucocorticoids, catecholamines and serum glucose levels are increased during the post-traumatic period. These factors may obviously act synergistically to induce the deiodination of T_4 in the direction of rT_3 production in the trauma group.[7, 15]

The reduction of the serum T_3 concentration in shock may have unfavorable metabolic effects. T_3 is capable of entering mitochondria, which have membrane receptors for this hormone.[16] Thus T_3 may be an important regulator of mitochondrial activity. The reduction in circulating T_3 levels may contribute to the functional impairment of mitochondria in shock and anoxia.[17]

Most circulating T_4 and T_3 are bound by thryroxine-binding globulin (TBG), with only about 5 % bound by albumin. Thus, changes in the serum albumin levels have relatively little effect on serum T_4 and T_3 values.[8]

Serum total T_4 levels are unaltered in patients who survive the trauma, but are significantly depressed in fatalities.[3] Becker found 84 % mortality in critically ill patients when the total T_4 declined below 3.0 μg/dl.[3]

Hypothyroxinemia with normal TSH levels was found to be associated with high mortality rate.[3, 9]

In our series, serum T_4 concentrations did not decrease to critical levels, so we did not establish this correlation. In our trauma group, increasing levels of TSH 12 hours after surgery would be a response to decreased levels of T_3. But TSH concentrations did not increase to higher levels, in spite of T_3 having reached its nadir.

Current techniques are inadequate to establish all the factors contributing to changes in thyroid hormone concentrations, but changes in T_4 and T_3 production rate, alterations in thyroid hormone binding protein capacity and alterations in cellular uptake, metabolism and degradation have all been implicated by various authors.[5, 7, 11, 12] Metabolic factors that initiate these changes and the mechanisms by which they occur remain unclear.

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The Comparison of Vaginal Yeast Flora of Mother and Oral Mucosa of Newborn

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Summary

Although Candida species may frequently be found in the vaginal flora of pregnant women, infestation of the baby during delivery is not well documented. In this clinical and mycological study, vaginal yeast flora of parturients and oral candida species of the newborn in the first and fifth days of life were compared. C. albicans, C.tropicalis and C. krusei were found in vaginal yeast flora of the mother and oral mucosa of the child. Other species were found only in vaginal flora. The correlation between them suggested that vaginal carriage of some yeasts may be a risk factor for the newborn.

Key Words: Vulvovaginal candidiasis (VVC), Oral Thrush, Candidiasis.

Introduction

Candida species can be frequently isolated from mucous membranes and stool of apparently healthy individuals. It is obvious that most infections have an endogenous source and occasionally the infection is contagious. The determination of the source of the infection is difficult.[1,2]

Vulvovaginal Candidiasis(VVC) is most often seen during pregnancy.[2,3,4] Although Candida species may frequently be found in the vaginal flora of pregnant women, fetal infection has not been commonly reported.[5,6]

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Oral Candidiasis (Oral thrush) is a disease of the newborn. Infection probably occurs during passage through the birth canal. The etiological relationship between VVC and oral thrush of the newborn is not clear.[1, 2, 7]

In this study, vaginal yeast flora of pregnant women was compared with oral candida species of the newborn.

Material and Methods

270 pregnant women (at term) and their newborn infants were examined clinically and mycologically. Specimens were taken with cotton swabs and examined with 20 % KOH. Cultures were made on Sabouraud Dextrose Agare using chloramphenicol. Candida species were differentiated by means of germ tube formation and oxidative-fermentative reaction on culture media containing glucose, saccharose, lactose and maltose.

Results

270 pregnant women (ages between 16-42) were examined clinically at the time of delivery and the following results were found: 89 cases (32.9 %) showed vaginal discharge; 49 cases (18.1 %) suffered from itching and a burning sensation; 17 cases (6.3 %) showed vulvovaginitis with erythema, excoriations and exudative lesions. None of the 270 babies showed mucosal lesion on the first day of life. Only 189 of them could be investigated at the fifth day of life. 37 infants showed oral thrush with typical creamy-white patches on tongue and buccal mucosa and the others were clinically normal.

On direct microscopical examination 66 vaginal specimens were found positive for fungal elements. The relationship between clinical findings and mycological results are shown on (Table I).

TABLE I

RESULTS OF CLINICAL AND MYCOLOGICAL EXAMINATION OF MOTHERS

			Mycological Examination			
			Direct		Cultural	
Clinical Examination	n	%	(+)	(−)	(+)	(−)
Symptomatic VVC	106	39.2	68	38	73	33
Asymptomatic VVC	164	60.7	11	153	16	148
Total	270		79	191	89	181

Direct microscopical examination of oral mucosa specimens of babies showed only five positive cases on the first day of life. Only 37 positive cases for fungal elements were seen out of the 189 infants that were examined on the fifth day (Table II).

TABLE II
RESULTS OF CLINICAL AND MYCOLOGICAL EXAMINATION OF BABIES

Clinical Examination	1st day	5th day	Mycological Examination: Direct 1st day (+)	Direct 1st day (-)	Direct 5th day (+)	Direct 5th day (-)	Cultural 1st day (+)	Cultural 1st day (-)	Cultural 5th day (+)	Cultural 5th day (-)
Oral Thrush	0	37	0	37	30	7	0	37	37	0
Asymptomatic	270	152	11	259	0	152	14	256	14	138
Total	270	189	11	259	30	159	14	256	51	138

There was a significant correlation between the maternal VVC and oral thrush of the newborn. The relationship between vaginal yeast carriage and oral thrush is shown in (Table III).

TABLE III
CANDIDA SPECIES ISOLATED FROM VVC AND ORAL MUCOSA OF NEWBORN

Candida Species	Mother n	Mother % (in general)	Mother % in (positive cases)	Baby n	Baby % in (general)	Baby % in (positive cases)
C.Albicans	59	21	67.8	46	24.3	90.1
C.Stellatoidea	11	4	12.6	–	–	–
C.Tropicalis	9	3.3	10.3	3	1.5	5.8
C.Krusei	6	2	6.8	2	1	3.9
C-Pseudotropicalis	2	0.7	2.2	–	–	–
No growth	183	67	–	138	51	–

C. Albicans in mother and baby % 77.9 $p < 0.001$
C. Tropicalis in mother and baby % 33.3 $p < 0.05$
C. Krusei in mother and baby % 33.3 $p < 0.05$

Discussion

Asymptomatic colonization of yeasts was found in 30-40 % of healthy pregnant women. We found 32.9 % candida colonization at the time of delivery.

Pathogenesis of VVC during pregnancy may be because of the abnormal glycogen content of vaginal epithelium under the stimulation of reproductive hormones.[3,4]

During pregnancy the symptomatic VVC is high and increases during the course of gestation.[4] In our cases symptomatic VVC was 39.2 % in the pregnant women.

It is not clearly understood whether VVC is a risk factor for the baby or not, because of the general rule that the Candida species are not transmissible.[1, 2, 4] It is suggested by many authors that the infection probably occurs during passage through the birth canal which is infected with yeasts.[5, 6, 7]

Candida Albicans is the most important pathogenic species of Candida and the usual etiologic agent of VVC and oral thrush,[1, 2] Occasionally the other species may also be pathogenic for humans.[2] We found 67.8 % C. Albicans in VVC and 90 % in oral thrush of the newborn.

We conclude that the vaginal carriage of some yeasts may occasionally be a risk factor for the newborn, and suggest that further research should be made to decide if Candida species are absolutely intransmissible in similar cases.

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Drug Therapy For Aplastic Anemias

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Summary

Four cases of aplastic anemia were treated with the combination of androgenic and corticoid steroids, folic acid, vitamin B12 and hydrochloroquine sulfate. This therapy was aimed at stimulating residual stem cells, suppressing autoimmune reactions and providing necessary hematopoetic cofactors. One patient with benzene-induced aplastic anemia has been in remission for about 20 years. Two patients with the idiopathic type of aplastic anemia had been in remission for 5 years before the therapy was discontinued by other physicians. The last patient has been in remission for 7 years, who also has the idiopathic type.

Key Words: Aplastic Anemias, Drug Treatment.

Although bone marrow transplantation has become the preferred mode of therapy for aplastic anemias during the past decade, it may not be within the reach of many patients. Furthermore, the problems peculiar to organ transplants would be additional obstacles.

The therapy protocol applied to the cases presented in the paper was initiated in 1967 with case 1, who is a patient with benzene-induced aplastic anemia. The hypothesis behind this approach has been to stimulate the residual stem cells in the bone marrow and to supply adequate hematopoetic cofactors and probably to suppress the autoimmune process.

Case Reports

Case 1: This was a 22 year old male draftsman who was hospitalized in February of 1967 with a chief complaint of ecchymotic spots over his lower extremities. These spots had come on 2 weeks prior to admission and the patient felt dyspnea on exertion and he had experienced generalized weakness for several months.

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His past history revealed that he had experienced rectal bleeding in 1966 which had again recurred four months prior to admission. He had worked in a tire company for about 3 years between 1962-1965. There he had been repeatedly exposed to benzene and benzol-type compounds (or solvents) when he handled the cloths soaked in benzene solution to clean the rubber sheets. On physical examination, he had several ecchymotic spots over his lower extremities.

Initial lab findings were: hemoglobin 7.0 gms %, WBC 3,850 with 38 % PMN's, 58 % lymphocytes, 3 % monocytes, platelets 64,000, reticulocyte count 1.4 %-corrected, urinalysis - trace proteinuria, heterophile titer-negative, direct Coombs-negative, indirect Coombs-slightly positive, LDH 540 units, elevated with high myocardial isoenzyme portion on electrophoresis, BUN, creatinine, alkaline phosphatase, blood calcium, blood uric acid were all within normal limits. Fecal urobilinogen was elevated to 150 Ehrlich units. Anti-leukocyte antibodies were negative. Fluorescent antinuclear antibodies (FANA) were negative. Anti-mitochondrial antibodies were positive on a dilution of 1:10. Plasma fibrinogen was 225 mg %. Cryoglobulins were detected at 172 mg % level. Bence Jones protein in the urine was negative. Serum protein electrophoresis was within normal limits. Urine protein ELP was also normal. Schilling test for B12 was normal. Diagnex blue test suggested the presence of anacidity in the stomach. Radioactive bone scan was within normal limits. Serum iron was high at the level of 300 mcg %. Bone marrow aspiration biopsy revealed the presence of marked hypoplasia in the red cell and white cell lines with absence of megakaryocytes. There appeared to be an abundant number of plasma cells, some of which formed clusters and some appeared to be of the "myeloma type". In fact, this led to the inital diagnosis of multiple myeloma. The final diagnosis was aplastic anemia probably due to benzene poisoning.

The patient was placed on a daily dosage of Prednisone 40 mg., Halotestin 20 mg, and folic acid 20 mg. with vitamin B complex. His reticulocyte count rose to 6.8 % on the 5th day and he was discharged on this regimen. Three months later he was re-evaluated; his hemoglobin was 12.5 gm % with normal red cell indices, and his WBC was 6,500 with a platelet count of 127,000. Bone marrow aspiration was repeated, which revealed hypoplasia with a myeloid - erythroid layer of 0.5 %, macrocytosis, lymphocytosis and thrombocytopenia. The reticulocyte count was 6 %.

As his hemoglobin level stabilized between 11 - 13 gm %, he was maintained with a daily dose of Prednisone 10 mg. Halotestin 5 mg, folic acid 3 mg. and Trinsicon 2 capsules.

In 1970, he underwent a splenectomy, as his hemolytic process seemed to be difficult to control. The spleen weighed 250 grams, and microscopically it showed extensive hemosiderosis. Because of his intermittent gross hematuria, a urological work-up was undertaken. There was an ulcer at the vesicle of the bladder neck which was fulgurated. A kidney biopsy showed marked hemosiderosis as studied extensively with light and electron microscopy.

In 1973, he underwent bilateral simple mastectomy because of gynecomastia. Hydrochloroquinine (Plaquenil) was added to his therapy at the dosage of 400 mg. daily, because he had positive nuclear antibodies at the low titer levels of 1:20 and 1:40. It was hoped that Plaquenil's steroid sparing action might reduce the patients steroid maintenance dose. By 1974, he was stable with a hemoglobin level of 12-13 gms %, a platelet count of 200,000 - 300,000 and a leukocyte count of 7.000 - 8,000. At this he was on a daily maintenance dose of 5 mg. of Prednisone, 5 mg of Halotestin, 1 mg. of folic acid, 1 capsule of Trinsicon and 200 mg. of Plaquenil. The Plaquenil was discontinued in 1977 when his hematology picture seemed to be stable.

In November 1985, he appeared to have developed cholestatic hepatitis, probably due to the Halotestin, which was then discontinued. His Prednisone was then increased to 10 mg. daily. The hepatitis has been subsiding gradually and his hemoglobin levels have been between 11 - 12 gms % with normal WBC and platelets.

Except during the above mentioned hospitalization episodes, this patient has worked full time and enjoyed the quality of his life.

Case 2: This was a 56 year old man who was a T.V. repairman at the time of diagnosis in 1970. In the fall of 1970, he was hospitalized and the diagnosis of aplastic anemia of the idiopathic type was established by the peripheral blood and bone marrow studies. He was placed on fluoxymesterone (Halotestin) 10 mg. tablets and folic acid 5 mg. tablets daily. His hemoglobin initially was 9.0 gm % which raised to 12.2 gm % in association with similar improvements in the leukocytes and the platelets. However, by May 1971, his hemoglobin declined to 6.9 gm % with platelets of 60,000 and a WBC of 5,800. His liver was palpable 10 cm. down on the anterior axillary line and his spleen, which was descended to the iliac crest, was markedly enlarged. There was generalized lymphadenopathy, in that the average lymph node seemed to be about 0.5 cm. in size. Other pertinent lab findings included a sedimentation rate of 95 mm/hr., a fecal urobilinogen of 349 Ehrlich units, a corrected reticulocyte count of 3.8 %, a serum LDH of 750 units with

an otherwise normal SMA-12 profile. Normal tests included an ELP, immunoelectrophoresis, A.N.A., rheumatoid factors, direct and indirect coombs tests, Schilling test and chest X-rays.

His treatment was changed to Prednisone 40 mg. daily, testosterone cypionate 100 mg. I.M. (Depo-Testosterone) weekly and Trinsicon capsules three times daily. Within four weeks, his hemoglobin was 14.8 gm % with a platelet count of 120.000 and a WBC of 7,500. He stayed in complete remission for about 4 years and 8 months on the above mentioned regimen, although the Prednisone was reduced to 15 - 20 mg. per day after he first month of therapy. His clinical signs and symptoms showed dramatic improvements; the spleen returned to normal size, the liver decreased in size to the 5 cm. palpable level at the anterior axillary line, and his lymphadenopathy completely disappeared. Then gradually his hemoglobin declined to 8.3 gm % with an increasingly enlarged spleen by February 1976. Depo-Testosterone was discontinued in favor of nandrolone decanoate (Deca-Durabolin) 100 mg. I.M. weekly which did not seem to induce any significant change in his hemogram. Another androgenic hormone (Methosarb 50 mg. one b.i.d. orally) was tried without much success. During this period, his spleen enlarged back to the 1971 level, i.e. down to the iliac crest. The patient was referred to the Mayo Clinic for a splenectomy. He died from infection and bleeding following the splenectomy in February of 1977.

Briefly, this was a case of aplastic anemia of the idiopathic type. The above outlined drug therapy produced 5 years of complete remission and $1\frac{1}{2}$ years of partial remission. The presence of marked hepatosplenomegaly was a striking clinical finding which had improved during hematological remissions.

Case 3: This was a 45 year old salesman for a food producing company. In January 1972, he presented with a hemoglobin of 4.0 gm %, a WBC of 4,200 and a platelet count of 48,000. The bone marrow biopsy done at this time was compatible with a diagnosis of myelofibrosis and aplastic anemia. His corrected reticulocyte count was 2.2 %. Twenty four hour urine studies for heavy metals was negative. He was placed on a daily dose of Prednisone 20 mg., which was later reduced to 10 mg. for maintenance; Halotestin 10 mg., Premarin 2.5 mg. (conjugated estrogens), folic acid 3 mg., and Trinsicon 3 capsules. He went into remission quickly as his hemoglobin rose to 14.5 gm % with normal values of leukocytes and platelets. This remission lasted for about 2,5 years. By August of 1974, his hemoglobin dropped to 4.2 gm % with a WBC of 4,100 and a platelet count of 23,000. He was transfused up to the level of 8 - 9 gm %. of hemoglobin and then he was given Depo-

Testosterone 200 mg. I.M. weekly instead of Halotestin. In addition, he was receiving Plaquenil 200 mg. tablets twice a day since his antinuclear antibody test was positive at the dilution of 1:40. The bone marrow biopsy was repeated, which again confirmed the original diagnosis of aplastic anemia. This time his liver was down 10 cm., and spleen was 5 cm. The patient went into another remission with these changes mentioned in the therapy program. The second remission lasted about one year. By August 1975, his hemoglobin had again dropped to 4.2 gm %, his spleen had increased in size, palpable 10 cm. down on the anterior axillary line. His liver also gradually increased to 12 cm. down on the anterior axillary line. Deca-Durabolin was tried in place of Depo-Testosterone without any significant results. However, the addition of Methosorb 50 mg. tablets three times a day seemed to help: his hemoglobin levels stayed between 8 - 10 mg % with near normal levels of leukocytes and platelets. Clinically, his spleen size did not change but his liver decreased in size, coming from 12 cm. to 5 cm. level. This remission also lasted for about 12 months. He died in April 1977 at the Minneapolis Veterans Administration Hospital from infectious complications.

Case 4: This was a 58 year old male office worker, whose diagnosis of aplastic anemia was established in January of 1979 following bone marrow aspiration biopsy, when the patient was hospitalized with cholecystitis and an abscessed gall bladder. He had no lymphadenopathy or hepatosplenomegaly. His hemoglobin was 9.2 gm %, his platelet count was 122,000 and his WBC was 2,100 with 64 % lymphocytes. The corrected reticulocyte count was 2.6 %. The patient had been leukopenic for at least 5 years before this 1979 hospital admission.

Following a successful cholecystectomy and suitable antibiotic therapy, he was placed on a daily dose of Prednisone 20 mg., folic acid 2 mg. and Trinsicon one capsule. As his hemoglobin rose to 13 - 15 gm % range with normal platelet and leukocyte counts, his daily medication was reduced to 5 mg. of Prednisone, 1 mg. folic acid, and one capsule of Trinsicon. He has remained in complete remission since February of 1979, up to the current time. Other significant laboratory findings at the time of diagnosis included a normal ELP and SMA-12 and a borderline elevation of the fluorescent nuclear antibodies (FANA) test, which was positive at the dilution of 1:20. His FANA has been negative during his remission.

It was of interest that while the patient was in the service at the age of 23, he had been stationed for 3 days within a 20 mile radius of Nagasaki 6 months after the bomb had been dropped.

Discussion

The term of "aplastic anemia" in adults embraces a group of hematological conditions which present peripheral blood pancytopenia associated with a reduction or depletion of precursor cells in the marrow. These conditions might be hematopoetic malignancies (such as acute leukemia, auto-immune diseases), radiation or drug-induced pancotypenias and pancytopenia induced by infections and industrial solvents. However, in the majority of aplasticanemia cases, no etiologic factor may be apparent and therefore the term of "idiopathic-type" has been adopted. In the U.S.A. and Europe, the idiopathic group may be 40 - 70 % of all of the aplastic anemia cases, whereas in Japan this is even higher: 90 % or more.[2] Our last 3 cases fall in the category of the idiopathic type since no definite cause could have been elicited. We believe that in due time the researchers will uncover the fact that the idopathic aplastic anemia cases are rather heterogeneous in etiology and pathogenesis. This may explain the discrepancies of therapeutic results with the same agents obtained in the treatments of these cases.

Our case 1 developed aplastic anemia following direct skin contact with benzene for a period of 3 years. However, the manifestations of aplastic anemia did not become obvious until 2 years after this exposure had been discontinued. This type of delayed onset is apparently not unusual for potentially toxic agents like benzene.[3] It is also of interest that the initial diagnosis of multiple myeloma in this case was made on the basis of increased plasma cells of the "myeloma type" which tended to form clusters in the bone marrow. This finding might also explain the presence of A.N.A., the positive indirect Coombs antibodies and the splenomegaly. It may be argued that the secret of this patient's successful long-term remission is due to the self-limited nature of benzene of the hemolytic process (hemoglobinuria, elevated retic count, etc.) with the intermittent lowering of hematological values would point to the continued activity of the disease process even after 23 years of exposure-free time. It seems as if once the initial destructive process triggers the immunological disease state, it becomes self-perpetuating. During the time of intercurrent infections, these events especially become more pronounced, perhaps suggesting a presence of antigenic stimulus from infectious agents.

The therapy outlined in this paper has been intended to accomplish:

a. To stimulate the residual stem cell population.
b. To suppress the auto-immune reactions directed against the bone marrow cells.

c. To provide an adequate amount of hematopoetic cofactors, such as vitamin B12, folic acid, liver, etc.

The therapy, with the agents like antithymocyte globulin may simply be working through immunosuppression.[4, 5]

Finally, this therapy is cost effective. The cost of a bone marrow transplant ($90,000 - $100,000) and antithymocyte globulin therapy ($15,000 - $20,000)[5] should also be considered in choosing therapeutic modalities.

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Microsurgical Re-Anastomosis of the Previously Ligated Fallopian Tubes*

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Summary

The author's experience with tubal reanastomosis by microsurgery is presented. Microanastomosis was performed in 35 patients and overall pregnancy rate was found to be 74.2 percent with an ectopic pregnancy rate of 8.5 percent. The best success rate was obtained with isthmic-isthmic anastomosis. Isthmic-ampullary re-anastomosis group included the 3 cases with tubal pregnancy. Overall term delivery rate was 57.1 percent.

Key Words : Microsurgery, tubal re-anastomosis, tubal ligation.

Introduction

A small but significant number of women who had tubal ligation, requested restoration of fertility because of death, disaster, divorce or psychological reasons. Traditional surgical techniques in tubal restoration offer a 30 percent pregnancy rate at best, while microsurgery offers an improvement over this number due to the more accurate reapproximation of tubal lumina.[1-4]

Materials and Methods

From 1979 until mid-1984, tubal re-anastomosis for reversal of sterilization was performed by the author in 35 patients. The characteristics of the patients who underwent microsurgical tubal re-anastomosis are

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shown in Table I. 42 percent of our cases were 25 years old or less at the time of sterilization. 65 percent were 30 or younger when they had their tubes ligated. There was only one case who had no gravidity at the time of tubal ligation. She had a left nephrectomy and tubal ligation at the age of 18 for unknown medical reasons. She was found to be in perfect health and underwent surgery which resulted in term delivery.

TABLE I

PATIENT PROFILE

No. of patients	Sterilization Data				Interval to Reversal (yr)
	Age	Parity	Type	No.	
35	21 – 40	0 – 5	Pomeroy Cautery	20 15	
mean:	34.0	2.8			6 mont.-10 yr

16 out of the 35 patients were of low parity (2 or fewer children). The surgical technique utilized in tubal ligation was Pomeroy in 20 cases, 18 of whom underwent sterilization at the time of cesarean section (51 %). 5 of the cases requested reversal in 1 year or sooner following tubal ligation. The interval was longest in a 38-year-old woman who was unaware that her tubes had been ligated during a cesarean section 10 years ago. She applied to our clinic for secondary infertility upon the death of her 3 children in an accident. At the time, of laparoscopy which was a part of infertility evaluation, it was found out that she had a tubal ligation, and this was the cause of infertility. The only statement she remembered at the time of her last delivery was her obstetrician's advice against another pregnancy. She fortunately conceived and delivered a healthy baby boy following the tubal re-anastomosis. The reasons for requesting tubal reconstruction are shown in Table II.

TABLE II

REASON FOR REQUESTING REVERSAL OF STERILIZATION

Reason For Reversal	No.	%
Remarriage	24	68
Infant Death	7	20
Regret	2	6
Depression	1	3
Medical	1	3

At the first meeting with a couple, a thorough discussion included the pre-operative evaluation procedures, the nature and scope of the operative procedure, the pregnancy rate after surgery, the risks of the surgery and possible subsequent ectopic pregnancy. None of the patients refused to try tubal reconstruction in this series. Each infertile couple was subjected to a complete infertility evaluation including laparoscopy performed by the author. The proximal segment of the ligated tube was evaluated by hysterosalphingogram.

Microanastomosis was done bilaterally in 55 percent of the cases. In the rest, one of the tubes was either absent or inoperable. The type of anastomosis in each case was variable (Table III).

TABLE III
SITE OF MICRO - REANASTOMOSIS

Type	No.
Isthmic - Ampullary	16
Isthmic - Isthmic	12
Cornual - Tubal	3
Ampullary - Ampullary	2
Ampullary - Infundibular	1
Ist - Amp. and Amp-Amp. (Combined)	1
Total	35

The microsurgical technique was utilized as published previously.[5, 6] For magnification, a Zeiss Opmi-6 type operating microscope with a motorized zoom system, with focusing operated by a foot panel, and with an objective lens of 300 mm focal length was used. Micro-bipolar and micro-unipolar cautery units were used for meticulous hemostasis. In only one case, a magnifying loupe was chosen for magnification because of the unavailability of the operating microscope at the time of surgery. The mesosalpinx was sutured with 6-0 or 7-0 vicryl or dexon suture material. End to end anastomosis was performed in two layers with either 9-0 or 10-0 prolene or nylon (Ethilone).

The average duration of surgery for a bilateral microanastomosis was 3 hours and the average hospital stay was 6 days. All of the cases were under prophylactic antibiotic suppression starting one hour prior to surgery until the second post operative day.

The first hydrotubation was performed while the patient was in the hospital on the 4th or 5th post-operative day. Hydrotubation fluid simply included 20-30 ml. of saline, 1 gm aqueous Penicillin G and 100 mg Hydrocortisone. The second hydrotubation was performed after the

first post-operative menses. During the last 2 years of the study, no hydrotubation was performed after surgery. The patients were allowed to attempt conception in the first post-operative cycle. In the case of no conception in 6 months, a hysterosalphingogram was suggested to document tubal patency. The patients were offered laparoscopy to evaluate the pelvis after 12-18 months following surgery.

Results

12 patients underwent isthmic-isthmic anastomosis and 10 of them conceived. In one of the cases magnification was obtained with the magnifying loupe (X 4.5) since the operating microscope was not available at the time (Table IV, V). She conceived shortly after surgery and uneventfully delivered a healthy baby. 7 had bilateral and 3 had unilateral reanastomosis. One of the patients showed reobstruction distal to the reanastomosis site in hysterosalphingogram. One aborted and one was lost to follow up. One of the patients, a 38-year-old woman, who conceived shortly after reanastomosis and delivered a healthy baby boy, had the interesting history of having had a tubal ligation without her consent. Among 16 patients who had isthmic-ampullary anastomosis, 2 were lost to follow-up. Another 2 showed reobstruction of the anastomosed tubes in the postoperative hysterosalphingograms. Tubal patency was detected in 2 cases who failed to conceive following surgery. Of the remaining, 11 patients conceived; 8 in the uterus and 3 in the fallopian tubes.

TABLE IV
SITE OF ANASTOMOSIS AND PREGNANCY OUTCOME

Anastomosis (interval surg.-preg. in months)		Total No. of Preg.	Term Preg	Ectopic Preg.	Spont. Abort.	Total No. of Patients
Ist-Ist.	No :	10	9	–	1	12
(8.3)	% :	83.3	75.0	–	8.3	
Ist-Amp. (7.7-term)	No :	11	8	3	–	16
(2.1-ectop)	% :	68.7	50.0	18.7	–	
Corn-Ist.	No :	2	1	–	–	3
(7.5)	% :	66.6	33.3	–	–	3
Amp-Amp.	No :	1	1	–	–	2
(8.5)	% :	50.0	50.0	–	–	
Amp-Inf.	No :	1	1	–	–	1
(14.0)	% :	100	100	–	–	
Ist-Amp. and Amp-Amp. (4.0)	No :	1	–	–	1	1
	% :	100	–	–	100	

TABLE V
OVERALL PREGNANCY OUTCOME (N: 35)

	No.	%
Total Pregnancy	26	74.2
Term Delivery	20	57.1
Ectopic Pregnancy	3	8.5
Spontaneous Abortion	2	5.7
Therapeutic Abortion	1	2.8

Cornual-tubal reanastomosis was performed in 3 cases. One of them became pregnant shortly after microsurgery. Most unfortunately, the pregnancy was terminated voluntarily following a divorce. One delivered a healthy term baby by cesarean section. The third case (age 40) revealed tubal patency by hysterosalphingogram and normal pelvic findings by laparoscopy, a year after reanastomosis. Corpus luteum deficiency was diagnosed and treated with Clomiphene Citrate postoperatively. She has not become pregnant to date. We performed ampullary-ampullary anastomosis in 2 patients. One become pregnant without delay after surgery and delivered a healthy baby boy following an uneventful pregnancy. The other one revealed tubal patency in the sixth post-operative month but was divorced and was lost to follow up. A term pregnancy was obtained following ampullary-infundibular anastomosis in one patient. The last case underwent right isthmic-ampullary and left ampullary-ampullary anastomosis. She failed to conceive although the tubes were observed to be patent in the post-operative hysterosalphingogram. The shortest length of the proximal segment was 0.5 cm and the longest was 3 cms. The length of the distal tubal segment varied from 1 cm to 8 cms. No significant correlation was established between the tubal length and the success rates. The length of the shortest tube was 4 cms in the study group presented. The interval between microsurgery and sterilization did not correlate with the results either. Reanastomosis-conception interval appeared to be related inversely with the length of the tubes after reanastomosis.

In intrauterine pregnancies, the average interval between microsurgery and pregnancy was 8 months whereas in tubal pregnancies, this interval was only 2 months. The longest interval between surgery and pregnancy, i.e. 14 months, was with the patient who had ampullary infundibular microanastomosis.

Discussion

Approximately 1 percent of the patients who underwent tubal sterilization requested a reversal.[7] Ages of the patients in this study group

ranged from 18 to 40 years and the time interval between the sterilization and the request for reversal varied between a few months to 10 years. 14 percent of the patients in this study requested reversal in 1 year or less following tubal ligation.

The principal reason for reversal of sterilization was a change in marital status (68 %). This is not surprising, considering that 42 percent of our cases were at the age of 25 or less (65 % were 30 or younger) at the time of sterilization. It is also significant that 7 out of 35 (20 %) had experienced the loss of a child after sterilization. The most tragic example of this was the death of all 3 children in a traffic accident in one of the cases. 8.5 % desired more children, expressing regret after tubal ligation. 16 out of 35 (45 %) were of low parity (2 or fewer children). A patient whose request for reversal was refused, therefore not included in this study, gave the most interesting reason for her regret. She was blaming the sterilization as the cause of her tinnitus which apparently started at the same time with the procedure. Depression was another reason for reversal in one of the cases.

These findings identify a group of women who are most likely to request sterilization reversal later. Women in this high risk group are young (less than 25 years old), of low parity (2 or fewer children) and those in unstable marital relationships.[8] Great caution must be exercised in recommending puerperal sterilization to young women. In 51 percent of our cases, tubal ligation was performed at the time of cesarean section. Except in cases with specific medical indications, it appears best to delay sterilization procedures for 8 to 12 months after the last delivery.

Despite the recent advances in tubal surgery and perfection of the technique, microsurgery cannot as yet restore vital physiologic tissue that has been irreparably damaged. Therefore, the surgical technique used for sterilization is of vital importance if one expects to obtain good results with tubal microanastomosis. In the present study, term pregnancy occurred in 13 out of 20 (65 %) and in 7 out of 15 (46 %) cases who were sterilized by Pomeroy and laparoscopic cautery methods, respectively. All of the 3 patients with ectopic pregnancies had had cauterization as the tubal ligation technique. Term pregnancy rates vary between 38 to 62 percent after Pomeroy and 34 to 68 percent after electrocoagulation methods in the literature.[3, 9-11] Clip and band were reported to have a reversal rate of 87 percent and 77 percent, respectively.[3 12, 13] (Table VI). It appears that a Pomeroy type tubal ligation or laparoscopic fallopian ring or clip application should be preferred to more destructive types of surgery like fimbriectomy, salphingectomy or laparos-

copic coagulation. The use of microsurgical technique in uterine tube reconstruction significantly improves pregnancy rate. It may not be the use of the microscope per se which is of value, but the exacting technique, careful attention to hemostasis, delicate handling of tissue, avoidance of tissue dessication and accurate suture placement for precise alignment of the lumina which allow for positive results.

TABLE VI

VARIOUS METHODS OF STERILIZATION AND TERM PREGNANCIES AFTER MICRO-REANASTOMOSIS

Author	No. of Patients (No.) and Intra-uterine Pregnancies (PR)							
	Pomeroy		Coagulation		Ring		Clip	
	No.	PR	No.	PR	No.	PR	No.	PR
Henderson[9]	47	27	33	14	6	5	–	–
Schlosser[11]	58	22	52	18	–	–	6	3
Seiler[10]	40	25	22	15	6	3	–	–
Rock[3]	31	17	48	17	22	17	1	1
Hulka[12]	–	–	–	–	–	–	85	74
Total	176	91	155	64	34	25	92	78
Percentage	51.7 %		41.2 %		73.5 %		84.7 %	

The isthmic-isthmic anastomosis was the most successful method for subsequent term pregnancy rates (75 %). In addition, isthmic-ampullary reanastomosis offered lower intrauterine and high ectopic pregnancy rates. Henderson suggests that the length of the longest tube remaining in centimeters multiplied by a factor of 10 gives a very close approximation of the term delivery rate (e.g., length of longest tube equals 5 cm, term delivery rate equals 50 %, actual observed rate equals 47 %).[9]

Collecting a series of 25 or more cases of sterilization reversal procedures from the literature since 1980, and analyzing 897 operations with regard to pregnancy outcome, we obtained intrauterine pregnancy rates ranging from 50 to 80 percent.[1-4, 9, 11, 13-17] This is significantly better than the 25 percent which was reported in a collected series of 201 cases who were operated by conventional surgical techniques reviewed by Siegler and Perez[18] (Table VII).

The ectopic pregnancy rate after tubal surgery is significant. In the present report, isthmic-ampullary anastomosis seemed to carry the highest risk for the development of tubal pregnancy. The interval between microsurgery and pregnancy was only 2 months in patients who ended up having ectopic pregnancy. This was 6 months shorter than the time period among patients who conceived and had a normal pregnancy

In summary, despite high success rates with microsurgical reanastomosis, counseling procedures should never suggest tubal sterilization as a reversible procedure.

TABLE VII

PREGNANCIES FOLLOWING REVERSAL OF STERILIZATION

Author	No. of patients	Pregnancy		Intrauterine		Tubal	
		No.	%	No.	%	No.	%
Winston[2]	126	76	60.4	73	57.9	3	2.4
Gomel[4]	118	97	82.5	96.	81.3	2	1.7
Rock[3]	125	81	65.0	76	60.8	5	4.0
De Cherney[1]	124	92	74.2	84	67.7	8	6.5
Schlosser[11]	119	72	60.5	55	46.2	3	2.5
Henderson[9]	102	69	68.0	64	62.7	5	5.0
Silber[14]	25	15	60.0	14	56.0	1	4.0
Grunert[15]	40	24	59.0	23	57.0	1	2.5
Diamond[16]	46	25	54.0	23	50.0	2	4.0
Gantt[13]	40	16	40.0	10	25.0	6	15.0
Wittich[17]	32	13	39.7	12	57.5	1	3.2
Total	897	580	64.6	530	59.0	37	4.1

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Percutaneous Balloon Valvuloplasty For Pulmonic Valve Stenosis

Ali Oto, M.D.* / Erdem Oram, M.D. / Aysel Oram, M.D.* / Şevket Uğurlu, M.D.****

Summary

Although the management of congenital pulmonary stenosis by surgery is very effective, transluminal balloon valvuloplasty has been suggested as an alternative for surgery in recent few years.

In this report, we present a patient with severe congenital pulmonic valvular stenosis which is relieved by a newly developed triple balloon as a first performance in our country.

Key Words: Pulmonary stenosis, balloon valvuloplasty.

Introduction

The management of pulmonic valve stenosis by surgery is very effective. On the other hand transluminal balloon valvuloplasty has been increasingly used for the treatment of this disorder with a promising follow-up evaluation.[1-3] However, transitory occlusion of the stenosed valve by the inflated balloon that may lead to systemic circulatory collapse has been the major problem related to the procedure.[4] To avoid complete interruption of blood flow during balloon dilatation a new balloon has been developed.[5] It consists of three identical 3 cm long angioplasty balloons mounted in a parallel fashion on a single catheter, filled simultanously, allowing for continued blood flow (Figure 1).

In this report we present a patient with severe pulmonary valvular stenosis who was treated successfully by a trefoil dilatation balloon as a first performance in our country.

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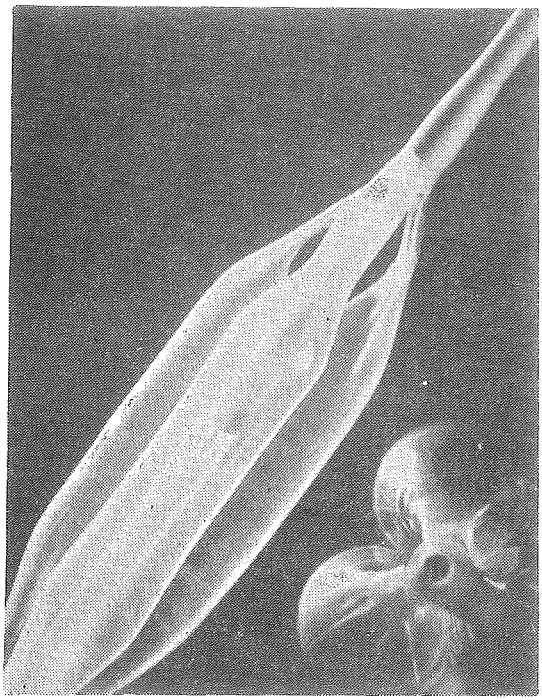


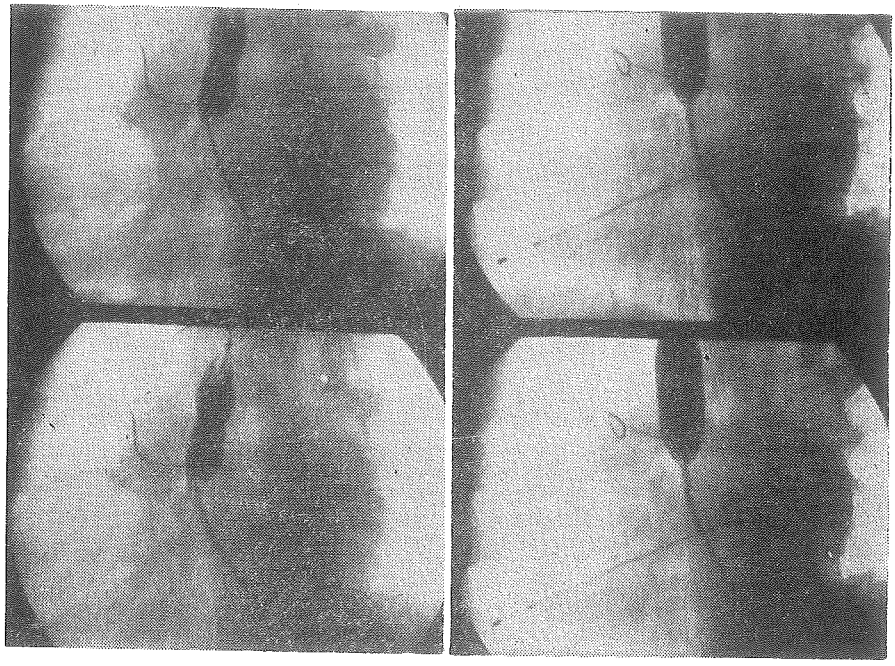
Figure 1
Trefoil balloon catheter for valvuloplasty.

Case Report

A-32-year old man admitted with a history of fatigue and exertional dyspnea limiting his daily activities. On examination blood pressure was 135/70 mm Hg and pulse rate was 80 beats/min and regular. He had a prominent left parasternal lift and systolic thrill along the left sternal border. A grade 4/6 systolic ejection murmur and widely splitted S_2 were also detected. The rest of physical examination was unremarkable.

An ECG showed sinus rhythm with a mean QRS axis of +110°. The chest X-ray was unremarkable beside a prominent main pulmonary artery. Cross-sectional and Doppler echocardiographic examination indicated a normal left ventricular function, normal aortic, mitral and triscuspid valves, but a stenotic non-calcified pulmonic valve with a peak systolic gradient of 70 mm Hg. A right heart catheterization with right ventricular cineangiography and oxygen saturation studies were performed and revealed a pure valvular pulmonary stenosis with a peak systolic gradient of 63 mm Hg.

The Procedure of Balloon Dilatation: The patient was premedicated with 50 mg meperidine IM given 30 minutes before the procedure. A



a Figure 2 b

a) Trefoil balloon inflated at the pulmonary stenotic area. Note the hourglass indentation. (b) Trefoil balloon at the third inflation. Note the disappearance of the indentation.

5-F catheter was introduced from the left groin for systemic pressure monitoring. Then, a 8-F Cournand catheter was positioned in the main pulmonary artery through a 9-F sheat from the right groin and replaced by a 0.035 inch guide wire which was stabilized in one of the left lobar pulmonary arteries. A 3x9 mm Trefoil Meier balloon catheter was advanced over this flexible tipped guide wire until the middle of the deflated balloon was positioned fluoroscopically accross the stenosed pulmonic valve (Figure 2). Additionally a 7-F Zucker electrode catheter was placed in the right ventricle to monitor the right ventricular pressure and to serve a potential side for pacing. After placement of the catheters the patient was given 100 U/Kg heparin IV. The balloon was then inflated with 50 % Ultravist^{R} solution three times, to the pressures 2,4, and 5 bar lasting 10,20 and 30 seconds respectively under continues fluoroscopic, hemodynamic and electrocardiographic control. The hour-glass appearence of the balloon disappeared and the right ventricular systolic pressure reduced to 40 mm Hg immediately after the third inflation. Even during maximal inflation there was only a slight decrease in systemic systolic pressure. No subjective symptoms was detected apart from

a slight retrosternal discomfort during the inflation of the balloon. Following the inflations a repeat right heart catheterization as well as a right ventriculogram were performed which revealed a peak systolic gradient of 23 mm Hg on the pulmonic valve.

Postprocedure physical examination showed no left prasternal thrill; a grade I-II/VI systolic ejection murmur on the left second intercostal space with a prominent systolic ejection click and splitted S_2 was detected. There was no disatolic murmur. Bouts of supraventricular tachycardia of short duration were observed during the procedure. A sinus tachycardia continued 12 hours following the dilatation which resolved without medication. A transient right bundle branch block was also recorded.

Discussion

Balloon dilatation has been used successfully to treat various forms of congenital heart diseases including pulmonary stenosis, coarctation of aorta, aortic stenosis.[1,3] Recently it has also been suggested as a promising method for acquired mitral and aortic stenosis.[6-8] Although a final consensus has not been reached, long-term results that have been reported recently by Kan, *et al*[9] and Kveselis, *et al*[3] have suggested the continued beneficial effect of balloon valvuloplasty in relieving the pulmonary valvular stenosis. Therefore balloon dilatation is now an accept-ed[1] and preferred method for the management of congenital pulmonary stenosis.

On the other hand the development of the trefoil balloons has opened a new era in this field.[4] The use of this type of triple lumen balloons lead to substantially less dramatic changes in systemic arterial pressures and cardiac output than single lumen balloons. The single lumen balloons often need to be inflated several times because maximal inflation cannot be maintained due to profound fall in systemic arterial pressure and severe bradycardia.

Using the triple lumen balloon we could not demonstrate a remarkable change of systemic pressure and we reached the maximum inflation without any untoward effect beside very short episodes of supraventricular tachycardia. As we demonstrated by a repeat cardiac catheterization we obtained a 40 mm Hg reduction of the peak systolic pulmonic valve gradient safely.

Therefore, we concluded that the triple lumen balloon valvuloplasty is an effective, safe and promising alternative for adult patients with congenital pulmonary stenosis. However the long-term results are to be determined by large series.

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Conservative Surgical Management of Torsion of an Ovarian Hyperstimulation Cyst

Kutay Biberoğlu, M.D.*

Summary

Ovarian hyperstimulation syndrome (OHSS) is well recognized clinically, in association with induction of ovulation. In the majority of cases, no active treatment is required and reassurance is all that is needed.

If surgery is needed in cases of rupture or torsion of an ovary, promptness in diagnosis and conservatism should be the milestones of management. An unusual case of unwinding of torsion of a cystic ovary by OHSS is presented.

Key Words: Ovarian hyperstimulation syndrome, Torsion of adnexa, Early unwinding.

Introduction

Ovarian hyperstimulation syndrome (OHSS) characterized by massive enlarged ovaries, may follow ovulation induction with clomiphene citrate, human menopausal and chorionic gonadotropins. Even the severe hyperstimulation cases with ascites, pleural effusion, hypovolemia, oliguria, and electrolyte disturbances will undergo gradual resolution in one or two weeks. In the majority of cases, no medication is required and reassurance is all that is needed.

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The rare occurrence of ovarian rupture or torsion must be considered in OHSS. In the case of emergency surgery, the ovaries are so cystic, enlarged, and brittle that wedge resection and repair are impossible, and an oophorectomy may be unavoidable in order to achieve adequate hemostasis.

The classical management of a strangulated and infarcted ovary is the removal of the ovary without untwisting the pedicle in order to avoid the possible release of thrombotic emboli.

Case Report

Y.G. (record : 793042), a 26-year-old, married and infertile woman was admitted to the Department of Obstetrics and Gynecology of the Hacettepe University hospital, on November 4, 1984, suffering from ovarian hyperstimulation. Gynecologic history revealed no pregnancy in the past and abnormal menstrual cycles occurring every 15 to 60 days. For over 4 years, she had unsuccessfully tried to conceive and was then evaluated for primary infertility and progressing hirsutism. She had undergone diagnostic laparoscopy in 1983, at Zekai Tahir Burak Maternity hospital and on ovarian wedge resection was recommended with the diagnosis of "polycystic ovarian disease".

Refusing surgical treatment, she was then given numerous medications to induce ovulation including human menopausal and chorionic gonadotropins without success, elsewhere.

Basic infertility studies at our infertility-reproductive endocrinology unit, revealed normal semen analyses and a normal hysterosalpingogram. Hormonal workup was compatible with anovulation and ovarian hyperandrogenism. Dexamethasone, 0.5 mg qs was started with a consequent drop in serum testosterone levels from 0.95 ng/ml to 0.60 ng/ml in one month, followed by improvement in the menstrual cycle pattern. When she failed to respond to dexamethasone alone and to the combination of dexamethasone and clomiphene (Dyneric Merrell, West Germany) using up to 200 mg/day for 5 days, for 6 months, human menopausal gonadotropin (Humegon-Organon, Holland) was administered (individualized regimen). Ultrasonographic serial monitoring of the ovarian follicles wa performed. In the first cycle of the triple drug regimen, 2 dominant follicles were matured and corpus luteum was demonstrated ultrasonographically. A resultant elevation of basal body temperature followed an injection of human chorionic gonadotropin of 10,000 units (Pregnyl-Organon, Holland).

Ten days later, on November 4, 1984, the patient was re-admitted, complaining of lower abdominal distention and pain accompanied by nausea and vomiting. On admission, the blood pressure was 100/70, and the pulse was 100/minute. The abdomen was tender but had no signs of rebound. Ultrasonic examination showed bilateral cystic ovaries, each measuring 8.0 cm in diameter, with no evidence of ascites. Serial blood hematocrit and electrolytes remained normal. On the second day, the patient had severe lower abdominal pain with signs of acute abdomen, but no evidence of hemoperitoneum. An emergency laparotomy was performed with the diagnosis of ovarian torsion (Figure 1). Torsion of the right ovary involving the infundibulopelvic ligament with no evidence of ischemia was observed. The ovary was carefully unwound and returned to its normal anatomic position. Postoperatively, the patient made an uneventful recovery. Hyperstimulation resolved spontaneously with the onset of menstruation, a week later.

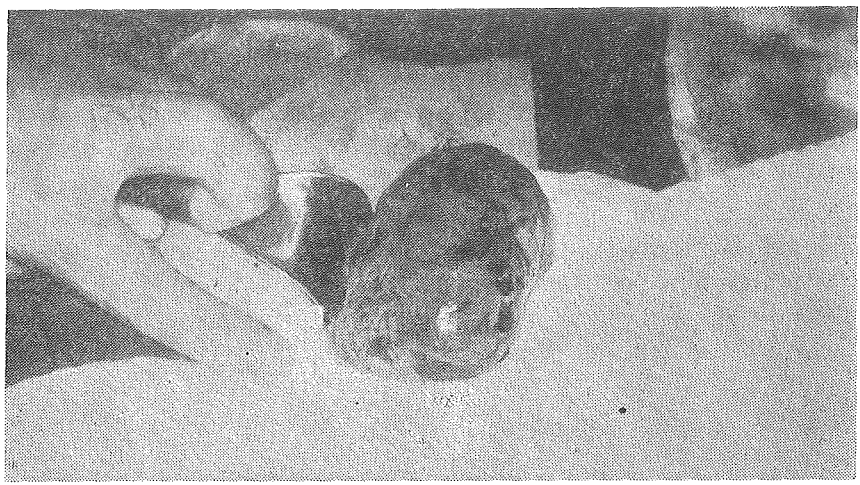

Figure 1

She reapplied to the clinic for the ongoing problem of infertility. This time,she had chosen an ovarian wedge resection an as alternative to the medical treatment because of fear of reoccurrence of hyperstimulation syndrome. On January 21, 1986, she underwent microsurgical wedge resection of the ovaries. During the following six months, no improvement was observed and anovulation persisted. The hormonal profile was unchanged. Clomiphene citrate was restarted and eventually she conceived in the second treatment cycle with the dose of 150 mg/day for 5 days (between 3 rd and 8 th days of the cycle), on September 14, 1985. A healthy baby girl weighing 3180 gm was delivered by cesarean section on June 9, 1985.

Discussion

The introduction of gonadotropins and clomiphene citrate into medical practice brought hope and realization of motherhood to women who were previously barren because of ovulatory dysfunction. But it also posed new problems and complications of treatment which were previously nonexistent. Experienced gynecologists may practice a lifetime without encountering a single case of OHSS if they do not get involved in induction of ovulation. Spontaneous occurrence of OHSS has not been reported. The hyperstimulation syndrome is an iatrogenic condition seen in conjunction with clomiphene citrate and gonadotropin therapy. Therefore, physicians managing ovulation defects should familiarize themselves with OHSS and be prepared to manage it.[1]

Although hyperstimulation can occur following any stimulation, it is usually associated with stimulations involving higher estrogen concentrations and multiple follicular development. In monitoring the treatment cycles, ultrasonic follow-up is preferred to achieve higher pregnancy rates, but serum estradiol appears to be superior to ultrasound as a predictor of OHSS.[2] Torsion of the ovary in OHSS is rare;[3] but it is a most serious complication, usually causing loss of an adnexa. The more severe the torsion, the more likely is the development of tissue necrosis. Early abdominal surgery may permit preservation of adnexa that might otherwise become gangrenous. Clinically, torsion is manifested by severe abdominal pain. General examination findings are usually normal with possible mild tachycardia or a slightly elevated temperature or both. Abdominal findings would be consistent with those of peritoneal irritation with or without slight rigidity. Diagnosis is especially difficult in OHSS because of abdominal distention and tenderness due to cyst formation in the ovaries.

In the above mentioned case, continuous hospitalization enabled prompt diagnosis and this promptness probably saved the ovary by letting us unwind torsion of the ovarian cyst instead of removing it.

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Meningioma Seeding

Nur Altınörs, M.D.* / Ayhan Türker, M.D.* / Nuri Arda, M.D.* / Engin Şenveli, M.D.* / Nusret Çınar, M.D. * / Işın Pak, M.D.**

Summary

The first definition of multiple meningiomas was made by Cushing and Eisenhardt.[1] Such a condition is one in which the patient harbors more than one meningioma but less than a diffusion of meningiomas. The latter condition is mostly seen in association with neurofibromatosis.

There is also a distinction between multiple meningiomas and recurrences.[2] On the basis of the above mentioned criteria, the incidence of multiple meningiomas is about 1-2 %.[3-9]

Remarkably higher figures have been reported by Horax (6.7 %)[10] and Lusins (8.9 %).[11] The incidence is even higher in Wood's[12] autopsy study (16 %).

In this paper a patient is presented who developed spinal meningioma eight years after the total removal of an occipital, intraventricular meningioma.

Key Words: Intraventricular meningioma, multiple meningioma, Spinal meningioma.

Case Report

A 39-year old male patient was hospitalized because of complete loss of movement in his lower limbs. Physical examination was normal. Neurologic examination revealed complete paraplegia, bilateral hypoesthesia up to the level of T 12, bilateral hyperactive patella and achille reflexes and bilateral Babinski and clonus.

A left occipital, intraventricular mass had been removed on October 11, 1977 in Bonn, Germany. The histopathologic diagnosis had been meningioma (Figure 1).

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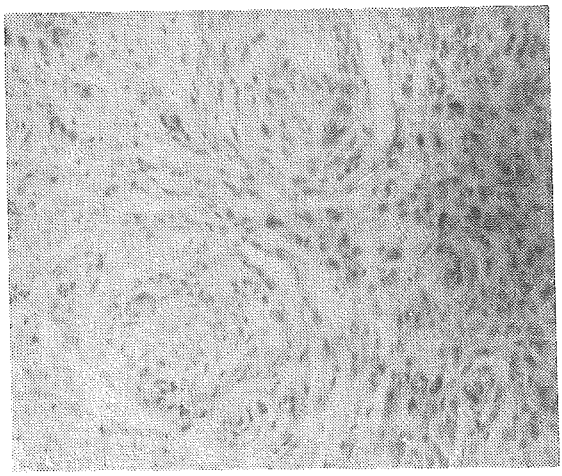

Figure 1

Meningothelial bands composed of cells similar to those lining the arachnoid are seen. These cells are moderately vesicular with oval and round nuclei. The mild eosinophilic cytoplasm have fine granules. H-E x 100.

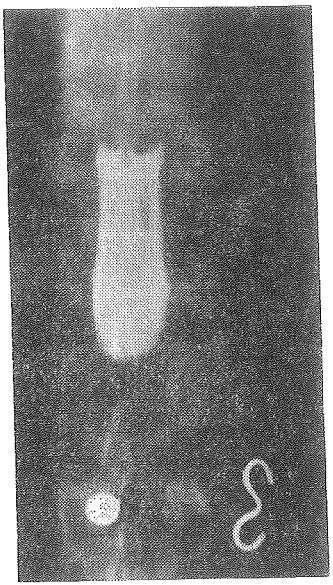

Figure 2

A myelographic complete block at the midst of T 11_1 vertebra.

Figure 3

Bundles and whirling shapes of cells lining the arachnoid along with meningothelial niches can be seen. In addition there are foam cells. H-E x 100.

A myelogram was performed and a complete block at T 11 (Figure 2) was seen. The patient was operated on August 19, 1985. Following T 10-11-12 total laminectomy, a left, laterally localized, intradural extramedullary mass, 1.5x1x2 cm. in size was totally removed. There was no definite attachment to the dura. The histopathologic diagnosis was meningioma (Figure 3). The postoperative period was uneventful. The patient improved rapidly and was discharged at the end of the second postoperative week. By that time he was able to walk. A control CT showed that there was no recurrence at the previous craniotomy site.

Discussion

There is not one single satisfactory explanation for the existence of multiple meningiomas. Among the hypotheses suggested for this phenemenon are: multifocal growth, CSF seeding, venous transmission, and hereditary factors.[11, 13]

The frequent invasion of dural sinuses by the meningiomas and the very low incidence of multiple meningiomas and extraneural meningiomas is a clear paradox. Hence the venous transmission mechanism becomes very questionable. Thus far the multifocal growth theory has gained more support than the others.[9, 14, 15]

We believe that this case is an example of the rare condition of meningioma seeding on the basis of the following analysis:

1. Histopathologic appearance: Both tumors were of meningothelial type. Although multiple meningiomas may be of the same veriety, there are reports where simultaneous intracranial[16] or spinal meningiomas 22 years after an intracranial one were of different histologic types.[15]

No dural elements were seen in microscopic evaluation of the spinal tumor suggesting primary growth.

2. Localization of the primary tumor: The intraventricular localization of the intracranial tumor makes it very easy for the tumor cells to break away and seed as a consequence of the surgical manipulation.

Schöpe[17] reported a patient with pterional meningioma in whom occipital meningioma and areas of meningioma in the pons, corpora quadrigemina and choroid plexus of the fourth ventricle, even in the absence of croniotomy, were found postmortem.

Kalm reported a patient harboring a malignant tentorial meningioma seeding itself in the subargchnoid space, again with no previous craniotomy.

There are other articles about malignant transformation and seeding by means of CSF.[19, 20]

Winkelman's case is another example of postoperative subarachnoid space and ventricular seeding. Neither the primary sphenoid ridge meningioma nor the implants showed malignant features.[21]

3. Time interval and localization: The development of most of the multiple meningiomas, even in different neuro-axial compartments, is either simultaneous or shortly after the first tumor.[5, 16, 22, 23] In our case, the time interval is eight years.

Another tendency of multiple meningiomas is to be intracranial and many of them are in the same hemicranium.[24]

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News

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Correction

In the article titled "High Dose Gamma Globulin in Childhood Acute and Chronic Immune Thrombocytopenic Purpura (ITP)" by Sümer, T., Abumelha, A. and Al-Muhim, I., which was published in the January 1987 issue (Hacettepe Med. J. 1987; 20: 11-16), the dose of gamma globulin in the Summary and in the Material and Methods is incorrectly printed as "400 mg/m²/day". The correct dose is 400 mg/kg/day".

We apologize for this error.

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Ultrastructure of Gastric Parietal Cells in the Mouse During Development

Esin Aşan, Dt.Ph.D.* / Aysel Şeftalioğlu, D.V.M.Ph.D.**

Summary

Development of gastric parietal cells in the 16,19 and 20-21 day old mouse embryos and new born mice were studied at the ultrastructural level. The parietal cells were first encountered in 19 day old embryos. Some primitive parietal cells were also observed during this period. On the course of development there was a marked enlargement of the intracellular canaliculi, which are necessary for HCl secretion. An increase in the number of mitochondria and a gradual reduction in the amount of free ribosomes were also observed. Multivesicular bodies, lysosomes and some unusual dense membranous structures appeared concomitant with the development of the intracellular canaliculi. These observations indicate that parietal cells actively participate in membrane synthesis and recycling during development.

Key Words: Gastric parietal cells, ultrastructure development.

Introduction

Cytological changes which take place during development give a great deal of information about the functional maturity of various cell types. The development of gastric parietal cells have been studied in some species previously.[1-6] But there are still a number of controversial issues regarding the ultrastructure of these cells during development.[1-6] In the present study the development of gastric parietal cells in 16,19 and 20-21 day old mice embryos and new born mice were studied at the ultrastructural level and an attempt has been made to correlate the functional maturity with the morphological development.

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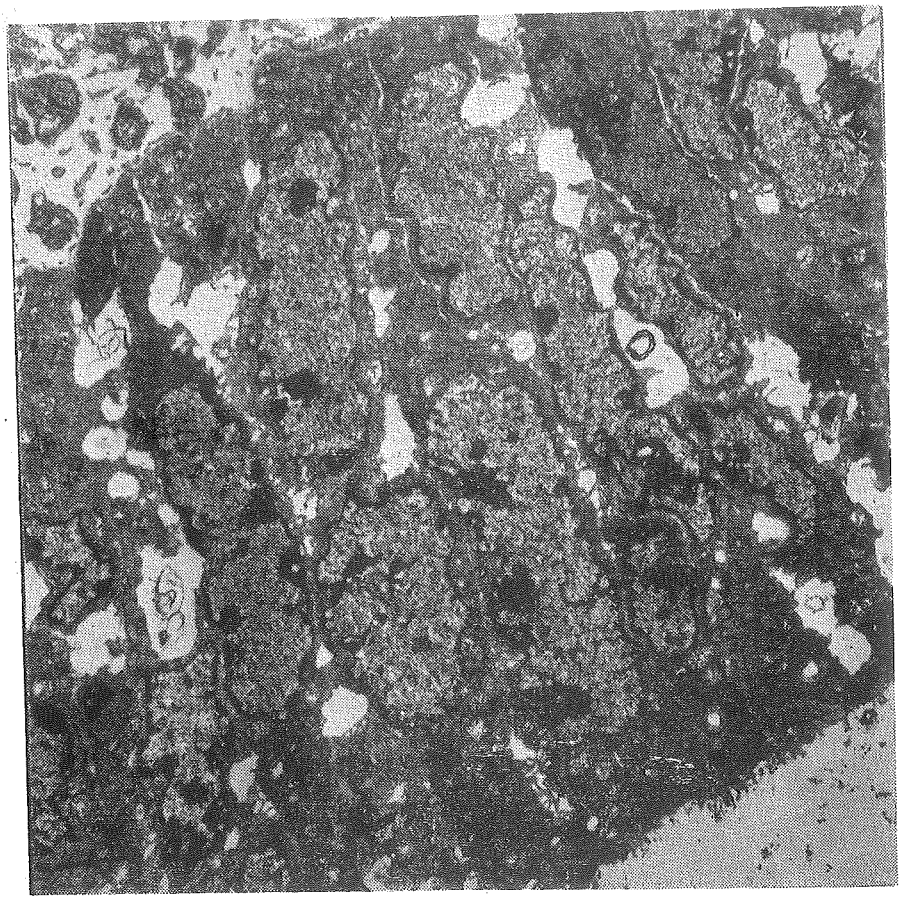

Figure 1
General appearance of gastric epithelium in 16 day old embryo. X 5700

Material And Methods

Female Swiss albino mice in estrus were mated overnight. The day following a successful mating, assessed by the presence of vaginal plug, was considered the first day of gestation. On the 16,19 and 20-21 day of gestation embryos were removed.

The embryonic and new born tissue samples were first fixed in 2.5% gluteraldehyde and then in 1 % osmium tetroxyde solutions. After dehydration in ethanol the tissue slices were embedded in araldite. Ultra thin sections were examined with a Zeiss EM 9S2 electrone microscope.

Results

On the embryological age of 16 days the gastric epitelium is generally stratified columnar. Cells are more or less same in ultrastructure.

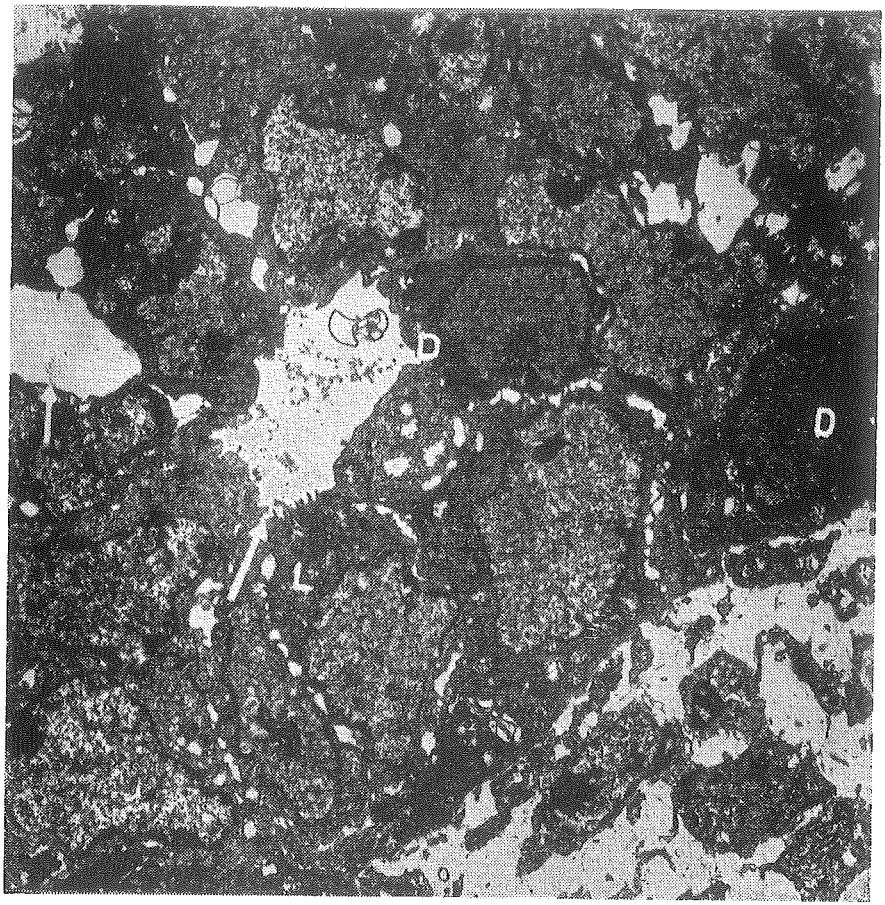

Figure 2
16 day embryo. Some gland lumina are evident (arrow). Dark(D) and light (L) cells are visible.

They are fairly undifferentiated epithelial cells. The large centrally located nucleus, occupies most of the cytoplasm. Ondulating nuclear envelope is well-developed and the nucleoli are prominent. These indifferentiated cells are not rich in organelles. Numerous free ribosomes are present.But there is only a very little rough endoplasmic reticulum. Large irregularly formed intercellular spaces separate the epithelial cells. Generally the epithelium is compact but in some cases small gland lumina, presumably originating from the intercellular clefts, are developed (Figures.1,2).

Some cells are very rich in ribosomes so they appear darker than the others. Light cells are usually rich in mitochondria and they also exhibit microvillous apical cell surface (Figure 2).

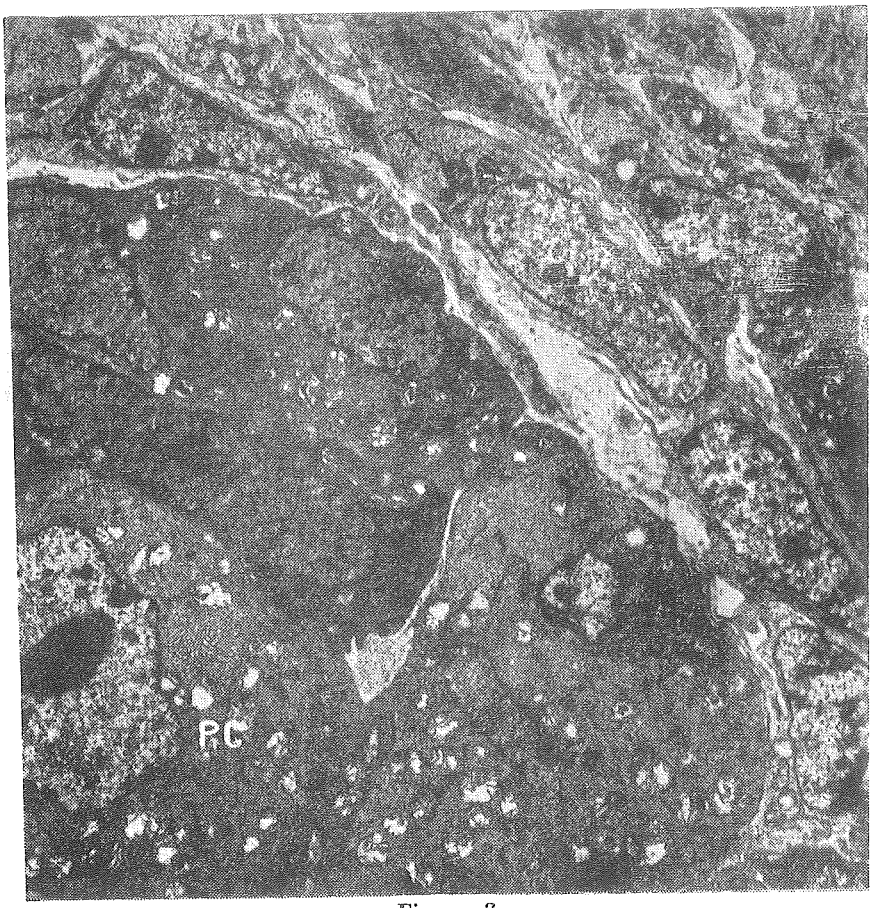

Figure 3
19 day old embryos, gland formation is appearent. Parietal cells (PC), are clearly visible.

19 days: On the 19th day, the appearance of gastric epithelium is greatly changed. Gland formation is now appearent. But these glands have not fully differentiated yet. The connective tissue elements circularly surround these primitive glands.

The cells of the gastric epithelium on the embryological age of 19 days is more mature than those in embryos on the 16th day. Parietal cells can be clearly identified for the first time, because of presence of intracellular canaliculi. These primitive parietal cells often occur in pairs, and they show a true parietal position in the glands (Figure 3). Generally the intracellular canaliculi have not fully developed yet. Numerous microvilli fill up these primitive canaliculi. The nucleus is large and often folded and occupies a large proportion of the cell cytoplasm. Mito-

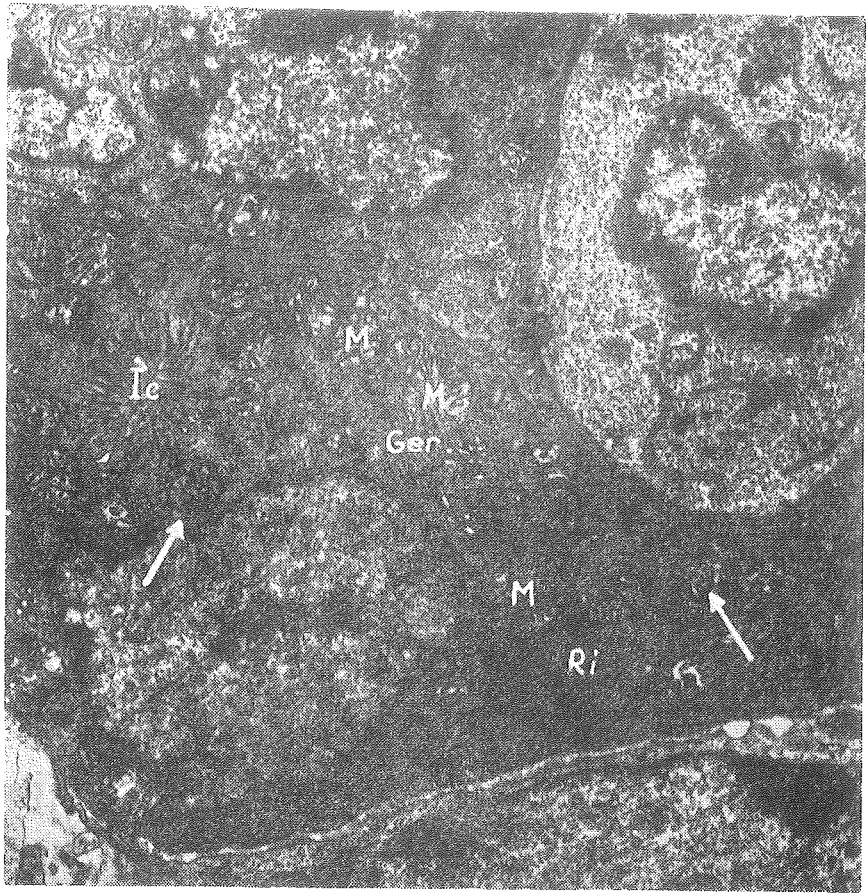

Figure 4

Parietal cell from 19 day old embryo. A small intracellular canaliculis (IC), many mitochondria (M), Rough surface endoplasmic reticulum (GER), and free ribosomes (Ri) are seen. Dense intramitochondrial granules. (arrows) are also present.

chondria are the most prominent and characteristic organelles at this stage of development. They are abundant, and are usually round or ovoid structures with a closely packed cristae, some of which have dense intramitochondrial granules (Figure 4). The rest of the cytoplasm is filled with ribosomes. Some rough surfaced endoplasmic reticulum tubuli are also seen between the abundant mitochondria.

Most the cells have a prominent golgi apparatus at this stage of development (Figures 4,5,6). Some small, dense, granule-like structures are observed while they are budding from the golgi membranes. Small coated vesicles are seen in the vicinity of the golgi complex (Figure 5). At higher magnifications microtubules and microfilaments are clearly seen in the cytoplasms of some cells (Figure 5).

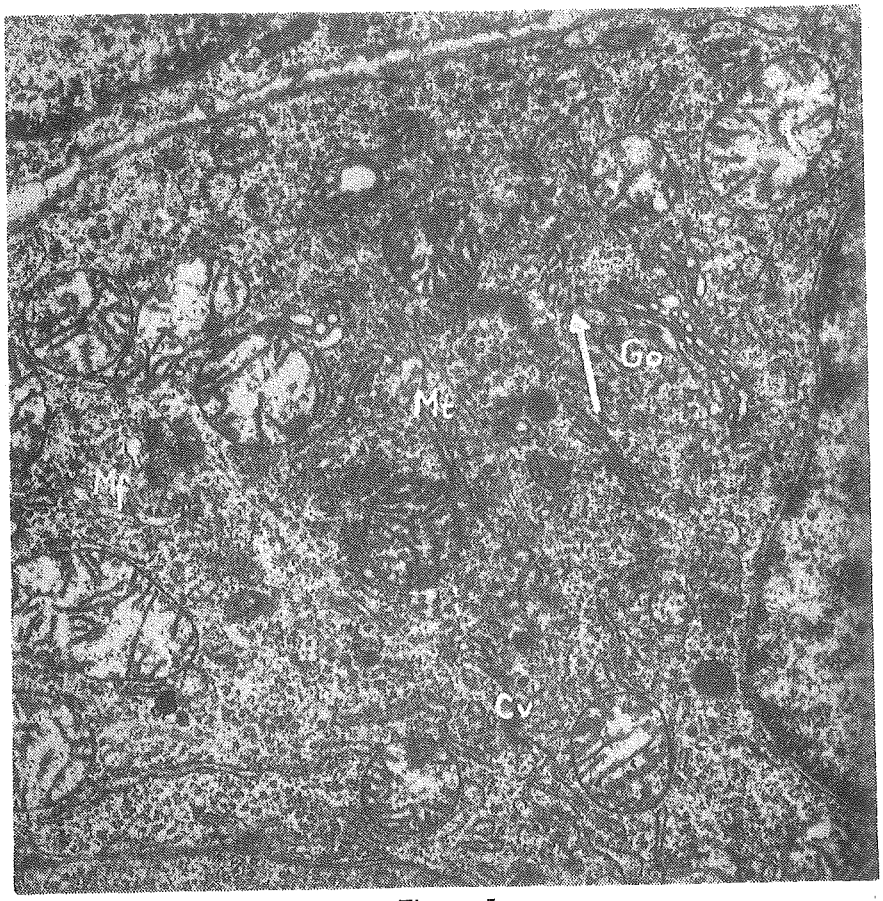

Figure 5

Fine structural details of a parietal cell from 19 day. Golgi Complex (Go), Small granule like bodies originated from the Golgi complex. (arrow) coated vesicles (Cv), Microtubules (Mt), and Microfilaments (Mf) are observed.

At the embryological age of 19 days, some mitochondria-rich cells are also observed. These cells have organelles typical to developing parietal cells at this stage, except that they don't have characteristic intracellular canaliculi. Cells of this type show irregularly arranged microvilli on their luminal surfaces. At the bases of the microvilli apical cell membrane shows some small invaginations.

20-21 days: Individual parietal cells appear to be nore mature than the previous stage. The intracellular canaliculus now penetrated more deeply into the cytoplasm. There are numerous, some times branching microvilli projecting into the lumen of the intracellular canaliculi. The number of mitochondria has increased considerably and their cristae

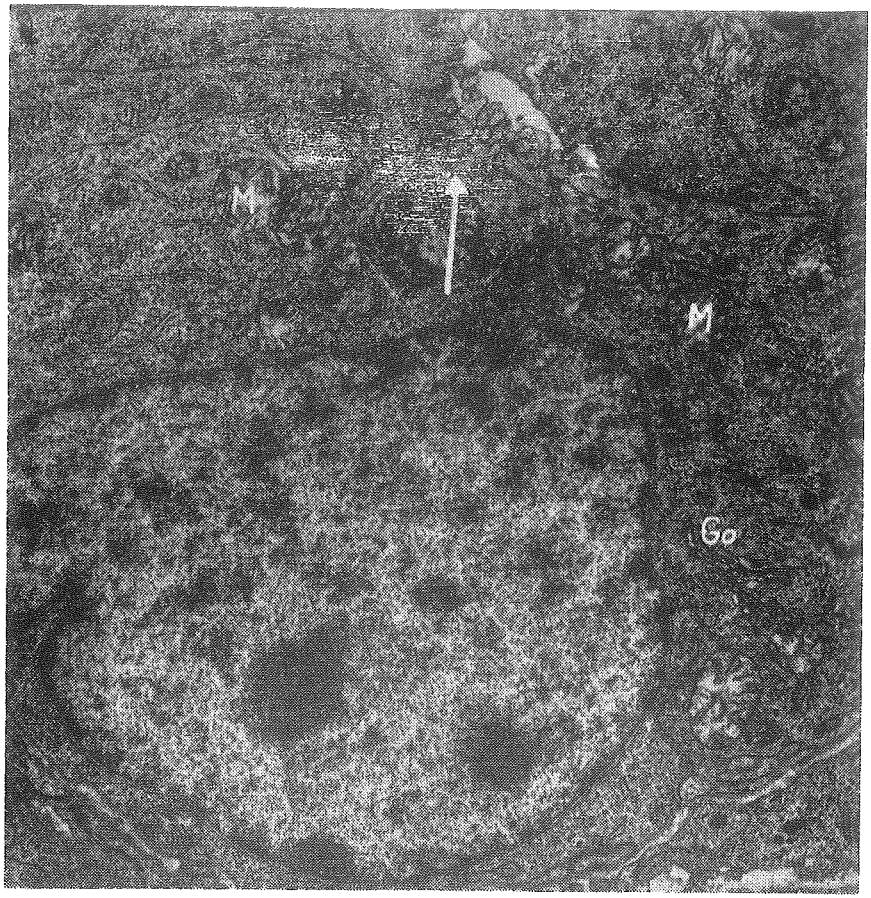

Figure 6

A primitive parietal cell from 19 days. Small irregular microvilli are seen on the apical surface (arrow). The Golgi Complex (Go), and the Mitochondria (M) are prominent.

are well developed and closely packed (Figure 7). There is a gradual decrease in the amount of both free and membrane bound ribosomes. Lipid droplets and some dense lysosomal structures are usually encountered in the cytoplasm. There are numerous membraneous structures scattered throughout the cytoplasm. But these structures are especially abundant in the vicinity of the intracellular canaliculi. Membranous structures are either circular or rod shaped and they seemed to arise by the fusion of the two contiguous plasma membranes. Golgi complexes are prominent in most of the cells. Some small dense granules and coated vesicles and a small number of lysosomes are also observed (Figure 8).

New born mice: In the new born mice parietal cells display one or several discrete intracellular canaliculi, bordered by numerous micro-

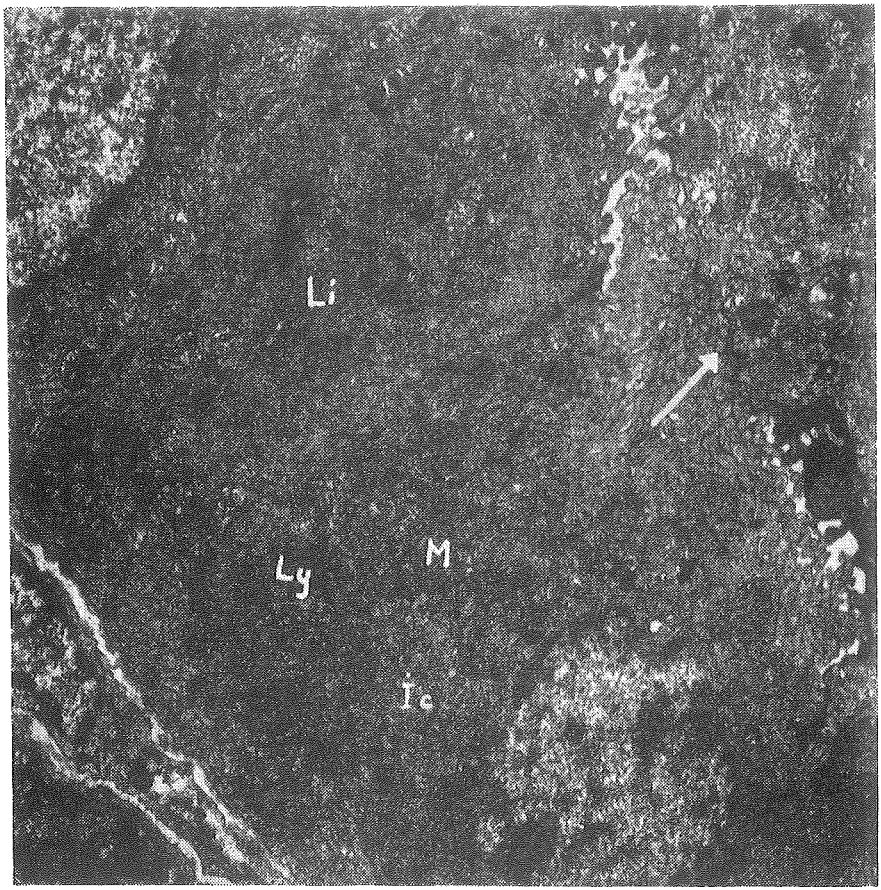

Figure 7

Electron micrograph of two parietal cells from 20-21 day old embryos. Intracellular canaliculus (Ic) and intercellular canaliculus (arrow) are prominent, many mitochondria (M), some lipid droplets (Li) and lysosomal bodies (Ly) are clearly seen.

villi. There is a substantial decrease in the amount of rough endoplasmic reticulum. But free ribosomes are still found. Mitochondria are larger and their cristae are nore closely packed than the previous stage (Figure 9). Lysosomes, multivesicular bodies and residual membraneous structures are usually encountered in the cytoplasm (Figure 9).

In some cells, the intracellular canaliculi with a very few microvilli are also seen. These small microvilli poor discrete canaliculi appeared to be the extensions of the main canaliculus. Cells of this type usually contain substantial quantity of membraneous structures. These structures are mostly in the form of vesicles. Secondary lysosomes and membraneous whorls and some membrane bound structures containing dense

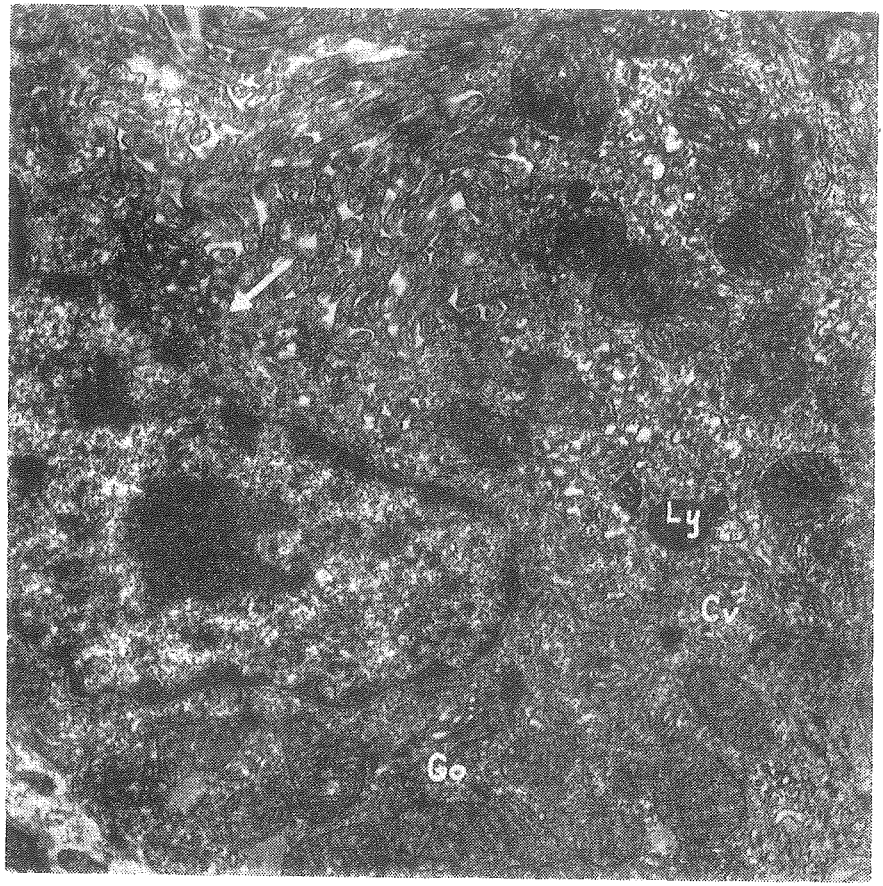

Figure 8

Electron micrograph of a parietal cell from 20-21 day old embryos. The cytoplasm contains substantial quantity of membraneous structures, which are especially abundant around the intracellular canaliculus (arrows), a small Golgi Complex (Go) some coated vesicles (Cv) and lysosoms are seen.

osmiophilic material are also very common. Structures of these type are preferentially located around the developing intracellular canaliculi (Figure 10).

Adult mice: The most prominent feature of adult parietal cells are the abundance of their mitochonrria. They are preferentially concentrated at the peripheral cytoplasm, some of which have dens intramitochondrial granules. Very few rough endoplasmic cisternae are observed but poly ribosomes are not sparse. Generally the intracellular canaliculi are well developed and bordered by numerous microvilli. Vesicular structures are the most characteristic feature of the adult cell. These structures are usually found on the inner (Nuclear) side of the intracellular

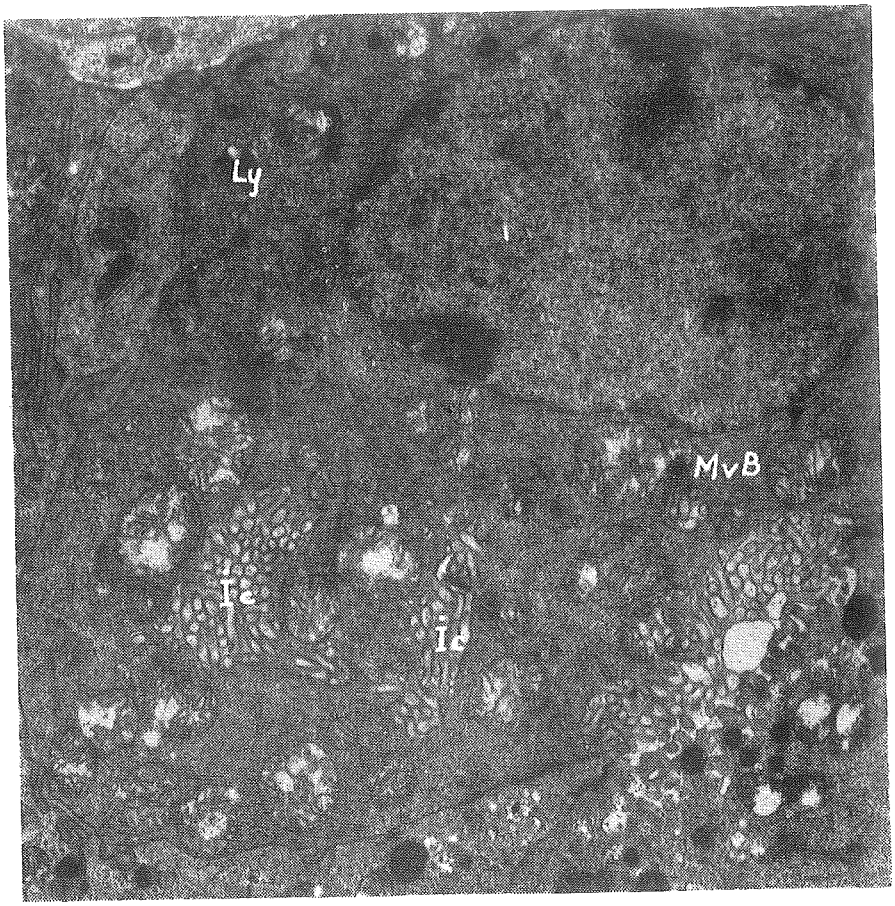

Figure 9

Electron micrograph of a parietal cell from a newborn mouse. The intracellular canaliculi (Ic) lysosomes (Ly), multivesicular bories (MvB) are seen.

canaliculi. The sizes and shapes of the vesicles vary considerably. Some are in the form of tubules, while some have short bulbous extensions at their one end. Multivesicular bodies are usually found in the vicinity of the vesicular structures.

Discussion

The results of the present investigation indicate that the parietal cells start to differentiate between 17-19 days of gestation. In the 16 day old embryos all gastric epithelial cells were poorly differentiated. There were still minor differences in the ultrastructure of these undifferantiated cells. Dark cells had more ribosomes than the light cells. Light cells were very rich in mitochondria, some of them had a microvillous apical

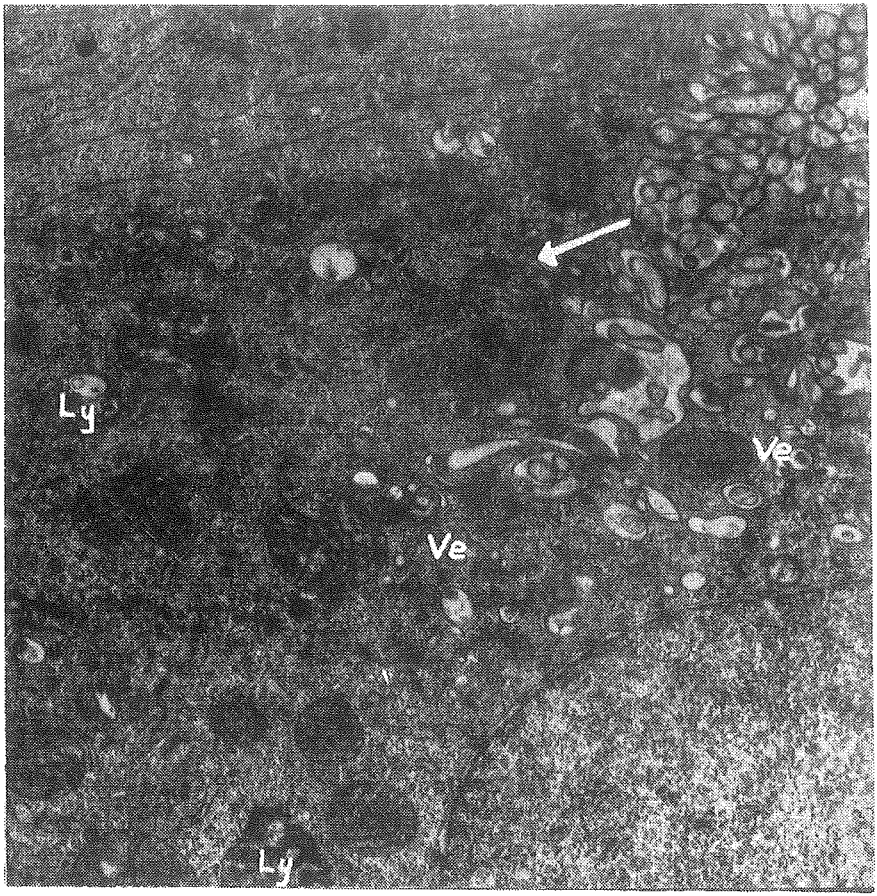

Figure 10

Cytoplasmic detail of a parietal cell from newborn mouse. Vesicles of varying size and density (Vc), lysosomes (Ly), membraneous structures (arrow) are seen.

surface. It was difficult to designate the light cells as the precursor of the presor of the parietal at this stage of development.

The earliest parietal cells was found at the 19th day of development. These cells appeared in pairs and were easily identified with their prominent and characteristic intracellular canaliculi. But these canaliculi were not well developed, only one or two small discrete canaliculi were seen in the cells. Common occurence of cells in pairs may indicate that they might actually the daughter cells of a single mitosis. These earliest parietal cells were identical to those described by Halender[1] in rat embryos on the 20th fetal day. Similar cells were also described in rat embryos on the 22nd, 23th days of gestation.

Some vacuolated primitive parietal cells have been described in rat embryos.[1] Menzies[15] also have reported preoxyntic cells in rabbit embryos. Such a cell could not be observed in our material at any stage of development. But we have also observed some immature parietal cells in 19 day old embryos together with rather well differentiated cells. Structurally these cells were quite different from those described before.[1, 5] They were very rich in mitochondria but lack of characteristic intracelluar canaliculi. The most characteristic feature of these cells were the microvillous apical surface. They were probably the first cells differentiating towards the parietal cells. Similar cells have also been reported in rabbit embryos in 23th day of gestation.[4]

The differentiation of parietal cells results in a decrease in the number of free ribosomes and increase in the amount of mitochondria. Both of these changes are concomitant with the development of intracellular canaliculi.[1-6]

In the present study positively identified parietal cells were found at 19 day old embryos. There was a prominent development in the intracellular canaliculi as the cells matured. The intracellular canaliculi have become more extensive and progressively lined by microvilli as the development proceeds. It has been felt that first a microvillous apical cell surface has been formed, then these surfaces evaginated into the cytoplasms to form a secretory or intracellular canaliculus. But the cellular details of this differentiation could not be observed.

It has been well known that in the adult parietal cell the degree of development of intracellular canaliculi is closely related to its HCl and probably intrinsic factor secretion.[8, 9] There is a complex smooth-surfaced intracytoplasmic membrane network termed tubulovesicular system in the parietal cells. When cells are actively secreting HCl tubulovesicles decrease in number while microvilli appear on their secretory surface. In the resting state however tubulovesicles increase in number as the microvilli on the secretory surface decrease. It has not been clearly shown at the moment that how these two different membrane systems interact each other during secretion.[8, 9]

There were no structures which could be defined as tubulovesicular membranes in our material at any stage of development. But in the 20-21 day old embryos and in the newborn, the parietal cells were very rich in membraneous structures which were quite different from the tubulovesicular membranes. These membraneous structures include irregular or rod shaped membraneous profiles, membraneous whorls and some membrane bound structures whit dense osmiophilic material. Beginning from 19 day old embryos all developing parietal cells had a concpicious golgi

complex and associated coated vesicles. Some cells also showed small secretory vesicles budding from the golgi membranes. So it would appear that during development the parietal cell is very active in membrane synthesis and reorganization. Prominent golgi complex, secretory vesicles and coated vesicles might be related to the membrane synthesis. Residual membraneous structures and lysosomes which were usually encountered in our material seemed to be related with the reutilization of the membranes for the synthesis of new membranes.

The intracellular canaliculus is reported to appear first on the 31 day of fetal life in rabbit[3] and on day 20 of fetal life in the rat.[1] In human fetuses however the intracellular canaliculi are still immature and require further development after birth.[3] The development of the parietal cells in perinatal rats is accelerated by the intake of milk. The enlargement in the lumen of the intracellular canaliculi is reported to appear in fetuses given milk. Woltrath[7] and Halender[1] had demonstrated carbonic anhydrase in parietal cells from rat embryos 19 days of age. The enzyme is known to be important in acid formation. But on the otherhand succinic dehydrogenase activity were found in parietal cells of the rat stomoch only from the 3rd day after birth.[7] Hayward[4] claimed that the parietal cell in fetal rabbit did not show any indication of acid secretion and Halender[1] could not be able to demonstrate HCl in the gastric contents of rat embryos.

Our pure morphological observations are not conlusive but would suggest that, during fetal life and even in new born (When food is not given) the parietal cells are not able to secrete HCl. Even though some cells showed a well developed canalicular secretory surface. But these cells were appearantly lack of tubulovesicular membranes wich are essential structures for secretion.

It is felt that, membraneous structures, which appeared during development are quite different from those seen in adult actively functioning cells at the ultrastructural level. It is quite probable that, these membraneous structures represent the synthesis and reorganization of new membranes rather than to be related with the secretion process.

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The Effect of In Utero Exposure to Furosemide on the Renal Maturation in Rabbits

Murat Yurdakök, M.D.* / Olcay Oran, M.D. / Tevfik Tekeli, D.V.M.*** / Melda Çağlar, M.D.**** / Hüdaverdi Erer, D.V.M.*******

Summary

Furosemide was given to the rabbits intramuscularly in a dose of 1 mg/kg body weight per day, from the first day of the pregnancy to the delivery. On the day of delivery the neonates of the furosemide group had a body weight and a kidney weight similar to those of the control group. But the number of fully developed glomeruli counted in a whole and median section was significantly ($p < 0.01$) lower in the furosemide group. The importance of furosemide on the maturation of fetal kidney in animals was stressed.

Key Words: Furosemide, Intrauterine drug effects, Kidney maturation, Rabbit.

Introduction

Diuretics have been widely used to treat hypertension due to various reasons during pregnancy. The effects of diuretics on the fetus

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have not been sufficiently investigated.[1,2] Since furosemide is known to be nephrotoxic and it crosses the placenta,[3] we planned to study the effects of furosemide on fetal nephronogenesis and nephotoxicity in rabbits.

Material and Method

Ten white female rabbits supplied by the Laboratory Animals Breeding Unit of Selçuk University for use in this study. The day of coitus was considered the first day of pregnancy, and they were divided into two equal groups randomly.

Normal saline was given intramuscularly every day to control rabbits through their pregnancies. On the same dates rabbits in the study group were given 1 mg/kg body weight/day of commercial solution of furosemide (Lasix^R) via the same route.

On the day of delivery, neonates were weighed, and their left kidneys were removed, weighed, and submitted to histological procedures. The sections were made sagittally and in these sections all fully formed glomeruli were counted in one median section is accepted as an index of maturation.[4]

Results were presented as mean $\pm$ standart error of the mean and comparisons were made with the Student's t test.

Results

The duration of the pregnancy was 30 days with no difference between the two groups ($p > 0.05$). The number of leverets was 21 for the control group and 23 for the study (furosemide) group ($p > 0.05$). No death occured in any group.

The neonates of the furosemide group had a body weight (BW) and a (left) kidney weight (KW) similar to those of the control group ($p > 0.05$). The ratio of KW x 100 / BW was 0.458 ± 0.021 for the controls and 0.552 ± 0.021 for the furosemide group ($p > 0.05$) (Table I).

The number of fully developed glomeruli counted in a whole and median section was significantly lower in the furosemide group as compared to the control group (21.379 ± 1.837 and 43.556 ± 5.513) ($p < 0.01$). The ratios of the number of differentiated glomeruli per section x 100 / KW or BW were significantly lower in the furosemide group ($p < 0.01$) (Table I).

TABLE I

BODY WEIGHT, LEFT KIDNEY WEIGHT AND NUMBER OF DIFFERENTIATED GLOMERULI COUNTED IN A WHOLE SAGITTAL SECTION IN STUDY AND CONTROL GROUPS

Group	Number of leverets	BW (g)	KW (g)	KWx100/BW	G	Gx100/BW	Gx100/KW
Furosemide	23	42.626 ± 1.173*	0.222 ± 0.011	0.522 ± 0.021	21.379 ± 1.837	51.770 ± 4.822	10.179 ± 0.944
Control	21	46.653 ± 1.944	0.213 ± 0.013	0.458 ± 0.021	43.556 ± 5.513	93.767 ± 11.276	20.650 ± 2.312
p		> 0.05	> 0.05	> 0.05	< 0.01	< 0.01	< 0.01

* Mean ± SEM.

BW: Body weight, KW: Left kidney weight, G: Number of differentiated glomeruli counted in a whole sagittal section.

Discussion

Since we have no serum levels of furosemide in both groups, we cannot say the delayed maturation of kidney is due to a direct effect of furosemide or its metabolite. Because neonatal disturbances of water and electrolyte balance after maternal administration of diuretics just before delivery have been reported.[5,6] Presumably these disturbances could result from direct action of the drugs on the fetal kidney or an effect on the placental transfer of water and electrolytes. Another indirect effect of furosemide could be possible. Diuretic effect of furosemide may cause a contraction of the circulating blood volume would have resulted in a diminuation of placental perfusion and then a hypotrophy.[7] But we have shown that there is no significant difference in the ratios of KW x 100 / BW between groups. Thus such an effect of furosemide seems to be unlikely.

The total number of differentiated glomeruli in kidneys is lower in the furosemide group than the control group in the present study. For this reason, it is concluded that there is a delay in utero nephrogenesis in newborn rabbits whose mothers received furosemide throughout their pregnancies. These findings support the hypothesis of Mallie *et al*[7] on the administration of furosemide to pregnant rats through the pregnancy delays the differentian of the glomeruli.

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Adult Non-Hodgkin's Lymphoma (NHL) in Turkey

A Histopathology Based Retrospective Survey on 211 Cases

Nükhet Tüzüner, M.D.* / Ümit İnce, M.D.**

Summary

This retrospective study undertakes the classification of our adult non-Hodgkin's lymphoma (NHL) cases material according to the new "National Cancer Institute" classification proposal to Lukes-Collins and Rappaport calassifications.

The results of our study show that both Lukes-Collins and Rappaport classifications can be used satisfactorily even when only morphological criteria obtained by routine histopathological methods are used provided that the technique is satisfactory. The application of the Working Formulation is no more than a task of bearing the scheme in mind or on the table. Since this proposal provides a uniform language through which any pathologist and clinician can communicate, it should invariably be used for every case in conjunction with whatever classification scheme is prefered.

Key Words: Non-Hodgkin's lymphomas, Working Formulation, Rappaport's and Lukes-Collins classifications.

Introduction

NHL represent a heterogeneous group of diseases with different natural histories and different responses to treatment. The identification of functionally separable human lymphoma cell populations has led to a conceptual revision of the origin and classification of malignant lymphomas. Thus traditional concepts of pathology and classification of malignant lymphomas based solely on morphologic descriptions have

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changed dramatically within the last 10 years.[1, 2, 3] Since that time, our knowledge of functional classification of malignant lymphomas has markedly increased resulting in several new lymphoma classifications and thus creating new controversy and misunderstanding. More recently, National Cancer Institute (NCI) organized a large scale international study to compare and evaluate the most prominent classification schemes for NHL and to attempt formulating a unifying concept. This has resulted in the "Working Formulation for Clinical Usage" (NWF).[4]

Our cases of NHL has been designated under a wide variety of names and therefore the collective data is useless for comparing our results with those from other centers and for any other scientific discussions. Thus this study was undertaken in which a reclassification of our NHL case material according to NWF, Lukes-Collins and Rappoport classifications is made with some emphasis on the applicability, comparability and value of these methods.

Materials and Methods

Adult NHL cases of 18 years of age or older, were chosen from the files of the Istanbul University Cerrahpaşa Medical Faculty, Department of Pathology from January 1976 to June 1985. The morphology of each case was reviewed and confirmed prior to being included in this study.

Histopathological classification was done according to Lukes-Collins, Rappaport classifications and the NWF. The clinical data were not known by us at the time of classification except for the age and the sex of the patients and the sites of the biopsy materials.

All the diagnoses were made on formalin or B-5 fixed paraffin-embedded sections, stained routinely with H-E, PAS and MGP. Immunoperoxidase (IPX) for demonstration of intracytoplasmic immunoglobulins (CIg) was also applied on deparaffinized paraffin sections as described by Taylor.[5] In 50 cases imprints were also available for interpretation and were stained for Giemsa, PAS, Acid Phosphatase (ACP) and Alpha Naphtyl Acetate Esterase (ANAE) as described previously.[6]

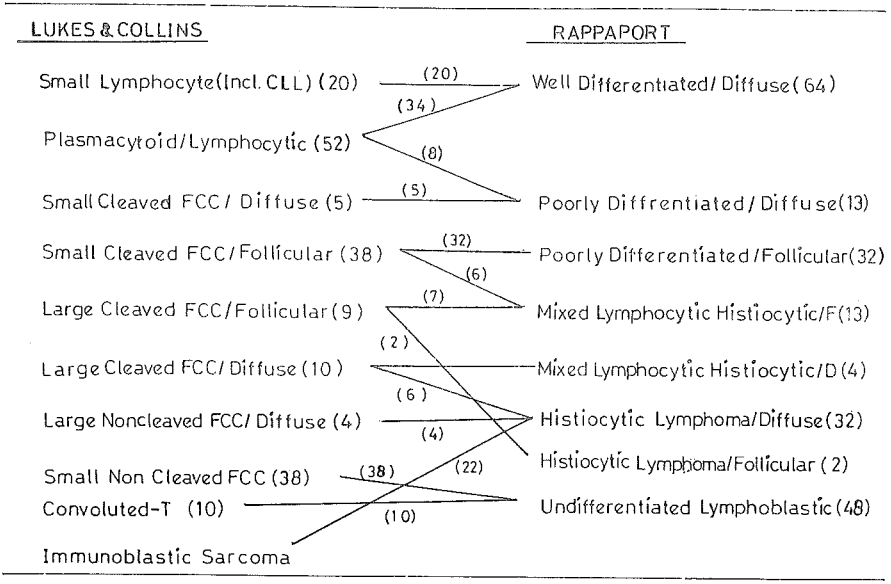
Results

A total of 404 cases of NHL were available for the study. Diagnoses other than NHL were made in 12 cases. The material was considered imperfect for technical reasons in 181 of the 392 available cases (46%) which were excluded from the study to avoid errors in interpretation.

Histological classification: The distribution of cases according to Lukes-Collins and classic Rappaport classifications is shown in Table I. Follicular Center Cell (FCC) lymphomas accounted for 104 cases (49.3%) and 47 of them represented follicular growth pattern (45.2%). Of the 38 cases in FCC lymphoma group with the small non-cleaved (Snclv) type, 5 were classified as Burkitt variant and 33 as non-Burkitt (NB). B cell lymphomas other than FCC accounted for 91 cases (43.1%). 72 cases in this group exhibited small lymphocytic morphology, of these 20 were classified as chronic lymphocytic leukemia (CLL), and 52 as plasmacytoid lymphocytic lymphoma. In the remaining 22 cases diagnoses of immunoblastic sarcoma (IBS) was established.

TABLE I

DISTRIBUTION OF CASES ACCORDING TO LUKES-COLLINS AND CLASSIC RAPPAPORT CLASSIFICATIONS



In T cell lymphomas diagnoses were established by morphology and punctate ACP or ANAE activity and negative staining for CIg. Ten out of 16 T cell lymphoma cases were classified as convoluted lymphocytic, 3 as IBS and 3 as Lennert's lymphoma.

Distribution of cases into prognostic groups of "NWF".

I- Low Grade Lymphomas:

1) Malignant lymphoma-Small lymphocytic (Small lymphocytic and CLL-Lukes and Collins, Well differentiated lymphocytic lymphoma (WDLL, D)-Rappaport):

20 out of 211 cases (9.5%) were diagnosed as CLL by using morphologic criteria (2,3). In all cases the structure of the lymph nodes was completely affected. The pattern of growth was principally diffuse, but in 8 cases, small or large areas of proliferating cells (pseudofollicles) were found. Morphologically, larger than normal small lymphocytes predominated. Lymphoblasts (paraimmunoblasts) were always present among the lymphocytes. In 9 cases imprints or peripheral blood smears were also available for confirming the diagnosis. All cases had peripheral nodal biopsy. Bone marrow involvement was present in all cases at the time of biopsy. 15 were male and 5 were female (3:1 M/F ratio). Median age was 62 with a range of 45-80 years.

2) Plasmacytoid lymphocytic lymphoma (Lukes-Collins), WDLL, D (Rappaport):

44 out of 52 cases of plasmacytoid lymphocytic lymphoma fill into the low grade malignancy group except for 8 cases of polymorphic variant. Histologically the pattern of growth was diffuse-in most cases, but a pseudo-follicular picture like that seen in CLL of the B-type was not uncommon. The clusters of proliferating cells included immunoblasts. Cytologically, lymphocytes and plasmacytoid cells predominated the histologic picture. Plasmacytoid cells contained PAS (+) globular inclusions in the nuclei and/or cytoplasm. (Figure 1) Protein masses probably representing extracellular immunoglobulin were usually encountered in the plasmacytic variant in our case material. They were found in 9 out of 44 cases and 3 of them revealed positive staining for amyloid. IPX stain was applied in 31 of 44 cases, all tested cases showed monoclonal CIg (Figure 2) (Data is not presented).

In 33 cases diagnosis was made of peripheral lymph node biopsy, 19 cases showed extranodal location. Gastrointestinal tract (GIT) was the most common extranodal location. Clinical data were not complete but at least 14 cases, had bone marrow involvement at the time of biopsy. 42 were male and 10 were female (4.2:1 M/F ratio). Median age was 55 with a range of 26-76 years.

3) Malignant lymphoma follicular, predominantly small cleaved cell (Small cleaved Sclv) FCC-Lukes and Collins, Poorly differentiated lymphocytic lymphoma, nodular (PDLL, N)-Rappoport) and Malignant lymphoma follicular mixed small and large cleaved (Lclv) cell (Sclv/Lclv FCC lymphoma-Lukes and Collins, Mixed lymphocytic, histiocytic nodular (MLH, N)-Rappaport.

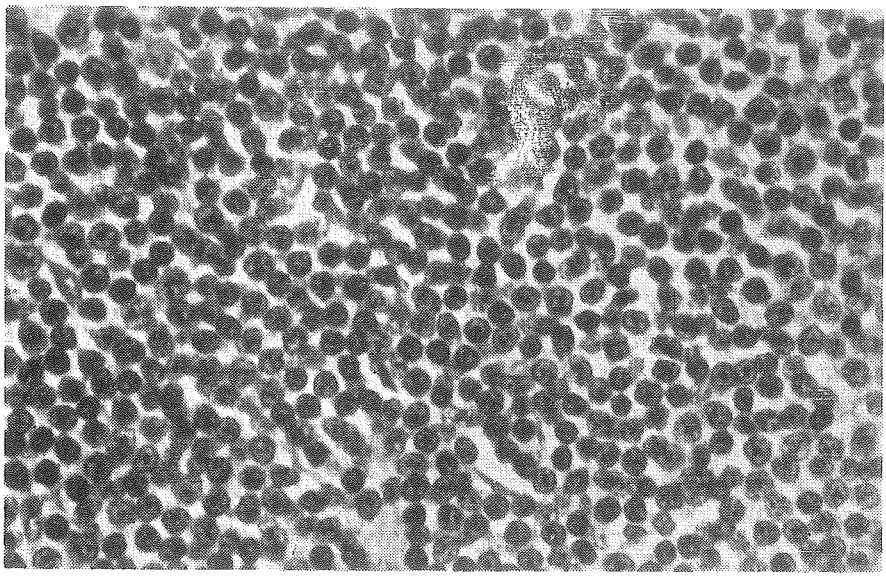


Figure 1

Plasmacytoid lymphocytic lymphoma-Lymphocytes and plasmacytoid cells with round nuclei, clumped chromatin (H & E X 500).

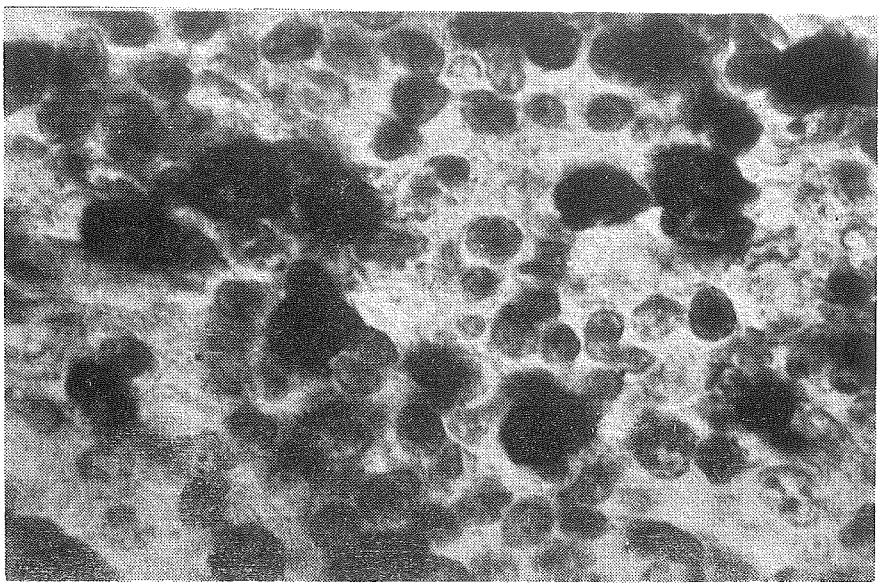


Figure 2

Plasmacytoid lymphocytic lymphoma-Monoclonal cytoplasmic staining for lambda light chain (immunoperoxidase-H & E counterstain X 1250).

Of the total 47 cases of follicular cleaved cell (FCC) lymphomas, 38 were classified as ML follicular, predominantly small cleaved cells. 13 out of 47 cases of follicular cleaved cell lymphomas were classified as ML follicular, mixed small and large cleaved cell. In these cases, histologically. The nodal architecture was destroyed by uniformly distributed neoplastic follicles lying "naked" in the interfollicular tissue. These neoplastic follicles consisted of predominantly small cleaved cells with compact chromatin, inconspicious cytoplasm and indented nuclei. In cases with mixed small and large cell morphology larger cells possessing granular chromatin, scant cytoplasm and indented nuclei were intermingled with small cleaved cells (Figure 3).

29 cases of follicular cleaved FCC lymphomas had peripheral nodal biopsy. 14 of 47 cases had extranodal involvement, again GIT was the most common extranodal location. 5 of follicular Sclv FCC lymphoma and 2 follicular Lclv FCC lymphoma cases represented trasformation into a high or intermediate grade malignancy category, in sequential biopsies (Snclv FCC and Lnclv FCC respectively). 27 were male and 16 were female (1.7:1 M/F ratio). Median age was 56 with a range of 38-71 years.

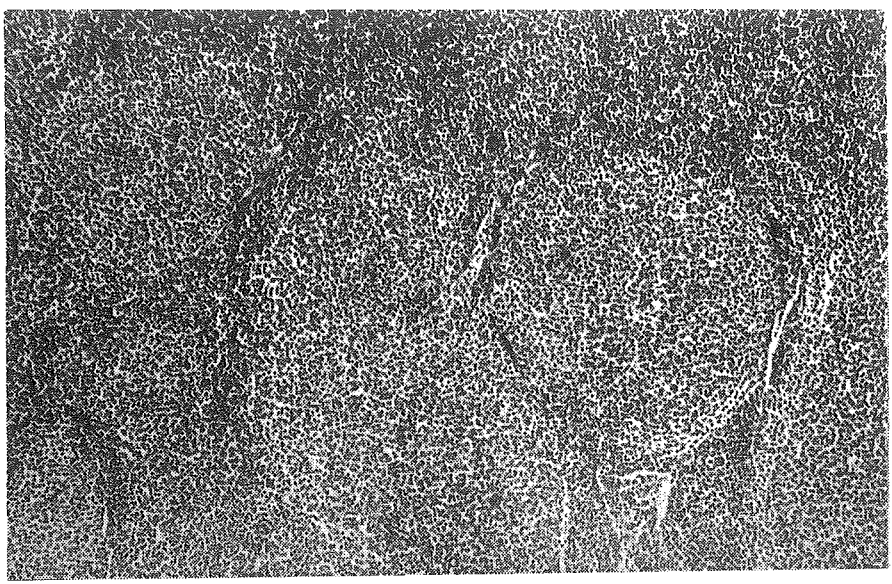


Figure 3

Malignant lymphoma predominantly small cleaved cell with follicular growth pattern (H & X 80).

II - Intermediate grade lymphomas:

1) Plasmacytoid lymphocytic lymphoma-Polymorphic sub-group (Lukes and Collins), PDLL, D-Rappaport:

8 out of 52 cases of plasmacytoid lymphocytic lymphoma were found to have polymorphic morphology. Histologically, in addition to the lymphocytes, plasmacytoid cells and immunoblasts there were also follicle center cells. IPX stain was made in all cases and revealed monoclonal cytoplasmic staining (data is not presented). All except one had peripheral nodal involvement. The remaining one represented GI tract involvement. All cases were male, median age was 50 with a range of 45-65 years.

2) ML diffuse, predominantly small cleaved cell (Sclv FCC-Lukes and Collins-PDLL, D-Rappaport):

5 cases of Sclv FCC lymphoma fall into this group. Histologically the proliferation was diffuse. Cytologically the lymphoma cells were small to medum sized the nuclei were polymorphic with irregularly angular or cleaved shapes. The cytoplasm was scanty.

All except one had peripheral nodal biopsies and in the remaining case diagnosis was made on cheek soft tissue and nasopharyngeal biopsies. All cases were male, median age was 42 with a range of 26 to 60 years.

3) ML follicular and diffuse predominantly large cleaved cell (Lclv FCC-Lukes and Collins-Histiocytic lymphoma, nodular or diffuse (HL, N/D)-Rappaport:

Of the total 19 cases of Lclv FCC lymphomas, 8 were classified as malignant lymphoma predominantly large cleaved cell and 2 of them presented follicular growth pattern. Cytologically, nuclei of these cells were greater than or equal to that of the nucleus of a histiocyte's nucleus, with an angular or indented shape, chromatin distribution was clumped and nucleolus was inconspicious. Cytoplasm was moderate in amount, IPX was done in all cases and revealed monoclonal cytoplasmic staining (Figure 4). Sclerosis was present in all cases.

4) ML diffuse predominantly large non-cleaved cell (Large non-cleaved-Lnclv-FCC-Lukes and Collins, HL, D-Rappaport):

Of the total large cell FCC lymphomas, 4 were classified as ML diffuse with predominantly large non-cleaved cell. Histology of this type resembles immunoblastic sarcoma, differentiation may be difficult in some cases but we chose to classify diffuse large cell lymphomas with large, round nucleus, fine chromatin, 2-3 small but conspicious nucleus

and moderate to large amounts of cytoplasm with less amphophilia as Lnclv FCC lymphoma. Absence of plasmacytoid differentiation is an important indicator. B cell nature of these lymphomas, as established by IPX stain in all cases. All had diagnostic nodal biopsies, 3 were male and one was female and the median age was 53, with a range of 45 to 67 years.

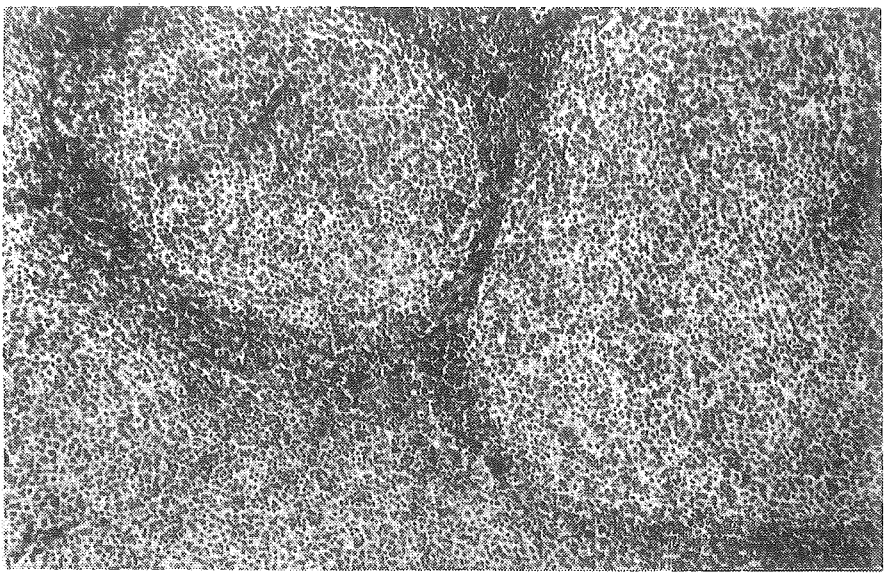


Figure 4
Malignant lymphoma follicular predominantly large cleaved cell (H & E X 80).

III- High grade lymphomas:

1) Small non-cleaved FCC-Burkitt's and non-Burkitt's type (Lukes and Collins), Undifferentiated lymphoblastic-Burkitt's and non-Burkitt's type (Rappaport):

38 out of 211 cases were diagnosed as Snclv FCC type, 5 of which had Burkitt morphology while non-Burkitt morphology was identified in the remaining 33 cases. Histologically in both groups of Snclv FCC lymphoma, the nuclei of tumor cells were equal to or smaller than that of nuclei of macrophages. In Burkitt's type, cell size variations were minimal, nucleus was round and contained granular chromatin and 1-3 basophilic nucleolus, cytoplasm was amphophilic and noderate in amount, in non-Burkitt's variant tumor cells exhibited greater variations in size and nuclear shape. The nuclei had prominent and centrally located nucleoli, chromatin was delicate, cytoplasm was scanty and pale

(Figure 5). IPX stain was done in 21 cases and revealed monoclonal CIg. Mitotic figures were prominent in both groups. 24 were male and 14 were female (2.2:1 M/F ratio). Median age was 50 with a range of 28 to 82 years. In 27 cases diagnoses were established on peripheral nodal biopsies, and on biopsies from extranodal sites in the remaining 11 cases.

2) Convoluted T cell lymphoma (Lukes and Collins), ML lymphoblastic (Rappaport):[10]

10 cases were classified as convoluted T cell lymphoma using the criteria described by Barcos and Lukes.[7] Morphologically, lymph nodes were involved by a diffuse proliferation of primitive looking cells with finely stippled nuclear chromatin, small or inconspicious nucleoli and scanty cytoplasm. The nuclei of these cells showed typical subdivisions (Figure 6). Imprints were steined for ACP in all cases and more than 75% of lymphoma cells demonstrated punctate ACP activity. All cases had peripheral nodal biopsies. 9 out of 10 cases presented with mediastinal mass. Bone marrow involvement was present in 4 cases at the time of biopsy. 6 were male and 4 were female. Median age was 32 with a range of 17 to 60 years.

3) Immunoblastic sarcoma (IBS-Lukes and Collins), HL,D(Rappaport):

The criteria used to distinguish T-IBS from B-IBS in the majority of cases was based on morphologic observations alone, as has been reported by Mauer *et al.*[8] Using these criteria we established the diagnosis of IBS in 22 cases, 19 of which represented B cell and 3 of T cell morphology. Morphologically IBS of B cells revealed a monomorphous proliferation of large cells with round vesicular nucleus with finely dispersed chromatin. Nuclei were basophilic and centrally located. Cytoplasm amphophilic and typically plasmacytoid (Figure 7). In T IBS tumor cells were both large and intermediate, had abundant pale, almost water-clear cytoplasm. Nuclei were prominent and often centrally located (Figure 8). In addition to morphological criteria, the B cell nature of the IBS was confirmed by IPX stain in all cases (Figure 9).

Initial biopsy was made from peripheral lymph nodes in all T and 14 B IBS cases. Extranodal involvement was present in 6 of the 19 cases of B IBS; Waldeyer's ring (1), breast (1) and GIT (4) respectively. Twelve of the patients were male and 10 were female (1.2:1 M/F ratio). Median age was 50 with a range of 25 to 76 years.

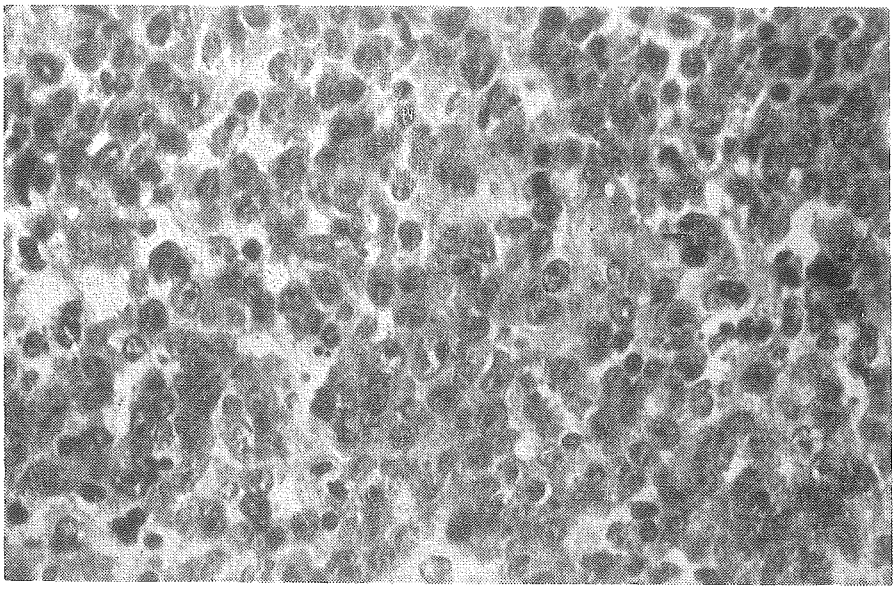


Figure 5
Small non-cleaved FCC lymphoma non-Burkitt's type-note greater variations in nuclear size and shape (H & E X 500).

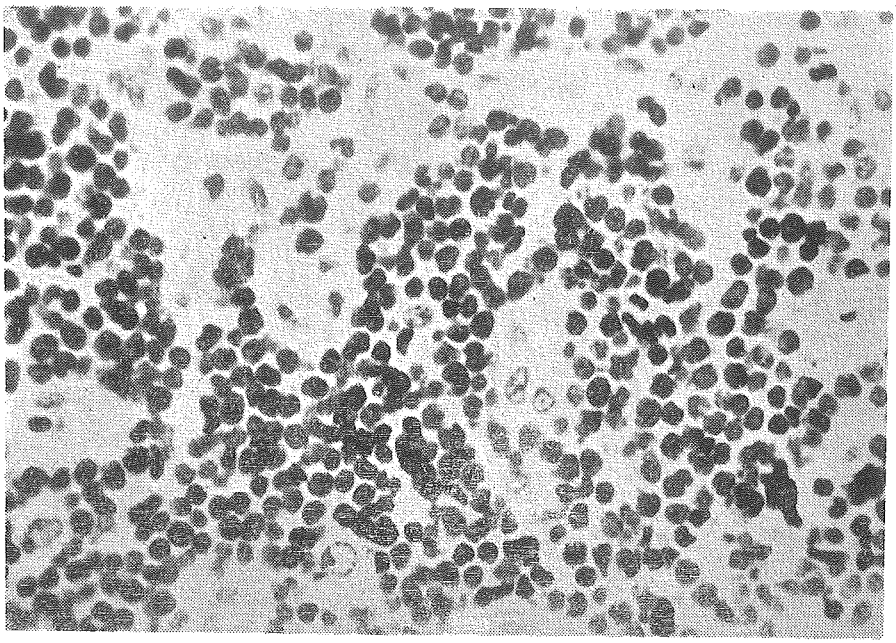


Figure 6
Convoluted T cell lymphoma-primitive appearing cells with finely stippled chromatin, small or inconspicious nucleolus and scanty cytoplasm (H & E X 500).

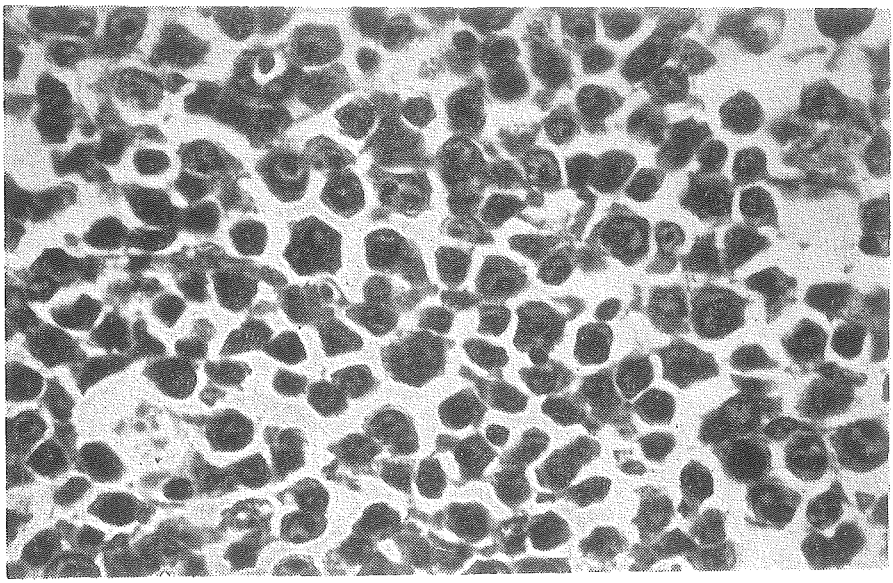


Figure 7

Immunoblastic sarcoma B cell type-Large cells with round vesicular nucleus and prominent nucleolus. Cytoplasm large and typically plasmacytoid (H & E X 500).

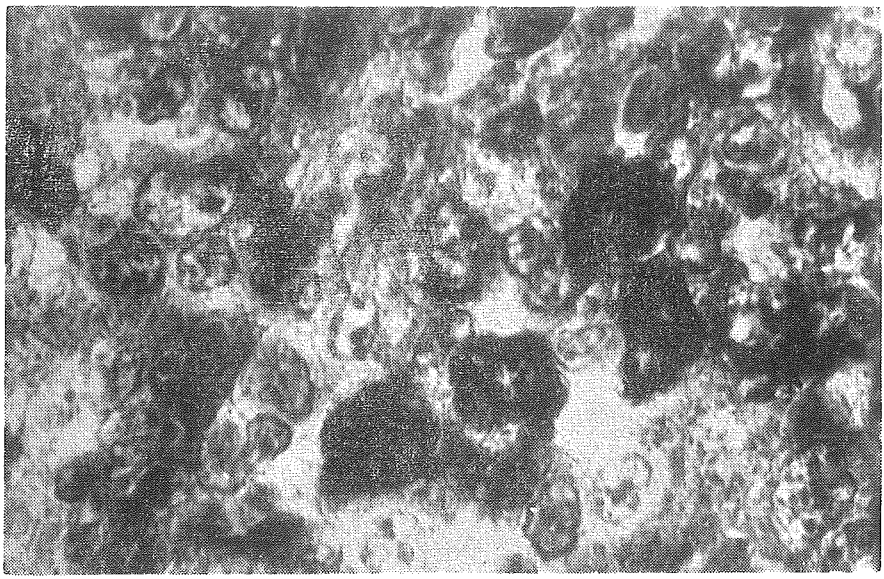


Figure 8

Immunoblastic sarcoma B cell type-Monoclonal cytoplasmic staining for kappa light chain (Immunoperoxidase- H & E counterstain X 1250).

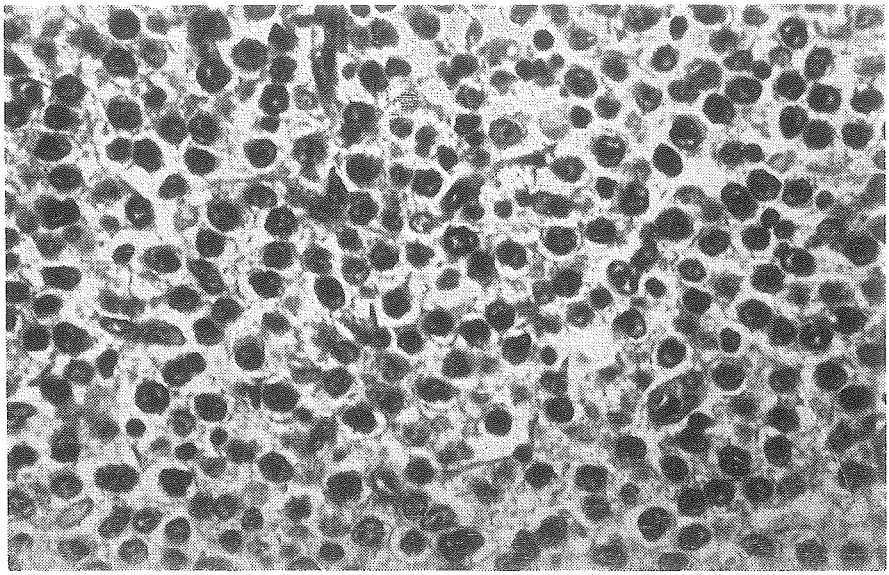


Figure 9
Immunoblastic sarcoma T cell type-both large and intermediate cells with abundant pale, water-clear cytoplasm and prominent nucleoli (PAS X 500).

4) Lennert's lymphoma: This is a rare lymphoma (only 3 cases in our series of 211) of a peculiar nature in that the primary neoplastic cell is believed to be a lymphocyte but associated with variable clusters of reactive histiocytes of epitheloid type. In all 3 cases diagnoses were made on peripheral nodal biopsies. 2 were male and one was female. Mean age was 57 with a range of 46-75 years.

Special lymphoma cases in our case material, including cutaneous T cell lymphomas, extramedullary plasmacytomas and malignant histiocytosis, were not included in this study.

Distribution of the NHL cases in NWF, according to Lukes-Collins and Rappaport classifications:

Histologic subtypes according to Lukes-Collins and Rappaport classifications as prognostic indicators for the various lymphoma types of the NWF are given in Table II and III. Low grade malignancy group comprised 111 cases or 52.6% of our case material according to Lukes-Collins, and 109 cases or 51.7% according to Rappaport classifications. The majority of these cases were plasmacytoid lymphocytic lymphoma (39.6 %) and malignant lymphoma follicular predominantly small cleaved cell and/or mixed small and large cleaved cell (42.3 %).

TABLE II
LUKES-COLLINS (WORKING FORMULATION)

Low Grade		
Small lymphocytic (Including CLL)	16	
Plasmacytoid Lymphocytic	44	
Small Cleaved FCC/Follicular	38	
Large Cleaved FCC/Follicular	9	
Total	109	(51.8 %)
Intermediate Grade		
Small Cleaved FCC/Diffuse	5	
Plasmacytoid/Lymphocytic (Polymorphic)	8	
Large Non-cleaved FCC/Diffuse	4	
Large Cleaved FCC/Diffuse	10	
Large Cleaved FCC/Follicular	2	
Total	29	(13.5 %)
High Grade		
Small Non-cleaved FCC	38	
Convoluted Lymphocytic	10	
Immunoblastic Sarcoma (B and T)	22	
Lennert's Lymphoma	3	
Total	73	(34.7 %)
Percents approximated		

TABLE III
RAPPAPORT-WORKING FORMULATION
(RECENTLY MODIFIED)

Low Grade		
Well Differentiated Lymphocytic $\bar{s}$ Plasm. Features	20	
Well Differentiated Lymphocytoc $\bar{c}$ Plasm. Features	44	
Poorly Differentiated Lymphocytic/Noduler	32	
Mixed Lymphocytic Histiocytic/Nodular	13	
Total	109	(51.8 %)
Intermediate Grade		
Histiocytic Lymphoma/Nodular	2	
Poorly Differentiated Lymphocytic $\bar{s}$ Plasm. Features	5	
Poorly Differentiated Lymphocytic $\bar{c}$ Plasm. Features	8	
Mixed Lymphocytic Histiocytic/Diffuse	4	
Histiocytic Lymphoma/Diffuse	32	
Total	51	(24.2 %)
(If HL/D = 0) Total	19	(9 %)
High Grade		
Histiocytic Lymphoma/Diffuse	32	
Lymphoblastic ($\bar{c}$/$\bar{s}$ Convoluted)	10	
Undifferentiated (Burkitt and non-Burkitt)	28	
Lennert's Lymphoma	3	
Total	83	(39.3 %)
(If HL/D = 0) Total	51	(24.2 %)

$\bar{c}$: with, $\bar{s}$: without, Plasm: Plasmacytoid.

The prognostic group of intermediate malignancy comprised 27 cases of our case material according to Lukes and Collinc and 51 cases or 24.2 % accorning to Rappaport if HL-D was included. When HL-D is separated from intermediate grade of malignancy group it comprised 19 cases or 9% of our case material.

The group having high grade malignancy included 73 cases or 34.6% according to Lukes-Collins and 83 or 39.3% according to Rappaport classifications.

A major problem arises in intermediate and high grade malignancy groups since HL-D may take place in both of these groups. In Rappaport classification with the NWF if HL-D is included into intermediate group, it will lead to some misunderstanding. When HL-D is included into high grade malignancy group a large degree of comparability of major lymphoma entities is reached in both classifications.

Discussion

Verification of major diagnostic types is important when dealing with NHL since a minor histopathological nuance represents a major change in prognosis. In the NWF, NHL have been divided according to survival data as a prognostic indicator for the various types, into those with an indolent course (low grade-favorable histology), those with an intermediate course (intermediate grade-less favorable histology) and those with a rapidly agressive course (high grade-un favorable histology). Verification of the less favorable and unfavorable histologic types in NWF is essentially based on Lukes-Collins system. It is well known that Lukes-Collins based their classification on cellular details rather than on growth patterns. In NWF growth pattern of follicular lymphomas gains prognostic importance primarily in large cell types.[4, 9] A follicular pattern in the categories of small and mixed small and large cleaved FCC lymphomas and a diffuse pattern in the categories of small lymphocytic and plasmacytoid lymphocytic lymphomas constitute favorable histology with low grade malignancy, only the polymorphic variant of plasmacytoid lymphocytic lymphomas exhibits less favorable histology with an intermediate grade of malignancy according to survival analysis of a large series of patients.[4] A diffuse pattern in the categories of Snclv FCC both Burkitt and non-Burkitt variants and IBS both B and T cell types and convoluted lymphocytic lymphomas constitutes unfavorable histology with high grade malignancy. Diffuse pattern in the categories of small and large cleaved FCC lymphomas, mixed small and large cleaved lymphomas and follicular lymphomas predominantly large cell, and polymorphic variant of plasmacytoid lymphocytic lymphoma represents the less favorable histology.

As outlined in the results a large degree of comparability of the major lymphoma types was reached in Lukes-Collins and Rappaport classifications. We have a personal preference for Lukes-Collins classification because it indicates lymphocyte function as well as distinctive cytologic variations. On the other hand, Rappaport classification does allow easy communication between pathologists and clinicians but the terminology of classic Rappaport classification is not adequate in tne light of our current knowledge. The current modification of Rappaport classification[4] is simple and subtyping into specific entities is included. However, pathologists must have a clear understanding in the specific histopathology of the diffuse lymphomas mixed small and large cleaved cell and diffuse histiocytic lymphomas when dealing with the Rappaport classification. Only immunoblastic sarcoma must be diagnosed as HL-D. Lymphomas consisting predominantly of large cleaved or large non-cleaved cells should be included into the less favorable histology group to avoid confusion, because it is now clear from recent studies that there is a distinct trend favoring the large FCC histologies as a favorable prognostic group compared to the IBS.[10, 11] On the other hand, Nathwani *et al*[12], reclassifying 202 patients of diffuse histocytic lymphoma into the Lukes-Collins classification, found that only large cleaved FCC demonstrated a favorable prognostic advantage compared to large non-cleaved FCC types, but only 6 patients of large cleaved FCC were available for comparison with 42 patients with large non-cleaved FCC histology, which does not appear to be convincing. They identified 6 patients with IBS and no survival differences were noted between these patients and patients with large non-cleaved cell type which is in agreement with our opinion stated above.

In our study, classification of NHL is primarily based on H-E stained sections, technical processing of the biopsied material is very important. Thick, inadequately fixed, dehydrated and stained sections frequently lead to errors in interpretation.[13] As pointed out in the results, we have excluded 181 out of 392 NHL cases due to technically poor and inadequate material. It could be thought that the relative percentage of different subtypes are not representative of the real distribution of cases. However no cases were excluded from diagnosis after 1982 due to improvement in our technique, and inclusion of IPX in the routine workup. When only these cases were taken into consideration the relative percentage of the different subtypes did not change.

In proper sections, it is easy to recognise follicular growth pattern and detailed cell morphology. Imprints have also proven detailed cellular morphology and diagnostic value. But in such studies it might be

difficult to differantiate cleaved cell lymphomas-small, large and mixed cell types-when they represent diffuse growth pattern. It is well known from several studies that in lymphomas, pattern recognition is more reproducible than identification of cell type. Three previous studies have showh that agreement on a nodular pattern is over 90%, whereas agreement on cell type ranged between approximmately 50-75 %.[14, 15] Warnke *et al*[11] found a great degree of discrepancy when four experienced observers attempted independently to subdivide diffuse large cell lymphomas into different cell types.

On the other hand, when NCI's surveillance Epidemiology and End Results (SEER) programme for the years 1977 to 1980 was applied to the language of WF, 64% of the cases in SEER fell into the intermediate grade, 32 were low grade and only 4% were high grade.[16] These figures are apparently in great contradiction with the results of the NCI sponsered NHL study which reflected the diagnoses of the authorities. The preponderence of cases in the intermediate-grade category, in our opinion, is certainly misleading. This is apparently due to misinterpreting the cell type.

The unfortunate point in the NWF is that the major lymphoma groups are not homogeneous from the standpoint of immunological concepts. In fact, recent studies using monoclonal antibodies have shown that small and large cell lymphomas other than FCC lymphomas are immunologically heterogeneous.[17] It is also well known that there are definite differences in the prognosis of T, B, pre-B and U cell lymphomas.[13]

As is apparent from the preceding writing, there is much confusion and controversy in the field of NHL in the world as well as in our country. NWF is an excellent attempt to solve these problems but still, since such investigations are newly published reports, the new set of knowledge has not been agreed upon among most pathologist yet, resulting in wide variety of lymphoma diagnoses, some of which are completely incorrect. Thus it is an urgent necessity to organise a briefing panel among the experienced pathologists in our country to conclude in a uniform lymphoma language in each major classification scheme.

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Motor Nerve Conduction Velocities in Sjögren's Syndrome

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Summary

Several investigations have suggested peripheral neuropathies in almost all of the connective tissue diseases. There are only a few studies dealing with motor nerve conduction velocities in Sjögren's Syndrome. This study was undertaken to examine the possible decrease of motor conduction velocity of median nerve in patients with Sjögren's Syndrome. The median nerves of fortyone patients ranging in age from twelve to seventy-two were evaluated electroneuromyographically and no significant change was observed.

Key Words: Sjögren's Syndrome, Peripheral neuropathy, Motor nerve conduction, Electroneuromyography.

Introduction

There is no absolute definition of Sjögren's Syndrome (SS), however, the diagnostic triad described by Bloch *et al.* serves most clinical purposes.[1, 2]

This triad includes keratoconjunctivitis sicca or dry eyes, with or without lacrimal gland enlargement, xerostomia (dry mouth), with or without salivary gland enlargement, and the presence of a connective tissue disease, usually rheumatoid arthritis.[3-6] More rarely systemic lupus

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erythamotosus, scleroderma (systemic sclerosis), polyarteritis nodosa, or polymyositis may be present.[7] In order to establish the presence of SS, at least two of these three criteria should be present. Patients having only the ocular and oral components are said to have the Sicca Syndrome, whereas those having associated connective tissue diseases categorised as SS with the spesific diseases. As in most autoimmüne diseases, there is a prediliction for middle aged and elderly females.[8]

Both primary and secondary forms of this disease are recognized. Approximately half the patients with an associated rheumatoid arthritis, are considered to have secondary SS, a small percentage may have another connective tissue disease accompanying the sicca complex. Primary SS is diagnosed in the absence of another connective tissue disease.[9]

Although the diagnosis can be made on the basis of clinical criteria along in many instances, the complete evaluation of a patient should include a lip biopsy. The advantage of this procedure over biopsy/ excision of major salivary glands is that the glands of the lower lip are "easyly accesible, they are above the muscle layer, seperated from the mucous membrane by a thin layer of fibrous connective tissue and the chance of excessive bleeding is minimal".[10]

In almost all of the connective tissue diseases the quantitative increase of the collagen fibres is the cause of sclerosis. This effects the peripheral nerves by means of compression and motor and sensorial conduction velocities decrease secondarily. There are only a few studies dealing with the peripheral neuropathies in SS in the literature and almost all of them deal with the decrease in sensorial conduction velocities. In the present study we have calculated the other component which is the motor nerve conduction velocity in forty-one patients with SS electroneuromyographically.

Materials and Methods

Forty-one patients (32 females, 9 males) with SS, ranging in age from 12 to 72 (mean:43.8, SD:14.273) were tested for nerve conduction velocities of median nerve using standardized methods described in the literature[11] between 1984 and 1986 in Physical Medicine and Rehabilitation Department. None of the subjects had a history of another disease known to cause neuropathy or were taking any drugs which would have affected the nerve conduction velocity.

All of our patients had oral and ocular dryness and chronic arthritis resembling rheumatoid arthritis so they were considered as secon-

dary SS. They were complaining of eye discomfort and difficulty with chewing. Episodic parotitis was observed in 19 (% 46.34) patients and lacrimal gland enlargement was observed in 8 (% 19.51) patients.

Except the chronic arthritis no extraglanduler manifestations were observed. The results of the neurological examination of all of our patients were normal and none of them had any sign or symptom of neuropathy.

We made lip biopsies of all of our patients and found typical changes for SS, like lymphocytic infiltration. Also Schirmer test as a primary screening test was applied and it was fond that tear producion was reduced in all of our patients.

Each patient was measured to dermine the median nerve distal latency, proximal latency, distance, amplitude and nerve conduction velocity. The tests were performed in a screened room in the elctrodiagnostic unit of our department and the ambient temperature during the tests didn't move outside the range 28°C to 30°C.

All subjects were asked to abstein from drugs of any kind, tobacco, alcohol and strongly caffeinated drinks for six hours prior to the test.

Their position was standardized as follows: The subject was made comfortable lying in bed in supine position, The right upper extremity was in natural position and no pressure was exerted on the median nerve. The right hand was in supination.

The equipment used to measure the median nerve conduction velocity was TECA Electroneuromyograph type 14, the stimulus was a 0.1 ms. rectangular pulse with repetition frequencies of 1 Hz for the motor stimulation. Sensitivity 10 milivolt, time base 30.

The distal latency of median nerve was determined using a supramaximal electrical stimulation at 6 cm. far from the bipolar needle electrode inserted in opponens policis muscle belly for the purpose of standardisation. The anatomical location of the distal stimulation site was lateral aspect of tendon of flexor digitorum superficialis muscle on the flexor carpal ligament at the wrist joint. The proximal latency of median nerve was determined using a supramaximal electrical stimulation at the antecubital region, approximately 5 cm. under the lateral epicondyl. The cathode electrodes were used as the active electrodes for both stimulation and recording. The right upper extremities of the patients were studied.

Normal values for motor conduction velocity and distal latency of median nerve are 57 $\pm$4 (lower limit of normal is 47) m/sec and 3.8$\pm$

0.5 (upper limit of normalis 5) msec.[12] Values that are less than 47m/sec for conduction velocity, and more than 5 msec for distal latency are considered to be pathological.

As a laboratory study sedimentation rates, alkaline phosphaeset values and latex tests were also calculated. Female and male patients are divided into two subgroups according to the results of the latex tests being positive or negative.

All results were subjected to mean, standart deviation, standart error and varians analysis to assess significance statistically in Hacettepe University, Faculty of Science, Department of Statistics.

Results

Mean age of 41 patients is 43.8, standart deviation (SD) 14.273, standart error (SE) 2.229, variation coefficient (VC) 32.6, mimimum age 12 and maximum age 72.

Mean value of the duration of disease is 6.2 years, SD:5.428, SE: 0.848, VC:87.6, min:1 year, max:25 years.

Values of the proximal latencies of 41 patients are given in Table I. Mean=7.2 miliseconds, SD=0.771, SE=0.120, VC=10.7, min=5.7 milisec, max=9.5 milisec.

TABLE I
VALUES OF THE PROXIMAL LATENCIES OF THE PATIENTS (MILLISECONDS)

Groups		N	Mean	SD	SE	VC	Min	Max
Females	Latex(+)	17	7.1	0.748	0.181	10.5	5.7	8.7
	Latex(–)	15	7.4	0.582	0.150	7.9	6.6	8.7
	Total	32	7.2	0.678	0.120	9.4	5.7	8.7
Males	Latex(+)	2	8.8	1.061	0.750	12.1	8.0	9.5
	Latex(–)	7	6.8	0.670	0.253	9.8	5.7	7.9
	Total	9	7.3	1.089	0.363	14.9	5.7	9.5
Total Prox. Lat.		41	7.2	0.771	0.120	10.7	5.7	9.5

Values of the distal latencies of our patients are given in Table II. Mean=3.3 miliseconds, SD=0.459, SE=0.072, VC=13.9, min=2.7 milisec, max=5.0 milisec.

Mean value of the distances between proximal and distal stimulation sites of the median nerves of our patients is 0.22 meter, SD=0.016, SE=0.002, VC=7.2, min=0.19 meter, max=0.27 meter.

TABLE II
VALUES OF THE DISTAL LATENCIES OF THE PATIENTS (MILLISECONDS)

Groups		N	Mean	SD	SE	VC	Min	Max
Females	Latex(+)	17	3.3	0.563	0.137	17.1	2.7	5.0
	Latex (-)	15	3.3	0.273	0.070	8.3	2.8	3.7
	Total	32	3.3	0.444	0.078	13.4	2.7	5.1
Males	Latex(+)	2	4.2	0.424	0.300	10.1	3.9	4.5
	Latex(-)	7	3.2	0.285	0.108	8.9	2.9	3.6
	Total	9	3.4	0.522	0.174	15.4	2.9	4.5
Total distal lat.		41	3.3	0.459	0.072	13.9	2.7	5.0

Values of amplitudes of our patients are given in Table III. Mean=8.0 milivolts, SD=2.795, SE=0.436, VC=34.9, min=2 milivolts, max=15 milivolts.

TABLE III
VALUES OF THE AMPLITUDE (MILLIVOLTS)

Groups		N	Mean	SD	SE	VC	Min	Max
Females	Latex(+)	17	9.4	2.396	0.581	25.5	5	15
	Latex(-)	15	7.5	2.542	0.656	33.9	2	11
	Total	32	8.5	2.601	0.460	30.6	2	15
Males	Latex(+)	2	3.8	1.061	0.750	27.9	3	4.5
	Latex(-)	7	6.9	2.854	1.078	41.4	4	11
	Total	9	6.2	2.850	0.950	46.0	3	11
Total amplitude		41	8.0	2.795	0.436	34.9	2	15

Values of nerve conduction velocities of our patients are given in Table IV. Mean=56.6 meter/sec, SD=9.638, SE=1.505, VC=17.0, min=47.0 meter/sec, max=88 meter/sec.

TABLE IV
VALUES OF THE NERVE CONDUCTION VELOCITIES (METER/SEC)

Groups		N	Mean	SD	SE	VC	Min	Max
Females	Latex(+)	17	57.3	11.039	2.677	19.3	47.4	88
	Latex(-)	15	55.0	6.550	1.691	11.9	47.0	67.5
	Total	32	56.2	9.142	1.616	16.3	47.0	88
Males	Latex(-)	2	49.6	2.263	1.600	4.6	48.0	51.2
	Latex(-)	7	60.4	12.339	4.664	20.4	54.0	88
	Total	9	58.0	11.732	3.911	20.2	48.0	88
Total velocity		41	56.6	9.638	1.505	17.0	47.0	88

Mean value of the sedimentation rate is 22.3 mm/hour, SD=17.668, SE=2.759, VC=79.2, min=2 mm/hour, max=88 mm/hour.

Mean value of alkaline phosphatase is 87.4 IU/liter, SD=59.295, SE=9.260, VC=67.8, min=20 IU/liter, max=361 IU/liter.

Discussion

According to Hollander the clinical presentation of SS is as the following: Sicca complex (Dry eyes and dry mouth), rheumatoid arthritis or other connective tissue diseases, salivary gland enlargement, purpura (non thrombocytopenic, hyperglobulinemic), renal tubular acidosis or other tubular disorders, polymyopathy, neuropathy (trigeminal), chronic liver disease, chronic pulmonary disease, lymphoma (local or generalized immunoglobulin disorder-cryoglobulinemia, macroglobulinemia).[9]

Severe proximal muscle weakness and rarely, tenderness may be early symptoms, leading to a diagnosis of polymyositis.[13] Weakness may also be associated with electrolyte imbalance,nephrocalcinosis and clinical findings of renal tubular acidosis. Peripheral or cranial neuropahy may cause symptoms of dysesthesia or paresthesia. Facial pain and numbness can accompany trigeminal neuropathy and contibute to the oral discomfort caused by the dryness.

The predominantly sensory characterastically mild, distal, symmetrical polyneuropathy occuring with SS was reviewed in 1966.[14] SS usually produces sensory neuropathy of "glove and stocking" type associated with paresthesias.[15, 16]Massey reported a patient with bilateral carpal tunnel syndrome, bilateral meralgia paresthetica, a right ulnar palsy from a block at the olecranon groove, and electrical evidence of mild sensory neuropathy without clinical symptoms to suggest such.[17]

The results of the previous studies suggest that the neuropathies predominantly sensorial type may be seen in SS. Only Massey mentioned about entrapment neuropathies especially carpal tunnel syndrome, so we evaluated only the motor conduction velocity of nervus medianus in patients with SS and our results showed that statistically no significant changes occured.

As a conclusion we can propose that although all of our patients had clinical, laboratory and histopathological positive criteria for SS no sign and symptom for neuropathy and no decrease in motor nerve conduction velocity was observed.

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Focal Colitis in Behçet's Disease*

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Summary

Rectal biopsy materials from 8 patients with Behçet's Syndrome were examined and these were compared with those from patients with ulcerative colitis. There was a marked mononuclear cell infiltration with increased numbers of eosinophils and mast cells in 4 out of 8 patients with Behçet's syndrome. In addition to the goblet cells and mucous retention, crypt abcess formation and fatty infiltration were also detected in these patients. Immunofluorescent staining using specific antisera against 5 subclasses of immunoglobulins, complement components (Clq, C_4, C_3, C_5 and C_9) and monoclonal T-cell specific antibodies (OKT 3,4,8 and 11) showed that the predominating cells in the infiltrates were IgM producing plasma cells in both Behçet's syndrome and also in ulcerative colitis. A few OKT-4 positive cells (inducer/helper) were seen in Behçet's syndrome but there were no OKT-8 positive (suppressor/cytotoxic) cells. However, the latter cells were found to be present in patients with ulcerative colitis. No capillary deposits of immunoglobulins or complement components were detected in Behçet's disease, whereas, these were present in ulcerative colitis. These findings indicate that focal colitis of distinct histological and immunohistological appearence might be one of the main features in Behçet's syndrome and it should be sought carefully in all cases.

* This work was presented in part at the International Conference on Behçet's Disease, London, 1985.

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Key Words: Focal colitis, Behçet's disease.

Introduction

Behçet syndrome (BS) is a multisytemic disease and in addition to the classical triad described by Behçet,[1] it may produce serious disturbances in various organs. Gastrointestinal manifestations have also been recognized in recent years, which may have fatal outcome in some patients.[2, 3]

Although some authorities regard GI involvement as a rare event in BS it has been reported that gastrointestinal symptoms like vomiting, diarrhea, abdominal pain and distention may be seen in as many as 50 % of the patients in Japan.[4] Furthermore, in a large series of 216 cases, 10 % had radiological erosions and ulcers in the large bowel and more than 25 % had intestinal ulcers.[5] Histologically, there was marked evidence of vasculitis of diverse morphology in the vessels in all these ulcerative cases. As might be expected, several cases of BS have been reported with intestinal perforation due to ulcer.[4, 6]

O'Duffy, however, described 5 patients amongst the 32 cases with BS, in whom inflammatory bowel disease resembling Crohn's was found.[7] Three of them had granulomotous colitis and two had ileocolitis.

Although GI manifestations are significantly low in Turkish patients,[8] due to the previous reports we now have proctoscopic examinations done routinely on all Behçet's cases complaining of non-specific GI symptoms and we have found that half of these patients had histological changes which could best be described as "focal colitis."

Materials and Methods

Patients: During the past two years 35 new patients were diagnosed as having BS in our clinic and most of them were seen more than once. Among 35 patients, 8 complained of nonspecific gastrointestinal symptoms and for this reason in addition to routine tests, proctosigmoidoscopic examination was performed.

All of these 8 patients (7 males and 1 female, aged between 23-47) had the complete type of BS according to the Mason and Barnes classification.[9] Two of them were under calchicine treatment. Two patients had slight diarrhea, 3 complained of abdominal distention with slight pain and the remaining 3 complained of a change in defecatory habits during the last 3 months.

Complete physical examination and routine laboratory tests were performed on the patients and all were found to be within normal limits. Stool examination for blood, parasites and pathogenic bacteria was negative. A barium enema was found to be normal.

Rectal biopsy specimens were obtained by rectosigmoidoscopic examination.

Control biopsy specimens were obtained from 3 cases with ulcerative colitis, 7 cases with irritable colon syndrome and two cases with carcinoma of the rectum. These specimens were examined using the same procedures.

Histological and Immunofluorescence Studies: Biopsy specimens of patients and controls were divided into two portions. One portion was fixed by 10 % formaldehyde solution and embedded into paraffin. Sections were stained by hematoxylin-eosin, PAS and Crystalviolet and were examined by two expert pathologists. Sections were also stained by Congo-red and examined under the polarizing microscope for any amyloid depositions.

The second portion was embedded into the gel of 5 % Tragacanth powder (Sigma Chemical Company, USA) prepared in phosphate buffered saline (PBS) (pH 7.4) and was rapidly frozen by CO_2. Cryostat sections were stained by the direct immunofluorescent technique using FITC labelled antisera to human IgM, IgG, IgA, IgD, IgE, C_3 (Wellcome Diagnostics, UK) and human Clq, C_4, C_5, C_9 (Behringwerke AG, W. Germany). Sections were also stained by sandwich immunofluorescence method using monoclonal antibodies, OKT 3,4,8 and 11 (Ortho Pharmaceutical Corp. USA) specific for human T cell subpopulations. Fluorescein labelled rabbit-anti-mouse-IgG (Miles Laboratories UK) was used as a second antibody.

The sections were examined by reflected light fluorescence microscope (Reichard-Jung, Microstar 110) equipped with an HBO 50 W Mercury lamp, exciter KW 418-BG 12, dicroic 500 nm and barrier 06505 filters.

Results

The rectosigmoidoscopic appearence of the mucosa was normal in all the patients with BS.

In histological examination, however, there was a marked mononuclear cell infiltration with increased numbers of eosinophils and mast cells in 4 out of 8 patients with BS. In addition to goblet cells and mucous retention, crypt abscess formation and fatty infiltration was also seen in these 4 patients (Figures 1 and 2).

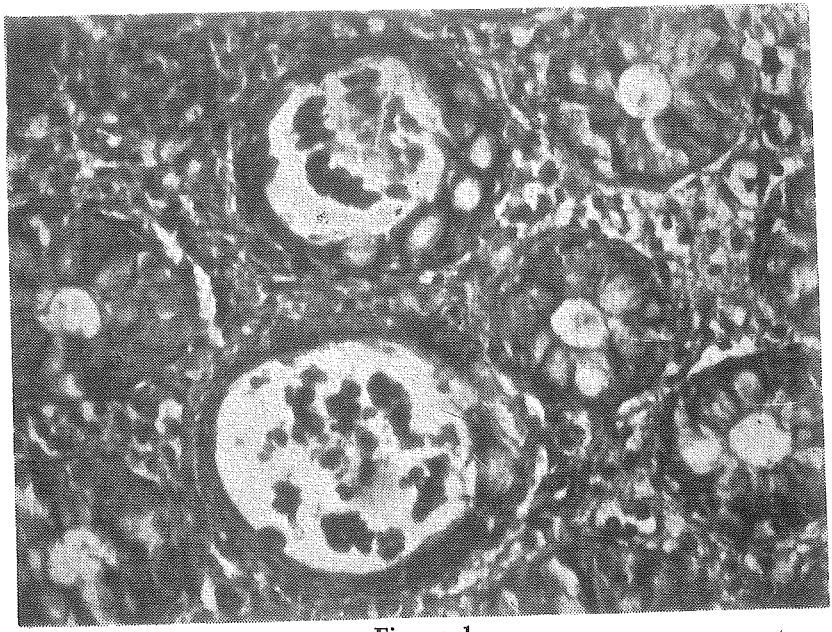

Figure 1

Large bowel biopsy of the BS showing cript abscesse formation, mononuclear cell infiltration, goblet cells hyperplasia and mucous retention. H and E. Original X 600.

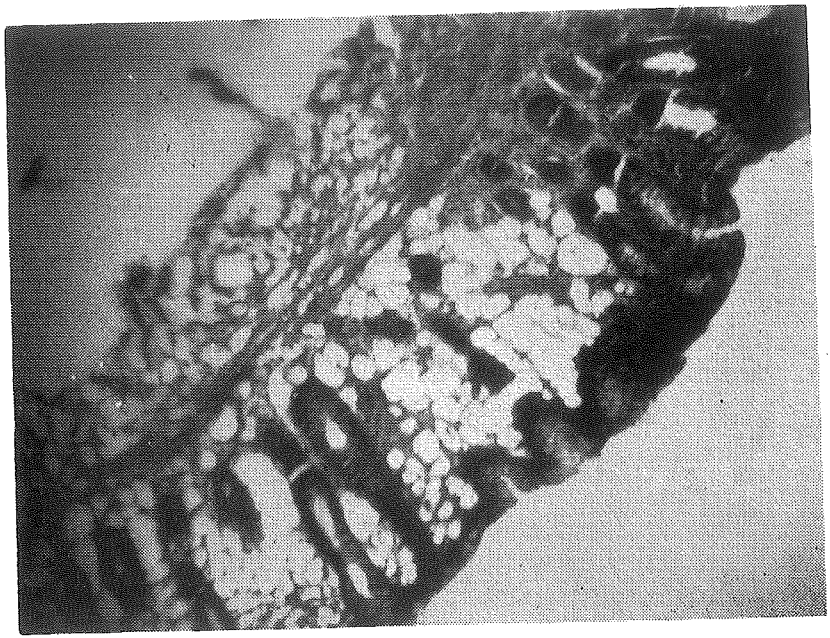

Figure 2

Large bowel biopsy of the BS showing focal area of fatty infiltration in the lamina propria. H and E. Original X 250.

There was no granuloma formation or any histological evidence indicatings vasculitis. The amyloid stain was also negative.

These findings were not specific for BS, since they were also observed, to a greater extent, in patients with ulcerative colitis, but were not in patients with irritable colon syndrome or patients with carcinoma of the rectum.

Reports from the Pathology Department indicated the histological evidence of "focal colitis" in these patients.

Immunofluorescence staining of the specimens by specific antisera revealed that the predominating cells in the infiltrates were IgM producing and C_3 binding plasma cells in BS (Figure 3). However, similar findings were observed in ulcerative colitis. One of the most distinctive variation between BS and ulcerative colitis was the absence of OKT-8 suppressor/cytotoxic cells in the BS infiltrates which were found to be present in ulcerative colitis. A few OKT-4 helper/inducer cells were seen in BS in contrast to the ulcerative colitis. But the total number of positive cells was low and this was not considered to be significant findings.

There was no capillary staining of immunoglobulins or complement components which indicates the presence of vasculitis in BS. However, focal capillary deposits of C_3 (in all 3 patients), Clq (in one of these patients), IgG (2 patients) and IgM (1 patient) were detected in patients with ulcerative colitis.

Figure 3

Direct immunofluorescence staining by the antiserum to human IgM showing increased numbers of IgM producing plasma cells in the lamina propria of the colon. Original X 800.

Discussion

Involvement of the small intestine and colon is a frequent manifestation of BS, especially in some parts of the world. Oshima *et al.*[4] have reported that 50 Japanase patients out of 85 had GI symptom. Radiological examination of these patients revealed various abnormalities mainly in small intestine but also in the colon.

In most of the patients the appearance of the gastrointestinal mucosa with ulceration, friability and pseudopolyps was found to be indistinguishable from the other forms of ulcerative colitis.

A low incidence of granulomotous changes resembling Crohn's disease has been also reported in BS.[7, 10] Nevertheless, Fukuda has shown that approximately one third of his 193 outopsy cases with BS had granulomatous changes in the GI system.[11]

In addition to the ulcerative or granulomatous changes, which were the main features in most of the Behçet's patients with GI involvement, other types of histological changes have been also described. Eng *et al.* have reported a case with colonic ulceration and perforation.[6] At operation the ulcers were visible on the serosal aspect of the colon. Microscopic examination of the resected bowel revealed sharply demarcated deep ulcers with destruction of the muscularis and the presence of predominantly mononuclear cell infiltration. It has been suggested that the patient did not fit well with the pattern either in ulcerative colitis or in Crohn's disease.[6]

Another case of BS have been reported by Reuben *et al.* who had episodic diarrhea, weight loss and pyrexia.[12] In the rectal biopsy material glandular atrophy with crypt abscess formation has been demonstrated. There were also goblet cell and mucous retention. Immunofluorescence studies were performed to the biopsy specimen obtained from the jejunal mucosa and found to be negative for IgA, IgM, IgG and C_3. Although this patient had markedly elevated ESR, the diffuse hypergammaglobulinaemia and pyrexia, which are very unusual for BS, histological appearence of the colonic mucosa described by Reuben *et al.* was similar to our patient's. Regarding with the histological changes, absence of OKT-8 positive cells in the infiltrates and also lack of capillary deposits of immunoglobulins and complement components we suggest that the pathology in the colonic mucosa of our patients does not fit the changes known to be present in ulcerative colitis or Crohn's disease.

The pathophysiological mechanism of these changes in BS is still obscure. As Behçet's syndrome is a multisystem disease with vasculitis and thrombophlebitis, Reuben *et al.* have suggested that their findings could be

explained by the ischemia of the colon.[12] The absence of bilateral radial pulses, ischemic changes effecting the thumb and the first finger of the right hand and approved left subclavian artery occlusion were all regarded as a sign for the presence of generalised ischemia in the patients.[12]

However, none of our 4 cases had clinical signs indicating venous or arterial occlusion. Furthermore, it is very difficult to explain rectosigmoidoscopic and histological appearence of the colon by the ischemia alone, because classical morphological criteria for ischemic colitis were absent.

Our patients had no significant GI symptom like bleeding, perforation etc. Therefore, histological changes we found, should be regarded as an early phase of a potentially more severe disturbances. Although focal colitis is a nonspesific description, we suggest that the follow up of these patients further, would give more information whether these changes represents early stage of granulomatous colitis, described by O'Duffy, or ulcerative lesions mainly described from Japan or finally and most probably represents entirely distinct phenomenon specific for BS.

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Recurrences After Various Therapies for Genital Warts

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Summary

Forty-one patients with condylomata acuminata have been analyzed retrospectively. All patients had histopathologically-proven genital warts. Their social-sexual status, clinical symptoms and findings, coexisting diseases, treatment and recurrences have been investigated and discussed.

Key Words: Condylomata Acuminata, Anogenital Warts, Human Papilloma Virus Infection, Recurrence.

Introduction

Condylomata Acuminata are anogenital warts which occur as a result of Human Papilloma Virus (HPV) infection.[1-4] Codylomata Acuminata are presently considered as one of the most important sexually-transmitted diseases, due to the great increase in prevalance and recently recognized relationship between HPV infection and the devolepment of genital malignancy.[4,5]

Genital warts are treated by the following methods; Podophyllin application, electrosurgical and laser destruction, cryotherapy, scissor exicision or shaving and systemic or local therapy with the following agents: Trichloroaceticacid, Bichloroaceticacid, 5-Flourouracil, Bleomycin, Dinitrochlorobenzene, Interferon, Levamisole, Autologous vaccines.[4] The purpose of this study is to investigate and discuss reccurrences in genital warts.

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*** Senior Resident.
**** Professor.

Material and Methods

This study included forty-one women diagnosed as having genital warts histopathologically, between the years 1975 and 1986 in Hacettepe University, Department of Obstetrics and Gynecology. Data were obtained from patients, pathology files, and follow-up forms.

Ages of the patients ranged from 20 to 75 years; the mean age being 33.2 years. Of 41 patients, 37 were married, two were widows and the other two were single and virgins at the time of diagnosis.

27 were multiparous, 9 were nulliparous and 5 were pregnant at the time of diagnosis. Four patients with genital warts were postmenopousal.

The most common symptoms seen in the patients are listed in Table I. Co-existing diseases and conditions are observed in 15 patients (Table II). The most common location being the vulva (Table III). Cervical smears were obtained in seven patients, one had mild dysplasia.

TABLE I
CLINICAL SYMPTOMS IN THE PATIENTS WITH GENITAL WARTS

Symptom	No. Cases	%
Vulvar Itching	25	60
Vaginal Discharge	9	22
Burning Sensation	9	22
Painful Intercourse	6	15
Palpable Mass	4	10
Asymptomatic	4	10

TABLE II
CO-EXISTING DISEASES AND CONDITIONS IN GENITAL WARTS

Disease or Condition	No. Cases	Recurrence
Allergic Asthma	1	1
Pseudocolinestherase Deficiency	1	–
Trichomonad Vaginitis	3	–
Gallbladder Dysfunction	2	–
OCP Users	1	1
Family History of Diabetes	7	–

TABLE III
LOCATIONS OF THE WARTS

Vulva	21
Vagina	4
Perianal reg.	4
Vestibule	8
Periurethral reg.	4

Various methods of therapy including electrosurgical destruction, Podophyllin application, surgical excision, simple vulvectomy, surgical excision together with electrosurgery, and only follow-up were performed (Table IV).

TABLE IV

No. Patients	Therapeutic Method	Recurrence
9	Podophyllin	2
19	Electrosurgical Destruction	2
6	Surgical Excision	–
3	Surgical Ex. and Electrosurg.	–
3	Simple Vulvectomy	–
1	Follow-up	Spontaneous Resolution

Results

The overall recurrence rate was approximately 10 % (4/41). Of these four patients two were treated with Podophyllin and the other two with electrosurgical destruction. (Table IV) One of the patients with recurrences had allergic asthma, one being an oral contraceptive pill (OCP) user (Table II).

Discussion

Condylomata Acuminata occasionally become resistant to therapy and most commonly may recur in patients with immune deficiency. Diabetes and depressed cell-mediated immune response are predisposing factors for HPV infections.[6, 7] Milder immune supressions which occur in pregnancy, with usage of oral contraceptives and individuals with the inherited atopic diathesis may also enhance the development of infection.[8, 9]

Most recurrences are not true lesions. Wart viruses are continuously shed from the surface of the lesions and may be autoinoculated into the surrounding epithelium.[4, 10] These result in the appearance of new warts during or shortly after treatment. In our series four patients had recurrences in six months to one year after therapy. Two recurrences had occured after podophyllin application. One of them was an OCP user.

In these two cases, only topical application had been performed. Reapplication of podophyllin to remaining warts for 6 weeks with 7-day intervals is advised in the recent literature.[4, 5] Hence, these two cases could be considered regrowths rather than recurrence.

One of the patients had a recurrence after electrosurgery. This patient had allergic asthma which might be an explanation for the recurrence. The other patient had no risk for recurrence but she needed retreatment eight months after the first therapy. All of the patients were retreated with electrosurgery. One was lost in follow-up; the other three patients had no sign of recurrence at the end of the 12-month follow-up period.

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Postero-Lateral Osteophytes and Differential Diagnosis in Thoracolumbar Radiculopathy with Computed Tomography

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Summary

In this article the diagnostic evaluation with computed tomography in one patient having radiculopathy in lower thoracal region has been presented.

The pathology of entrapment in the neural foramen, epidural fat tissue displacement and the conditions that cause root compression in the neural foramen or in lateral recess are diagnosed precisely by CT.

In our case the entrapment is due to the presence of a posterolateral osteophyte.

The other causes that gives the same clinical picture (disc protrusion, superior articular facet hypertrophy, spondylolysthesis, lateral recess stenosis, tumors, abscess, cysts) are detected by CT even if the myelography is negative.

Key Words : Back pain, Thoracolumbar radiculopathy, Computed Tomography.

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Introduction

In the cases of back pain, with radiculopathy, after the presence of entrapment is shown, by several diagnostic methods, the patients are being treated by clinicians. Either conservative or surgical, sometimes these procedures are being ineffective. One of the reasons of inefficiency is that the diagnostic methods are insufficient for differential diagnosis, or, pathology can not be localized accurately by them.

The lesions in the neural foramen are caused by focal inflammatory lesions, lateral disc herniations, spondylolysthesis, lateral recess stenosis, superior fascet hypertrophy, tumors, vertebral osteophytic formations, subluxations or by cystic formations.

Although the contrast myelography is very important in the establishment of the diagnosis, if the lesion is at or below the level of the foramen it is being inadequate.

In this article, the CT findings of a case having foraminal neural entrapment due to the presence of an osteophyte is presented and the differential diagnosis of the other lesions with CT in accordance with the literature are emphasized.

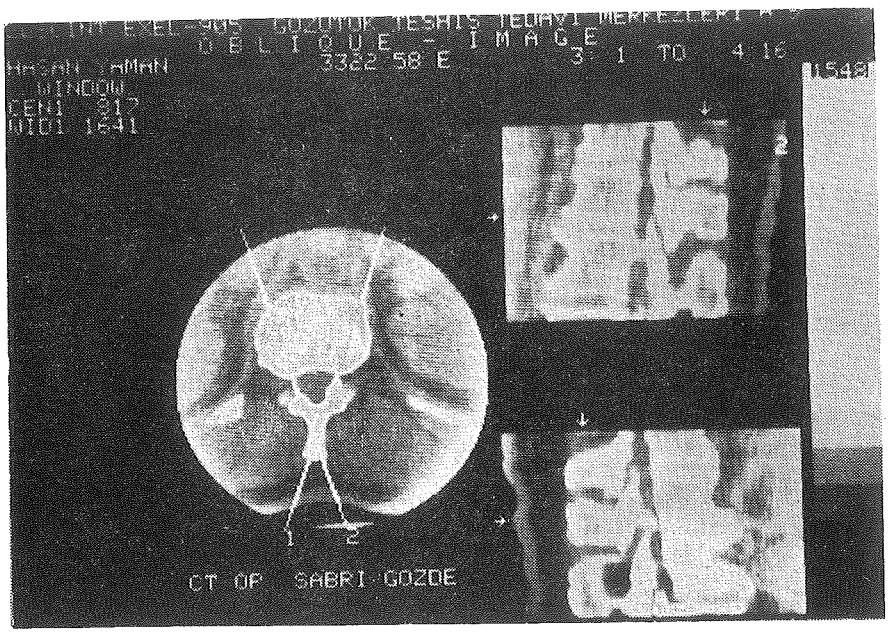

Figure 1

Sagittal CT. Bony spur formation at lower thoracal region.

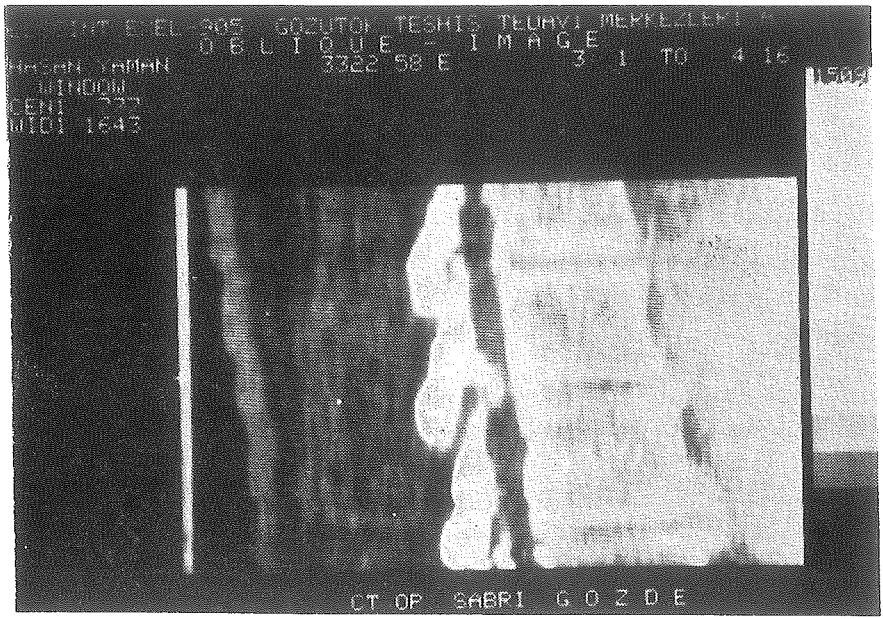

Figure 2

The spur formation developing from lamina towards left foramen and occluding the canal, in sagittal CT, the association with superior fascet hypertrophy is seen.

Case Report

An osteophyte with posterolateral localization and hypertrophy of superior fascet: A 58 year old male complaining about severe back pain radiating down to the left region of his low back. Although his complaints are chronic in nature, they have increased recently. Neurologic examination revealed sensoriel impairment, over the left part of the 9th and 10th thoracal dermatome area. In the following CT (Figures 1,2) spur formation with posterolateral localization towards the foramen (Th_9) and osteophytes over the anterior aspect of corpus vertebrae, and superior fascet hypertrophy at 10th thoracal level are detected. Later the follow up examinations, couldn't take place.

Discussion

According to the recent developments in CT neural foramen and its contents are being exposed very briefly.[1, 2] Therefore CT is accepted as an additional and very precious diagnostic method in these cases.[3]

Also it has been able for us to establish the spesific diagnosis in these type of lesions.

In the differential diagnosis of the lesions, in the neural foramen and in the extradural structures next to it, the following must be considered; lateral intervertebral disc herniations, superior articular fascet hypertrophy, lateral recess stenosis, benign and malign tumors, localized infections, granulomas, spondylolysthesis, congenital and cystic lesions.[1, 4-8]

The hypertrophy of supeior articular fascet involves the root around the neural foramen or lateral recess. In these cases, CT, reveals bony and ligamentous hypertrophy near the superior articular fascet.[1,5,9,10] Degenerative interlaminar joint disease is frequently present and narrowing of the joint space, irregularity over the faces of fascet joint, subchondral and synovial cysts are seen.[7]

Lateral recess stenosis is another pathology related with the nerve root.[4, 11, 12] Michael *et. al.* have found the depth of lateral recess approximately 3-4 mm in 35 cases. In none of the cases, the depth was more than 5 mm. Though lateral recess stenosis is present radiologically, in some cases, that appear asymptomatic, it is accepted that, as the root is localized at the medial of the recess, the compression findings are not revealed.[13]

Although it is known that posterolateral vertebral osteophytes which develop at the vertebral body can cause root compression, the development of osteophytic formation at the posterior region is quite rare as it is seen in our case (Figures 1,2).

In the cases of spondylolysthesis the nerve root is entrapped by the interarticular part and in the superolateral direction by the vertebral body. This condition requires the examination of the neural foramen by sagittal CT image, otherwise it is quite difficult to measure the diameter of the neural foramen by conventional axial CT images.[6, 14]

The tumors, either benign or malign, the edema in paravertebral structures infectious cases, and the calcifications in chronic cases, air-liquid densities around the neural foramen are all detected by CT.[6,15-17]

If this advantage of CT will be accepted as a routine diagnostic method in the cases of radiculopathy, a new therapeutic approach will be gained, on the behalf of Physical Therapy and Rehabilitation patients. Thus the patients who'll undergo surgical intervention will be distinguished more easily and accurately.

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Epidemiological and Practical Aspects of Enzyme Mediated Aminoglycoside Resistance

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Summary

The aminoglycoside antibiotics are one of the most interesting group of antibiotics, because of their complex interrelated chemical structures the large number of modification sites and their clinical importance. Several studies have implicated greater aminoglycoside resistance among gram-negative bacteria. Practical aspects of the aminoglycoside resistance is discussed in this review.

Key Words: Aminoglycoside antibiotics, Aminoglycoside resistance.

Introduction

The aminoglycosides antibiotics are one of the most interesting group of antibiotics, because of their complex interrelated chemical structures, the large number of modification sites and their clinical importance.[1-3] However, several studies have implicated greater aminoglycoside usage at a given institution with the development of progressive resistance among *Enterobacteriaceae* and *Pseudomonas aeruginosa*.[4-10] Mechanisms of resistance to aminoglycosides, in general, can be divided into two broad classes:

1. Resistance conferred by mutations in chromosamal genes. Resistance of this type may arise either due to alterations of the ribosomes[3, 11] or due to mutations that interfere with active transport of aminoglycoside antibiotics.[4, 5, 12]
2. Resistance specified by R-plasmids. Enzymes associated with these plasmids inactivate the aminoglycosides through covalent

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modifications. These modifications involve N-acetylation (aminoglycoside acetyltransferase (AAC)); O- phosphorylation (aminoglycoside phosphotransferase (APH)) and O-nucleotidylation (aminoglycoside nucleotidyltransferase (ANT)) at amino or hydroxyl of the aminocyclitol or aminosugar[2, 11, 13] (Table I). The sites of modification by aminoglycoside modifying enzymes are shown in Figure. 1.

TABLE I

AMINOGLYCOSIDE - MODIFYING ENZYMES

Modification	Enzyme	Total Substrates
Phospharylation	(APH)	
	APH (3′)	
	APH (3′)-I	Kanamycin, neomycin, lividomycin
	APH (3′)-II	Kanamycin, neomycin, butiresin
	APH (3′)-III	Kanamycin, neomycin, butirosin, lividomycin, amikacin
	APH (″)	Streptomycin
	APH (2″)	Gentamicin
	APH (5″)	Ribostamycin
	APH (6)	Streptomycin
Acetylation	(AAC)	
	AAC (2′)	Gentamicin, tobramycin, neomycin, netilmicin
	AAC (6)[a]	Gentamicin[b], tobramycin, neomycin, netilmicin, kanamycin, amikacin
	AAC (3)	
	AAC (3)-I	Gentamicin
	AAC (3)-II	Gantamicin, tobramycin, netilmicin
	AAC (3)-III	Gentamicin, tobramycin, neomycin, kanamycin
	AAC (3)-IV	Gentamicin, tobramycin, neomycin, kanamycin, netilmicin, apramycin
Adenylylation	(AAD)	
	AAD (4′)(4″)	Amikacin, tobramycin, kanamycin
	AAD (2′)	Gentamicin, tobramycin, kanamycin
	AAD (3″)(9)	Streptomycin, spectinomycin
	AAD (6)	Streptomycin
	AAD (9)	Spectinomycin

a. Subdivisions into types AAC(6′) I to AAC (6′) -IV was suggested.

b. Gentamicin C_{1a} and C_2 are substrates for AAC (6′), but not Gentamicin C_1.

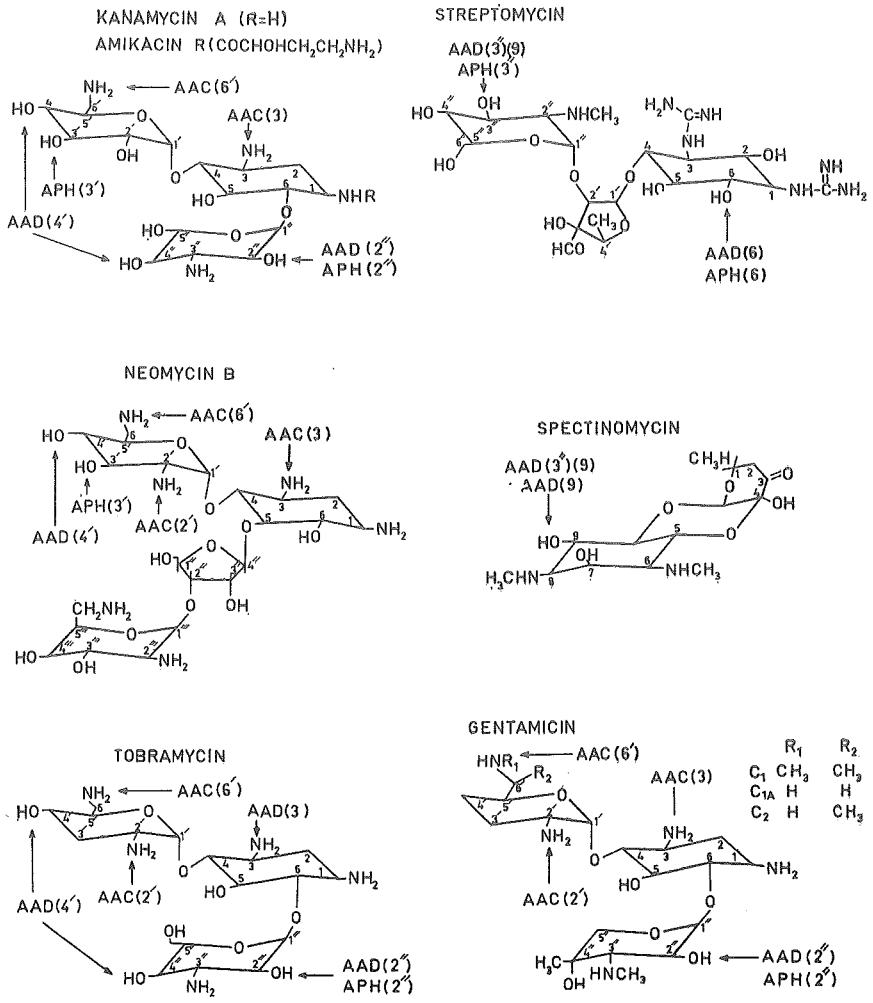

Figure 1
Structures of Some Aminoglycosides and Spectinomycin. The arrows indicate the sites of attack by aminoglycoside-modifying enzymes.

The resistance mechanism due to aminoglycoside modification is clinically the most singificant one.[11, 14-17] It is this form of resistance we emphasize in this review, not only because widespread occurrence of plasmid-determined resistance in clinical isolates as well as producing organisms, but because such studies have added more information to the understanding of genetic and biochemical aspects of biological systems and provided pharmacologists and chemists with information concerning the possibility of controlling bacterial infections.

Moreover, the discovery of aminoglycoside modifying enzymes in *Streptomyces* that appear to catalyze the same reactions as the enzymes that have been studied in clinical strains, raised interesting possibilities about the origin of the enzymes in these organisms.

Plasmid Association of the Structural Genes of Aminoglycoside Modifying Enzymes: The tendency for structural genes of modifying enzymes to be located on R-plasmids (Resistance Plasmids) greatly enhance their intergeneric and and intrageneric disseminations.[11, 18, 19]

These plasmids have a considerable strategic role in building up and maintaining resistant bacterial populations since many of them have the capacity of self-transmission (conjugative).[16, 20, 21] In some instances, a transferable R-plasmid can also promote the transfer of non-conjugative or non-transferable plasmids that are present in the same host by a process known as "mobilization".[12, 22, 23] On the other hand, if the determinants are chromosomal, this type of resistance cannot participate in facile transfer between different bacterial species or genera and resistance cannot spread throughout a bacterial population. By definition, resistance due to mutation, in any given set of circumstances involves a single bacterial species emanating from a single parent. Furthermore, mutationally altered bacteria are often metabolically defected (sick), and are at a selective growth disadvantage compared to their wild-type neighbours under normal growth conditions. In case of plasmid-determined resistance, the host cell is provided with new and often novel biochemical selective advantages which cannot necessarily be assumed as an extra genetic load. In fact, plasmids are often stably maintained in drug-free conditions, although their full genetic potential may not be expressed.[11]

In addition, plasmid replication is independent from that of chromosomal DNA and plasmid copy number per cell division may vary between 3 to 50.[20] That is why, plasmids, especially those of relaxed type (multi-copy plasmids) confer an increased level of drug resistance through "gene-dose effect".[2]

From epidemiological site, two features of conjugal transfer of drug resistance are specificially important:

(i) Conjugal transfer can result in a drug-sensitive pathogen becoming resistant by contact with a drug resistant non-pathogenic member of the normal gut flora. There are evidences that healthy members of the public are carrying R plasmid infected enteric bacteria, and it is highly probable that, they may produce refractory disease if they become established in the urinary tract, or alternatively, act as a source of R plasmid infection to a superinfecting, but sensitive pathogen,[24]

(ii) Pathogens can readily acquire new R plasmid associated resistance during the course of epidemics.[20]

Antibiotics appear to act in two ways to aid this transfer: (a) by providing an environment in which only drug-resistant bacteria can grow. That is, antibiotic usage maintains a selective pressure which helps a resistant bacterial population to emerge, (b) by destroying most of the resident gut bacteria, which are more suited to the conditions of gut and normally compete effectively against invading organisms.

Transposable Nature of the Structural Genes Coding for the Aminoglycoside Modifying Enzymes: The phenomenon of transposition is undoubtedly one of the most active factors in plasmid evolution; because it is the only known mechanism that allows exchange between non-homologous DNA molecules. During transposition, resistance determinants, essentially, translocate from one plasmid to another; or from a plasmid to the chromosome or to a bacteriophage.

Their discoveries give us a better understanding of construction of resistance plasmids from various genetic sources in nature and of assortment of resistance determinants among plasmids in the hospital environments. It now seems clear that multiresistance plasmids have emerged by the transposition of several discrete units to the same plasmid[25, 26] (Figure 2).

In the past few years, reports on the phenomenon of transposable multiresistance have accumulated and led to a detailed analysis of the elements involved. For example, a mobile genetic element designated Tn 1545, was detected in the chromosome of *Streptococcus pneumoniae* BM 4200 a clinical isolate multiply resistant to antibiotics. The 25.3 kb element was conjugative and conferred resistance to kanamycin and related aminoglycosides by synthesis of a 3-aminoglycoside phosphotransferase type III (aph A-3), to macrolide-lincosamide-streptogramin B-type antibiotics (erm A M), and to tetracycline (tet M).[27] These multiresistance transposons are of substantial clinical interest as they lead to a dissemination of several resistance genes "en bloc" and make the spread of such genes more efficient. The epidemiological significance of these elements was demonstrated by comparisons of their structural genetic relationship, and it was found that multiresistance transposons consist of a basic back bone structure.[28] This includes the genes encoding resistance to mercuric chloride, sulphonamids, and streptomycin/spectinomycin (AAD-3″) as well as the functions essential for transposition (tnp R and tnp A). In addition several different genes coding for important resistance mechanisms were connected to this backbone, such

as aminoglycosidemodifying enzymes like AAC (6′) (Tn 2424) and AAC (3) (Tn 1696) as linked to chloramphenicol resistance. General spread of multiresistance transposons within numerous plasmid incompatibility groups, on the other hand, may implay an important role of these elements in the evolution of large R-plasmids.

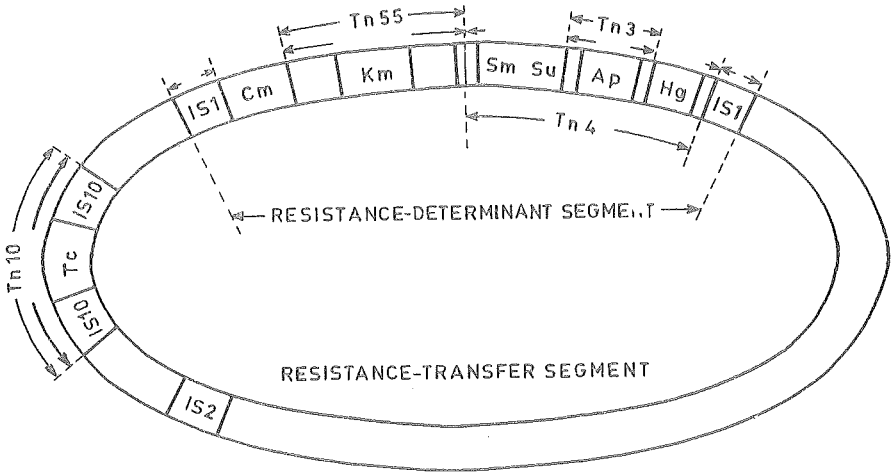

Figure 2

Role of Transposable Elements in the Evolution of Antibiotic-Resistance Plasmids. The plasmid appears to have been formed by the joinining of a resistance-determinant segment and a resistance transfer segment: there are insertion elements (ISI) at the the junctions, where the two segments sometimes dissociate reversibly. Genes encoding resistance to the antibiotics chloramphenicol (Cm), kanamycin (Km), streptomycin (Sm), sulfonamide (Su) and ampicillin (Ap), and to mercury (Hg) are clustered on the resistance-determinant segment, which consists of multiple transposable elements; inverted-repeat termini are designated by arrows pointing outward from the element. A transposon encoding resistance to tetracycline (Tc) is on the resistance-transfer segment. Transposon Tn 3 is within Tn 4.

Genes coding for aminoglycoside modifying enzymes, ANT (2″), AAC(3)-I, AAC (3)-IV, AAC (3)-III, AAD (3″) (9), APH (3′)-II and APH (3′) (5″)-I are located on transposons.[29, 31] The latter enzyme is one of the modifying activities most commonly found in nature and at least six different transposons encoding this type of enzyme have been reported (Table II).

These elements contain closely related structural genes.[29] Nucleotide sequence analysis revealed that a number of genes for aminoglycoside modifying enzymes (e.g., AAC (3)-IV, hygromycin B phosphotransferase) were expressed under the control of a same promoter; –35 region of which resides within IS elements in natural plasmids, such as R-plasmids

pWP 76, pWP14 a etc.[32, 33] The existance of such portable promoters in natural plasmids indicates that genes might become more effectively expressible by increasing the production rate of the gene product. Furthermore; genes could acquire by this type of natural "genetic engineering" new types of regulatory mechanisms or could adapt to the expression apparatus of a new host organism after conjugal or other transfers. Thus, gene expression seem to be no barrier to gene exchange between diverse group of organisms.

TABLE II

TRANSPOSONS ASSOCIATED WITH APH (3′) (5″) ACTIVITY

Transposon	Original Host
Tn 6	*E. coli*
Tn 601	*E. coli*
903	
Tn 602	*B. ochraceus*
Tn 1521	*H. influenza*
Tn 1699	*K. pneumonia*
Tn 2350	*S. paratyphi*

Cross - Resistance: Broad substrate ranges of aminoglycoside modifying enzymes lead to a well known problem "cross-resistance" in medicine.

Some enzymes only modify the structurally related compounds. For example, the APH (2″) enzyme from Gram-positive cocci inactivates extensively the 4,6 disubstituted deoxystreptamines.[33]

Other modifying enzymes can detoxify structurally unrelated aminoglycosides; 3″(9)-0-nucleotidyl transferase (AAD (3″) (9)) modifies both streptomycin and spectinomycin and determines resistance to both drugs.[3] This unusual cross-resistance pattern was explained when it was realized that, these apparently unrelated compounds share a small structural moiety including an hydroxyl group that is enzymatically adenylylated by the enzyme.

Due to overlap in the substrate ranges of the enzymes, different activities can modify the same compound. Kanamycin B can be detoxified by seven enzymes: AAC (3), AAC (2′), APH (3′), AAD (4′) (4″), AAC (6′), AAD (2″), and APH (2″).[29] A single aminoglycoside molecule can therefore, select various aminoglycoside modifying enzymes and some of the commercially available aminoglycoside antibiotics are a mixture of compounds (Table III). The problem of in-vivo selection and subsequent spreading of resistance is complicated by the fact that in most

cases aminoglycoside resistant strains harbour one or several plasmids that encode more than one modifying enzyme. The possibility exist therefore of selecting various enzymes with an antibiotic that is not even a substrate.

TABLE III

APPROXIMATE COMPOSITION OF COMMERCIALLY AVAILABLE AMINOGLYCOSIDE COMPLEXES

Gentamicin	C_{1a}	26 %
	C_1	41 %
	C_2	33 %
	C_{2b}	very low
Kanamycin	A	96–99 %
	B	4 – 1 %
Neomycin	B	80–90 %
	C	20–10 %
Lividomycin	A	99 %
	B	1 %
Butirosin	A	85 %
	B	15 %

Alternatively, cross-resistance to unrelated antibiotics may develop because of the presence of R-plasmids that encode multiple drug resistance as a result of linked genetic markers or by the transposition of linked resistance elements carrying more than one resistance gene.

A typical example is the trimethoprim-streptomycin resistance transposon[12], the two drugs are completely unrelated in structure, in mode of action, and in their resistance mechanisms. The use of one antimicrobial agent can lead to the development of resistance to totally unrelated antibiotic. This is probably the reason why R-plasmid coded streptomycin resistance is still common in bacteria, even though the drug is rarely used in clinical situations. Another unexpected cross-resistance pattern is macrolide-lincosamide-streptomycin (mcs) resistance in gram-positive organisms.[34]

Besides its enhanced capacity to resist many antibacterial agents, such an endemic population of cross-resistant bacteria, also would resist antibiotics which have not yet introduced into standard clinical practice.[5, 35, 36]

Improvements in Aminoglycoside Therapy:

One of the most important recent developments in the chemotherapy of bacterial infections is the introduction of antibiotic derivatives which evade existing resistance mechanisms.

New aminoglycoside antibiotics which are refractory to commonly encountered plasmid-specified modifying enzymes are now available. The discovery of 4-amino-2-hydroxybutyryl (AHB) side chain in butirosin and related antibiotics led to synthesis of a vaiety of 1-substituted derivatives (such as amikacin and netilmicin) which have excellent therapeutic potential, since they are all inert to a number of aminoglycoside modifications.[3]

Another improvement in this field has been the synthesis of 5-epi-isomers of gentamicin B and sisomicin. A more detailed study of the modifying enzymes in susceptible strains showed that thay had much increased Km values (100 times in some cases) for the 5-epi derivaties; the 5- epi compounds were much less effectively modified than the parent antibiotics.[37]

Since the establishment of aminoglycoside resistance by plasmid determined modifying enzymes is so dependent on the Km value for the antibiotic, it seems reasonable to propose that the development of competitive inhibitors for the aminoglycoside-modifying enzymes could have important chemotherapeutic application. Such inhibitors need not in themselves have antibacterial activity, but used in combination with other aminoglycosides. They could sufficiently reduce the Km value for the drug to render resistant bacteria susceptible to antibiotic treatment. For example, 7-hydroxytopolone, which interferes with AAD (2″) activity was suggested to increase aminoglycoside longevity.[37]

However, it seems unlikely that aminoglycoside resistance will be completely contained in this way. The drugs have several sites presenting potential targets for modifying enzymes. It may prove impossible to block or remove all potential modification sites without detracting from antibiotic activity. It seems likely that bacteria will evolve resistance to most, if not all, antibiotics with which they are challenged.

Modification Enzymes and Genetic Engineering: Aminoglycosides, with broad inhibition spectra have proved as cross-species selective agents in gene cloning experiments involving bacteria, plant and mammalian cells. For example, *Escherichia coli*-yeast shuttle vectors containing kanamycin (and G 418)-resistance gene of Tn 903 (encodes APH (3′) I) were constructed and used for the transformation of *Kluyveromyces lactis*, G 418 resistance being a direct selection marker for transformants.[38, 39] Another recombinant plasmid with wide-host-range transfer functions, carried the same transposon (Tn 903), transferred kanamycin resistance to a number of plant associated pseudomonads.[40] In last few years, there has been some attempts for transformation of higher-plant pro-

taplasts, to regenerate aminoglycoside-resistant clones. A chemeric vector pKR 612 B 1 was developed by Balazs *et al*, containing the neomycin phosphotransferase (APH) gene from the Tn 5 transposon under the control of the gene VI promotor of cauliflower virus (CaMV).[41] A large number of turnip and tobacco protoplasts were transformed and proved to be resistant to kanamycin (Km). From tobacco protoplasts whole Km-resistant plants were regenerated and shown to contain the integrated foreign gene. In a different study, liposome mediated transformation of tobacco mesophyll protoplasts by *E.coli* recombinant plasmid (pLGY 23 neo) conferring kanamycin resistance (APH (2′)) was achieved.[42] Kanamycin resistant plants regenerated from resistant clones carried in their genome sequences homologous to the plasmid used for transformation. All these effords are promising for broad-host-range vector development, and use of aminoglycoside resistance markers to select for intergeneric gene transfer.

Recently DNA hybridization methods were described to follow specific modification determinant in clinical strains. For this purpose a DNA restriction fragment, isolated from a modification enzyme gene cloned into a suitable vector and radiolabelled, was used as probes for rapid screening large numbers of clinical strains for this resistance determinant. DNA probes for the APH (3′) II structural genes[43] and for ANT (2″) gene[44, 45] were developed and proved to be highly specific and was more sensitive than enzymological techniques.

The development of a bank of specific DNA probes for a variety of aminoglycoside resistance genes could be important in epidemiological studies of antimicrobial resistance and could provide insight into the modulation of such resistance within hospital environments.

Modification Enzymes in Aminoglycoside Producing Organisms: Many aminoglycoside producing microorganisms contain modifying enzymes that are similar to those that determine antibiotic resistance in clinical isolates. Benveniste and Davies[3], and Walker and Skorvaga,[46] first time, proposed that aminoglycoside resistance determinants ancoded extrachromosomal elements of clinical isolates had their origins in antibiotic producing organisms. In favor of this hypothesis, for example, the R- plasmid specified enzyme APH (3′) of neomycin and kanamycin resistant bacteria may be related to the *Streptomyces fradiae* enzyme, the APH (6) of streptomycin resistant *Streptococci* may be derived from the *S.griseus* and *S.bikiniensis* determinants; and the APH (4) of hygromycin-resistant *E.coli* may be related to the *S. hygroscopicus* enzyme.[2] Recent DNA sequencing studies have indicated strong homologes at the protein sequence level in several domains of the APH (3′) enzymes from *Strep-*

tomyces fradiae and *E.coli*.[47] It is also possible that some acetyltransferases evolved in this way. For example, an extract of *S.spectabilis* (which makes spectinomycin) had an enzyme like gentamicin acetyltransferase II, and *S.kanamyceticus* (which makes kanamycin) contained an enzyme like kanamycin acetyltransferase.[2] Although plasmid determined nucleotidylation of aminoglycosides appear to be fairly common in clinical isolates, such activities have not been detected in any of the antibiotic producing strains so for examined.[48, 49]

The precise role of the aminoglycoside modifying enzymes in producing strains is obscure. One of the possible roles ascribed to modifying enzymes in *Streptomyces* spp is that they are involved in the biosynthesis of the antibiotics they produce. Davies *et al.*[48] Suggested that 3'-phosphotransferase is required for neomycin production in *S. fradiae*. N-acylated and O-phosphorylated aminocyclitols have been isolated from several producing organisms, although in small quantity. It is not known if production of these compounds indicates a role for modifying enzymes in the biosynthetic pathway to produce suitable intermediates protected or blocked or whether they are non cleaved inactiveted products or both.

An attractive hypothesis for the role of aminoglycoside modifying enzymes in producers has been the protective mechanism to avoid suicide. Most of the aminoglycoside producing organisms possess enzymatic activities which modify the antibiotis they produce[50] and certain of the such organisms have ribosomes which are fully sensitive to tha drug, for example, the neomycin producing *S. fradiae* [51, 52]. Besides with standing the effects of extracellular drug, producing organisms must also protect themselves against intracellular metabolites during aminoglycoside biosynthesis. Those which experience ribosomal modification face no such problem, but others must either confine toxic products within discrete subcellular compartments or produce them as inert derivatives to be activated during or following export. The latter procedure is probably quite common as, for example, in the biosynthesis of streptomycin.[50] As shown by Walker and Walker[53] streptomycin is made in the cell as streptomycin-6-phosphate and cleaved by a phosphatase on secretion so that the main medium component, is unmodified streptomycin.

An additional aspect, is the role of the resistance mechanism in antibiotic production. There are evidences supporting a causal relationship between resistance and production levels. High level aminoglycoside resistant strains are often overproducers[54], and loss of resistance is associated with loss of production.[55] Hotta *et al*[49] have characterized 200 Actinomycetes in terms of resistance to 11 different aminoglycosides

and noted that soil actinomycete isolates in Japon had a wide varieties of multiple aminoglycoside resistance which might be the likely candidate for production of aminoglycosides. In their subsequent reports they have demonstrated that aminoglycoside resistant actinomycetes had a higher probability of antibiotic production than aminoglycoside susceptible isolates thus establishing a relationship between aminoglycoside resistance and aminoglycoside productivity.[56] Under the light of these and other experimental results. Davies and Crameri[57] postulated that increased production of the modifying enzymes leading to the increased levels of resistance to the drug may provide a means of augmenting production of the drug. For this purpose, they have transformed *S.lividans* and *S.kanamyceticus* protoplasts with cloned AAC (6′) gene. Transformants carrying AAC (6′) gene showed 3-4 times more resistance towards the aminoglycosides compared to untransformed wild-type. Fermentation of transformants of both strains produced 3-4 times more antibiotic (e.g. kanamycin or neomycin). But, it has also been stated that it might not be correct to rely upon antibiotic inactivation as sole means of self production in high-level producers. In the absence of a reasonably effective permeability barrier, such organisms would need to inctivate the bulk of the antibotic produced which, in addition to precluding their status as high-level producers, might be energetically unfeasible.[50]

Concluding Remarks: The uses of genetical and biochemical analysis of enzyme mediated aminoglycaside resistance are several fold, especially from pratical point of view. Firstly, identification of the particular enzyme present may aid the rational choice of alternative chemotherapy and it is also important because the genes encoding for certain enzymes may be trasferred between species and between the plasmids. Secondly, such studies provide information for the development of antibiotics that are refractory to enzymatic modifications and active against some or all resistant strains of bacteria. Thirdly, a detailed analysis of the resistance determinants (on the molecular basis), both in clinical strains and antibotic producers, could serve for a better understanding of the possible origin of R-factor. Finally, by use of the techniques of recombinant DNA technology, genes for modification enzymes could serve as selective and insertional inactivation markers which inturn add more to the effords of the development of a rationale vector.

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Immunopathogenesis of the HIV-I Infection and Immune Defects in Patients with AIDS

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Summary

The etiologic agent of AIDS, HIV-I causes various quantitative, qualitative and serologic abnormalities in both cellular and humoral components of the immune system. The selective tropism of the virus to the helper/inducer T lymphocyte subset is the main cause of these abnormalities because of its central role on regulating the effector responses of other cell types of the immune system. The lack of helper function(s) on B lymphocytes and inducer function(s) on cytotoxic T lymphocytes, natural killer cells, monocyte/macrophages causes many functional abnormalities. However today it is well known that the virus may also effect monocytes, B cells and cytotoxic T lymphocytes. In spite of survival of these infected cells, loss of their functional abilities are also responsible for the immunologic abnormalities. On the basis of accumulated scientific knowledge, pathogenesis of this syndrome and immunologic abnormalities are outlined.

Key Words : Acquired Immune Deficiency Syndrome, HIV-I, Humoral immunity, Cell mediated immunity.

Introduction

The etiologic agent of AIDS, HIV-I, is a human lymphotropic retrovirus. This virus has al selective tropism to the helper/inducer T lymphocytes.[1] Helper/inducer T lymphocytes contain CD 4 (T4) antigen on their surfaces.[2] This CD 4 antigen or possibly a closely associated structure is the site of virus binding.[4]

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After the cells of the immune system are infected with HIV-I, a cytolytic effect and/or modification of the normal cell function will occur. This effect depends on the nature of the infected cell.[1] The virus causes a cytolytic effect on the helper/inducer T lymphocytes. It also effects monocytes, B cells and cytotoxic T lymphocytes in such a way that in spite of their survival they lose their functional abilities.[1]

HIV-I proteins may also have direct inhibitory activity on the immune system. These proteins effect their inhibitory activity presumably by interfering with the production of IL-2 or its interaction with T cells.[1, 5]

Various quantitative, qualitative and serologic immunological abnormalities involving both the humoral and cellular components of the immune system have been reported in patients with AIDS.[6]

Quantitative Abnormalities of T Lymphocytes

Lymphopenia is one of the first and most frequently reported clinical observation. This lymphopenia is especially selective to the T4 cell subset.[6] On the other hand prior infection with CMV or HIV-I or coinfection causes an increase in cytotoxic cells, an increase in the number of activated T cytotoxic/suppressor cells and a marked increase in the natural killer cells and their presumed precursors.[7] At the advanced stages of the disease however the T8 cell subset may decrease, and therefore the T8 cell subset varies in number depending on the stage of the disease. In all situations the ratio of T4/T8 cell decreases because of the prominent decrease of the number of the T4 cell subset.[6]

The mean CD4 (T helper/inducer) cell levels however, are decreased relatively little (about 20%) at the time when antibody was first found. Mean CD4 cell levels continue to fall in serial testing of the seroconvertes. AIDS develops in those seropositive individuals with the lowest CD4 and highest CD8 levels. Those individuals that are seropositive for several years but free from disease, show reduced CD4 levels but many do not show further progressive reduction of CD4 cell numbers. These individuals may have significant resistance to HIV-I damage or may lack cofactors such as other viral infections, or may not indulge in behavioral and sexual practices, which promote damage.[8] Furthermore many other cell types are now known to be more or less susceptible to HIV-I. The retrovirus may also effect directly monocytes, macrophages, B cells, and even the hematopoeitic stem cells. The functions of these cells are also effected by the lack of helper and inductive function of CD4 cell for these cells.[1, 9] These cells may serve as a viral reservoir.[9]

Functional Abnormalities of T Lymphocytes

At the begining of the disease the T cell functional defects may not be detected easily. In this setting, however, T cell colony assay shows a T cell proliferation defect in response to PHA and PHA +IL-2 stimulation during asymptomatic HIV-I infection.[10] Recently it has been shown that there is a direct relationship between the decrease of the lymphocyte proliferation in response to PWM (pokeweed mitogen) and the development of clinical symptoms in HIV-I antibody positive individuals.[11] Recently a functional defect in the clonal expansion of the purified T4 inducer/helper and T8 suppressor/cytotoxic cells has been found in patients with AIDS.[6] On the other hand Lane and associates have very recently shown that, mitogenic response to the spesific soluble protein antigen, tetanus toxoid, is markedly reduced while the mitogenic response to PWM is normal in patients with AIDS.[6, 12] There are also defective autologous mixed lymphocyte responses in the helper/inducer subset in patients with AIDS.[13] These autoreactive cells are believed to represent an antigen reactive cell.[14]

There is defective natural killer cell activity in the syndrome and this defect can be shown by the decrease of natural killer cell lysis of K 562 tumor target cells.[15] On the other hand there also is defective T cell mediated virus spesific cytotoxicity and this defect can be shown by the decrease in specific killing of CMV infected target cells.[15] This decreased virus spesific cytotoxicity is not due to a decrease in the number of circulating cytotoxic cells. Precursors to these effector cells circulate in the peripheral blood of patients, but maturation of these cells into cytotoxic cells is arrested during an interleukin-2 dependent phase. It has been shown that the sera from these patients contains an inhibitor of interleukin-2 production. Although the nature of this inhibitor is not known, it is not interferon, an immune globulin, immune complex, lymphotoxin or viral protein.[16] Furthermore it is probable that the defective T4 inducer function results in cytotoxic defects, because cytotoxic cells require an inductive signal from the T4 cell to express optimal function.[15]

Murray and associates have reported that spesific interferon production was diminished in mononuclear preparations stimulated with mitogens and even absent in cells stimulated with spesific microbial antigens.[17] Lack of these lymphokines may be responsible for the observed defects.[6] On the other hand interleukin-2 receptor expression in bulk T cell preparations have been found decreased.[18] Bowen and and associates however, have found normal total interferon and interleukin-2

production in mononuclear cell preparations in the early stages of the syndrome.[6]

Functional Abnormalities of B Lymphocytes

Hypergammaglobulinemia associated with polyclonal B cell activation is commonly seen in HIV-I infected states.[6, 19] This activation causes an elevation of serum Ig G and Ig A, an elevation of circulating immune complexes and an inability to produce an antibody response to in vivo immunization with primary and secondary antigens. The polyclonal B cell activation also causes numerous autoimmune phenomena.

In addition, many functional B cell defects have been identified in vitro. These defects include increased numbers of spontaneous plaque-forming cells, enhanced responsiveness to T cell dependent (PWM) and T cell independent mitogens (Staphylococcus aureus Cowan 1) and enhanced responsiveness to B cell growth factors in the absence of exogenous activation signals, elevated levels of spontaneous proliferation and a propensity for the development of transformed B cell lines.[6, 19]

In a study by Pahwa and associates, both glycoprotein rich and glycoprotein poor nonviable antigenic components of HIV-I have been found to exert stimulatory as well as inhibitory influences on the B lymphocytes.[20] Such influences may be partially responsible for B lymphocyte dysfunction in addition to the defective helper function on the T4 cells.[20]

Functional Abnormalities of Monocytes

There are also monocyte functional defects in patients with AIDS. These defects occur partly due to the direct effect of the virus and partly due to the lack of inductive function of T4 lymphocyte for the monocyte effector functions.

Defective in vitro chemotaxis and extracellular killing of the parasite giardia lamblia has been demonstrated. This monocyte cytotoxicity is not reversed by the addition of interleukin-2 to the culture. Intracellular killing of Toxoplasma gondii in vitro by monocytes has also been found defective but this defect can be enhaced by the addition of gamma interferon to the culture.[6]

It has been shown that spontaneous secretion of interleukin-1 and prostaglandin E_2 is elevated. Moreover there is a depressed response to the usual inducers of interleukin-1 secretion in monocytes from patients with AIDS.[21]

TABLE I

THE REPORTED ABNORMALITIES OF IMMUNITY IN THE AIDS,[6,22,24]

Quantitative Abnormalities of T lymphocytes
- Decreased numbers of peripheral T4 (Leu-3) inducer/helper cells
- Variably altered numbers of T8 (Leu-2) suppressor/cytotoxic cells

Functional Abnormalities of T Lymphocytes
- Host susceptibility to opportunistic infection
- Host susceptibility to unusual neoplasms
- Diminished delayed-type hypersensitivity responses
- Elevated spontaneous proliferation
- Decreased proliferative responses to mitogens and antigens in mononuclear preparations in vitro
- Normal proliferative response to mitogens in purified T4 and T8 lymphocyte subsets with abnormal responses to spesific antigen
- Decreased cytomegalovirus-spesific cytotoxic lymphocyte function
- Decreased ability to provide help to B lymphocytes for immunoglobulin production
- Diminished lymphokine production, especially in response to spesific antigenic stimulation
- Depressed clonal expansion of both T4 and T8 lymphocyte subsets

Functional Abnormalities of Natural Killer Cells
- Decreased in vitro cytotoxicity for the tumor cell line, K 562
- Responsiveness to short incubations with interleukin-2 in the enhancement of natural killer-mediated K 562 cell lysis

Functional Abnormalities of B Lymphocytes
- Elevated serum immunoglobulin levels
- Circulating immune complexes
- Inability to mount a serologic response following de-novo immunization or new infection
- Autoantibodies against various tissues including platelets, peripheral nerve tissues
- Elevated spontaneous proliferation
- Elevated numbers of spontanous plaque-forming cells in the peripheral blood
- Refractoriness to T-cell-dependent (pokeweed mitogen) or independent (Staphylococcus aureus Cowan 1 mitogen) in vitro signals for B cell activation, proliferation and/or differentiation
- Severely diminished in vitro response to de-novo antigen, keyhole limpet hemocyanin

Functional Abnormalities of Monocyte/Macrophages
- Diminished chemotaxis
- Decreased in vitro extracellular killing of Giardia lamblia
- Decreased in vitro intracellular killing of Toxoplasma gondii, enhanced by in vitro culture with gamma-interferon
- Lack of response to usual inducers of interleukin-1 production
- Spontaneously increased interleukin-1 secretion
- Spontaneously enhanced prostaglandin E_2 production

Serologic Abnormalities
- Supressor factors in sera
- T-cell-derived suppressor substances
- Possible antilymphocyte antibodies
- Acid-labile alpha interferon
- Elevations in beta-2-microglobulin
- Elevations in alpha-1 thymosin
- Decreased serum thymulin levels
- Increased erythrocyte adenosine deaminase activity
- Decreased erythrocyte C3b receptor, CR 1

Serologic Abnormalities

Various serologic abnormalities have been described in patients with AIDS. These abnormalities include elevated beta-2 microglobulin, elevated alpha-1 thymosin, decreased serum thymulin levels, and the presence of an acid labile form of alpha interferon.[6]

Suppressive factors have also been found in patients with AIDS. Although the nature of these suppressive factors is not yet known they suppress mixed lymphocyte reaction, T cell mitogenic and antigen spesific responses and B cell immunoglobulin differentative function.[6]

Very recently it has been observed that, there is an increase in erythrocyte adenosine deaminase (ADA) activity in patients with AIDS, ARC and asymptomatic patients at risk of infection with HIV-I. This increase may occur prior to seroconversion in asymptomatic patients.[22]

The C3b receptor, CR 1, on erythrocytes has also been found to be decreased in patients with AIDS and ARC when compared to normal.[23]

The reported abnormalities of immunity in the Acquired Immunodeficiency Syndroma can be outlined as Table I.

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The Effect of Air Pollution on Respiratory Functions

Oğuz K. Başkurt, M.D.* / Dicle Balkancı, M.D.* / Selma Yörükan, M.D. / S. Orhan Andaç, M.D.*****

Summary

Respiratory function tests were carried out on students who resided in and received their education at the Hacettepe and Beytepe campuses of Hacettepe University in December 1984. Tests were repeated in April 1985 on some of the students who had participated in the study.

Air pollution levels of these campuses were studied between December 1984 and April 1985. The mean sulfur dioxide levels of Hacettepe and Beytepe campuses were 412 $\mu gr/m^3$ and 91 $\mu gr/m^3$, respectively.

In the non-smoker group, the forced vital capacity and maximum mid-expiratory flow rate values of the students living in the heavily polluted region were found to be low when compared with the same values of the students living in the clean air region. On the other hand, in the smoker group, the flow rate values determined in the heavily polluted region were found to be high when compared with the values which were measured in the clean air region. This finding cannot be accounted for completely, but a possible adaptation phenomenon is discussed.

When test results obtained in December 1984 and April 1985 were compared, the differences were found to be more prominent in the Hacettepe group.

Key Words: Air pollution, Respiratory function tests, adaptation.

Introduction

Air Pollution, one of the most important problems of modern cities, has arisen largely as a result of rapid and disorganized urbanization.

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Various substances which contribute to air pollution are generally known as irritants. Since the repiratory system is most closely associated with the atmosphere, it is the system which will obviously be most seriously affected.

Irritant substances are classically described as those which have an injurious effect on tissues and cause inflammatory response.[1] However, it has been observed that in order for pollutants to cause injury and produce inflammatory responses in the respiratory system, they must be present in high concentrations and affect people for long periods. Since these pollutant doses are generally much higher than the levels measured in air pollution caused by urbanization, more sensitive methods must be used in measuring the effects of air pollution. This necessity has led the investigators to employ functional rather than pathological parameters.

As a result of these trends, the meaning of the term "irritant" has undergone a change. Irritants are now being defined as those substances which cause functional changes as well as injury and inflammation.[1] As for air pollutants, substances which cause changes in the respiratory function parameters have begun to be regarded as irritants.

Hence, respiratory function tests are the most frequently used method for investigating the effects of air pollution on urban populations. Among the respiratory function tests, those most sensitive to air pollution are the air flow rate tests.[2]

In this study, the effect of air pollution on respiratory functions was studied during the Winter of 1984-1985 with special emphasis on air flow rates.

Materials and Methods

This study was conducted on 147 volunteer male students attending Hacettepe University, 78 students who resided on the Hacettepe Campus in the center of the city comprised the group predicted to be exposed to air pollution. 69 students who resided at the Beytepe Campus situated on the outskirts of the city were taken as the control group.

Average ages of the student groups of Hacettepe and Beytepe Campuses were 19,5 and 20,2 respectively. 12 % of students in both groups regularly took part in sporting activities, 41 % of the Hacettepe student group and 49 % of Beytepe student group smoked cigarettes. Of the smokers in the Hacettepe group 59 % smoked 15 or more cigarettes a day, while in the Beytepe group this percentage was 82 %, and 47 % of the smokers in the Hacettepe group and 75 % of those in the Beytepe group had been smoking for 3 years or longer.

Respiratory function tests of the students were carried out on the two campuses using a Collins 9 Liter Respirometer. The tests were carried out in the sitting position, using a nose clip. The guidelines set out by the European Community for Coal and Steel, Standardization of Lung Function Tests Working Party,[3] were followed in the evaluation of respiratory functions. Measured volumes were corrected to body temperature and saturated pressure (BTPS).

The FVC values were compared with the tables prepared by Baldwin *et.al*,[4] and expressed as percentages of the standard value (FVC N %). The FEV_1 values were shown as percentages of the FVC of the same subject (FEV_1 /FVC %), $FEF_{200-1200}$ and FEF_{25-75} % values were expressed as liters per second.

The first tests were carried out between the 5th and 14th of December 1984 on both campuses when the air pollution in the Hacettepe Campus was at a high level. Between the 18th and 29th of March 1985, during a period of lower air pollution in Hacettepe, the procedure was repeated on 29 students from the Hacettepe group and on 22 students from the Beytepe group.

In addition, the sulfur dioxide content of the air in the Hacettepe campus (chosen as a polluted area) and the Beytepe campus, (chosen as the control area) were measured during the period between the 5th of December 1984 and the 15th of April 1985, employing the Hydrogen Peroxide - Acid Titration technique.

The results were evaluated by computer using the Student's "t" test.

Results

Air Pollution: The mean weekly sulfur dioxide values measured in the Hacettepe and Beytepe campuses are shown in Figure 1. The values measured in both campuses correlated well with each other (r: 0,89), but the sulfur dioxide level in the Beytepe campus did not exceed 200 $\mu gr/m^3$ at any time. During the study period, the mean sulfur dioxide level of the Hacettepe campus was found to be about 4.5 times the level measured in the Beytepe campus.

Between the 5th and 14th of December 1984, when the first respiratory function tests were carried out, the mean sulfur dioxide levels were 690 $\mu gr/m^3$ in the Hacettepe campus and 144 $\mu gr/m^3$ in Beytepe. Between the 18th and 29th of March 1985, when the respiratory function tests of some of the students were repeated, the mean sulfur dioxide levels were 256 $\mu gr/m^3$ and 52 $\mu gr/m^3$ respectively.

Respiratory Function Tests: The results of respiratory function tests which were carried out in December 1984, are shown in Table 1.

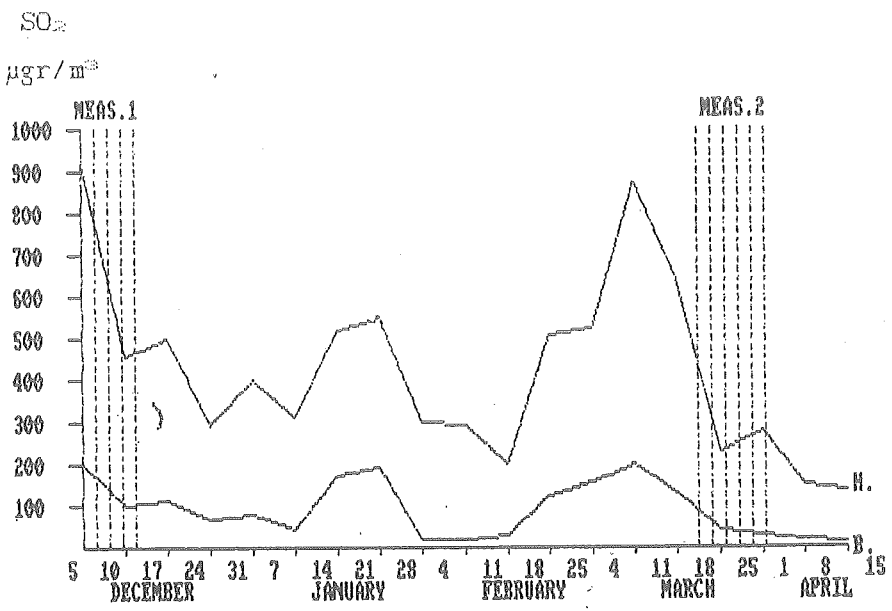

Figure 1
Sulfur dioxide levels of Hacettepe and Beytepe campuses during the research period.

TABLE I
COMPARISON OF RESPIRATORY FUNCTION TESTS OF SMOKERS AND NON-SMOKERS CARRIED OUT IN DECEMBER 1984

		Hacettepe	Beytepe	
FVC N % (%)	Non-Smoker	110.5 ± 2.86 n: 46	117.6 ± 1.99 n: 35	p = 0.022
	Smoker	116.7 ± 2.99 n: 32	119.4 ± 2.28 n: 34	p = 0.237
		p = 0.069	p = 0.273	
FEV_1/FVC % (%)	Non-Smoker	85.0 ± 1.53 n: 46	86.6 ± 2.17 n: 35	p = 0.274
	Smoker	88.5 ± 1.42 n: 32	83.2 ± 1.98 n: 34	p = 0.016
		p = 0.048	p = 0.090	
$FEF_{200-1200}$ ($L.sec^{-1}$)	Non-Smoker	5.43 ± 0.28 n: 46	6.03 ± 0.28 n: 35	p = 0.066
	Smoker	6.03 ± 0.30 n: 32	5.63 ± 0.28 n: 34	p = 0.166
		p = 0.073	p = 0.158	
FEF_{25-75} % ($L.sec^{-1}$)	Non-Smoker	4.78 ± 0.22 n: 46	5.44 ± 0.24 n: 35	p = 0.023
	Smoker	5.88 ± 0.29 n: 32	5.08 ± 0.26 n: 34	p = 0.022
		p = 0.001	p = 0.320	

The FVC N % and FEF_{25-75} % values of the non-smoker students residing at Beytepe were significantly higher ($p < 0.05$) than the same values of non-smoker students residing at Hacettepe. In the smoker group, the air flow rate parameters in the Hacettepe group were higher. Differences between FEV_1/FVC % and FEF_{25-75} % values were significant ($p < 0.02$).

With the measurements made on the Hacettepe Campus, it was found that FEV_1/FVC % and FEF_{25-75} % values of the smokers were significantly higher than the values of non-smokers.

Respiratory function test results obtained from some of the students at a repeat session in March 1985 are shown in Table II. According to these results, none of the respiratory function parameters measured in Hacettepe and Beytepe groups were significantly different either in December or March. Also, in the Beytepe group, the differences between March and December measurements were small and not significant. However, in the Hacettepe group the differences were more prominent and for the $FEF_{200-1200}$ values the difference was significant ($p < 0.05$).

TABLE II

COMPARISON OF RESPIRATORY FUNCTION TESTS CARRIED OUT IN DECEMBER 1984 AND MARCH 1985

		Hacettepe	Beytepe	
FVC N % (%)	December	109.5 ± 3.71 n: 29	114.4 ± 2.88 n: 22	p = 0.074
	March	105.7 ± 3.38 n: 29	108.6 ± 2.67 n: 22	p = 0.251
		p = 0.226	p = 0.073	
FEV_1/FVC % (%)	December	88.0 ± 1.55 n: 29	86.7 ± 2.17 n: 22	p = 0.314
	March	91.0 ± 1.47 n: 29	88.2 ± 1.49 n: 22	p ± 0.093
		p = 0.082	p = 0.285	
$FEF_{200-1200}$ ($L.sec^{-1}$)	December	5.65 ± 0.35 n: 29	5.98 ± 0.42 n: 22	p = 0.274
	March	6.45 ± 0.31 n: 29	6.87 ± 0.42 n: 22	p = 0.212
		p = 0.046	p = 0.070	
FEF_{25-75} % ($L.sec^{-1}$)	December	5.12 ± 0.33 n: 29	5.54 ± 0.33 n: 22	p = 0.186
	March	5.57 ± 0.29 n: 29	5.57 ± 0.28 n: 22	p = 0.500
		p = 0.155	p = 0.472	

Discussion

The major contributing factors to the type of air pollution seen in Ankara are sulfur dioxide and smoke mainly produced by heating fuel.[5] The effects of sulfur dioxide on the respiratory system was extensively investigated both in experimental and epidemiological studies.

It was established both by animal experiments[6, 7] and by research on human subjects[8-11] that inhalation of sulfur dioxide causes in increase in airway resistance. This response develops immediately on inhalation and rapidly reaches a maximum level.[11] When inhalation of sulfur dioxide is stopped, airway resistance returns to its initial level in a very short time.[12, 13] The degree of airway resistance was calculated to be directly proportional to the concentration of sulfur diodixe inhaled.[6] It was reported that exposure of even the upper respiratory airways only or an isolated segment of the trachea to the effects of sulfur dioxide can cause an increase in airway resistance.[13] These features of response to sulfur dioxide inhalation show that it is primarily caused by bronchoconstriction, secondarily to changes in smooth muscle tone. Nadel *et. al.*,[14] demonstrated that the response was basically a vagal reflex.

In addition to the bronchoconstriction, constriction also occurs in the bronchial and pulmonary veins, and this limits the absorption of the pollutant.[15] The changes which take place in the bronchi and the blood vessels of the respiratory system are regarded as part of the defense mechanism against air pollutants.

The respiratory effects seen in healthy persons who are exposed to urban air pollution are usually reflections of these defense mechanisms. Therefore, respiratory function tests which are most sensitive to air pollution are those which are concerned with air flow rates, since they are most evidentely affected by bronchoconstriction.

In epidemiological studies, it was shown that air pollution affected the FEV_1 and $FEV_{0.75}$ values.[16, 17] In research on air pollution it was reported that, investigations of volume-flow relations would yield the most significant results[2] and that flow rates especially at end-expiratory lung volumes were the most sensitive parameters for investigating the effects of air pollution.[8]

In our study, it was found that the values of FVC N% and $FEF_{25-75\%}$ for non-smokers -who were expected to be affected solely by air pollution- were significantly lower than the values obtained in the control area. Of the values of air flow rates which are obtained from the spirogram, the $FEF_{25-75\%}$ parameter reflects flow rates at relatively low lung volumes. Therefore, although it was not possible to evaluate volu-

me-flow curves in our study, the significant difference in $FEF_{25-75\%}$ values makes our results consistent with the views presented above. In his investigation on two groups consisting of adolescents and adults in Ankara, Kolaçan proposed that air pollution essentially affected FVC N % and $FEF_{25-75\%}$.[18]

Kagawa[9] however, found that in normal persons exposed to various air pollutants, changes in FVC values were statistically significant, while changes in FEV_1 values were not. Furthermore, another study carried out in Ankara,[19] reported that the vital capacities of children living in polluted air regions were significantly decreased. In a long-term study, Rom *et. al.*,[20] found that exposure to industrial sulfur dioxide caused a decrease in both FVC and FEV_1. On the other hand, Ware *et. al.*[21] reported that air pollution did not lead to significant changes in FVC and FEV_1 values. The reason for the discrepancy between these studies lies in the lack of standardization of techniques in respiratory function investigations and in the different characteristics of the groups studied.

In the second measurements carried out on some of the students participating in our study, the values of FEV_1/FVC %, $FEF_{200-1200}$ and $FEF_{25-75\ \%}$ in both regions were higher than those obtained in the smaller group during the December period. However, the differences are more marked in the polluted region. While no significant difference was found in any of the parameters between the two periods in the control group, there was a statistically significant difference in $FEF_{200-1200}$ values between the two periods in the polluted region. Taking into consideration that the March measurements were obtained during a period when air pollution had decreased, this result was interpreted as showing that students living in the polluted region were affected more. That is, this difference is the result of the return of the air flow rates to normal due to the decrease in air pollution effects. Although the second measurements were obtained during a period of low sulfur dioxide levels, relatively heavy pollution had preceded this period (Figure 1). Thus, the changes in respiratory functions seen in the more polluted period seem to be reversible. This means that the changes in the respiratory function parameters were not due to pathological changes. The impairement in these parameters should be the result of a transient reflex bronchoconstriction. We suggest that this response is purely a reflection of the action of defense mechanisms in the respiratory system which were discussed above.

Cigarette smoking is also known to affect respiratory functions, particularly flow rates.[22] However, the results obtained from our investigation seem initially to contradict this. When the smoker student group

was examined, it was found that the values of the flow rate parameters obtained in the polluted region were higher than those of the control region. This finding seems to be in complete contrast to the finding obtained from the non-smoker group. The high percentage of heavy smokers in the smoker group of the control area may partly explain this apparent contradiction. On the other hand, it is not easy to explain the finding that the flow rate values of the smoker group in the polluted region are higher than those of non-smokers in the same region.

It is important to bear in mind that this study is dealing with the interaction between air pollution and cigarette smoking on respiratory functions. On the other hand, as mentioned above, the observed effects are thought to be due to the action of defense mechanisms of respiratory system. So, if the lower flow rates measured in the non-smoker group in the polluted region are taken as reflecting the defensive reflex against air pollution, then the higher values obtained in the smoker group in the same region should be considered as the result of impaired defensive response. Obviously, this is not regarded as an advantageous effect of cigarette smoking.

The mechanism of this interaction is not clear. In their experimental studies of sulfur dioxide inhalation in human subjects, Frank *et. al.*,[11] reported that airway resistance started to increase from the first minute onwards, reaching a maximum in the fifth minute. After thirty minutes, however, although inhalation continued, airway resistance was seen to decrease. These findings indicate that adaptation to various irritants in the respiratory system may develop. This adaptation is probably related to changes in autonomic reflex activity. It has been proposed that the changes are due to adaptation in the central nervous system rather than in peripheral receptors.[23] The existence of a kind of adaptive mechanism had been shown in repeated experimental exposures to various irritants[24, 25] but such an adaptation cannot explain the high flow rates obtained for the smoker group in our study. Moreover, to our knowledge, such an adaptation to cigarette smoking has not been reported. Suzuki *et. al.*,[26] reported that the bronchoconstriction response following a single cigarette was no different in smokers and non-smokers. However, it is possible that in persons whose respiratory system is constantly exposed to an irritant such as cigarette smoke, the defense mechanisms against pollutants may be deficient.

Another possible explanation for the significant difference between smokers and non-smokers is the difference in their socioeconomical status. But, since for various reasons students failed to provide reliable

information, we were unable to obtain the necessary data to evaluate these factors.

As a result, in this study, findings from the non-smoker group show that air pollution causes a decrease in respiratory flow rates. It is proposed that the low flow rates found in the polluted area are related to an increase in bronchial smooth muscle tone.

The findings from the smoker group are not clarified completely, but the possibility of adaptation is discussed. Obviously, this adaptation is not regarded as a healthy response. The increased airway resistance is a response aimed at preventing irritants from reaching the lower regions of the respiratory tract. If this defense mechanism is suppressed in smokers, these persons may be more subject to the deleterious effects of air pollution.

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Intravenous Digital Subtraction Angiography (DSA) in the Evaluation of Vascular Diseases

B.Münci Oran, M.D.* / Fahrettin Hasırcıoğlu, M.D. / Salih Güran, M.D.** / Muzaffer Eryılmaz, M.D.****

Summary

Intravenous Digital Subtraction Angiography (DSA) allows visualization of the arterial system after intravenous injection of contrast material. DSA is a safe, accurate method of diagnosing a broad range of vascular abnormalities.

Seventy four patients were evaluated. The DSA studies were performed on a commercially available unit with a largefield-of-view image intensifier. Imaging was done on the 14 inch (35.6 cm), 10 inch (25.4 cm) or 6 inch (15.2 cm) modes of the image intensifier. Contrast material was introduced via percutaneous puncture of an antecubital vein or, rarely, a femoral or jugular vein.

Key Words: Digital Subtraction Angiography (DSA).

Introduction

Although the subtraction of bones and soft tissues which are superposed on vascular structures in angiographies and obtaining arteriography by giving intravenous (IV) contrast material had previously been defined, this method was not very practical because of the usage of concentrated contrast material in large amounts and the lack of high qua-

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lity image.[1, 2, 3] After the development of computers which record images at a high velocity, such difficulties have been overcome.

The digital subtraction angiography (DSA) is an angiographic system developed to enhance the image of the low concentrated opaque material in the vessel to as visible level and to subtract bone and soft tissues superposed on the examined vessel.[4] It is digital, it forms images by making subtraction and its field of application is angiography. The acronym, DSA, stands for the three most important characteristics of this system.[5]

Materials and Methods

In the department of Radiology, Faculty of Medicine, Hacettepe University, the first seventy four patients on whom angiographic investigation with digital subtraction angiographic (DSA) was performed, were evaluated. Of these 48 were male, and 26 were female. Their ages ranged from 4 to 74 years with an average of 42.

The images were taken with digital subtraction technique. (Philips DVI-V was used). One or two images were obtained every second, they were converted to digital form with a converter of 256 x 256 matrix and 8 bytes. Suitable image areas for the purpose with widths of 14 inch (35.6 cm), 10 inch (25.4 cm) or 6 inch (15.2 cm.) were used for the part examined. An angle was given to the C-armed X-Ray and image tube for the part which was then examined under fluoroscopy.

The catheter, which was especially prepared for DSA was introduced into one of the antecubital veins and was guided into the right atrium by the Seldinger technique. After the first 39 patients, it was introduced into one of the antecubital (preferably into the right v. basilica) with number 18 intracat having a length of 5 cm. for the vessels other than the cerebral ones. The catheter or intracat was connected to the pump. The contrast material was injected by the pump with a velocity of 20-30 ml/sec when the catheter was placed in the right atrium, and with a velocity of 10 ml/sec when the intracat was used in periphery. The average contrast given was 2 ml/kg and the average single injection amount was 30-50 ml.sodium methyl glucamine diatrizoate (urovison). However, the total contrast material amount was increased up to 4 ml/kg dose in cases where the examination of several vessels at one time was necessary.

Intestinal clearing was obtained one day prior to abdominal aortic and renal angiographies. 40 mg intravenous scopolamide was used to prevent intestinal peristaltism unless there were any contraindications.

After the IV contrast material was given, the time required for the passage of this material through the arteries (which would be examined) was predicted according to the clinical situation of the patient and was given to the computer as delay time. At the end of this period, the first image was taken as mask before the contrast material reached the part which would be examined and was put into one of the two memories of the computer. The image taken after the contrast material had arrived were converted to digital form and the mask was obtained from these images. In this way, 20 subtraction images, following each other in a serial manner, was put into the second memory of the computer. These images were evaluated later by various processes such as following, enlargement, pixel shift and filming of the image.

Results

Arteriography was performed on 74 patients by giving contrast material through venous injection and by digital subtraction technique. Cerebral arteries of twenty two patients, neck arteries of six patients, thorax arteries of two patients, abdominal aorta and distal parts of twenty one patients, renal arteries of twenty patients, brachial arteries of two patients were examined.

Cerebral arteries: Catheter was put into the right atrium in cerebral angiographies in all, expect four child patients. Cerebral angiography was performed by inserting intracat into one of the antecubital veins in child patients. On the twenty two patients where cerebral angiography was performed, the breakdown of diagnosis was as follows: IV DSA'in one patient, aneurysm of a carotis interna; in three patients occlusion of a cerebri media; in two patients, occlusion of a. carotis interna; in one patient, stenosis of a. carotis interna; in one patient, oligodendroglioma; in two patients, hemangioma, and two patients, atherosclerotic changes in basillar artery. The results of nine patients were normal. A result suitable for making any comment could not be obtained in the angiography made to one of the patients (Figure 1).

Neck arteries: Angiographic examination was carried out on the patients in right, left anterior oblique and antero-posterior positions. Additional images were taken in some of the patients by giving different angles to the tube because of superposition of the bifurcations of the common carotid arteries on vertebral arteries. The neck arteries of six patients were examined. The results in two patients were normal, while in one patient, stenosis of left a. carotis interna; in two patients, glomus jugulare tumor, and in one patient subclavian steal syndrome were diagnosed.

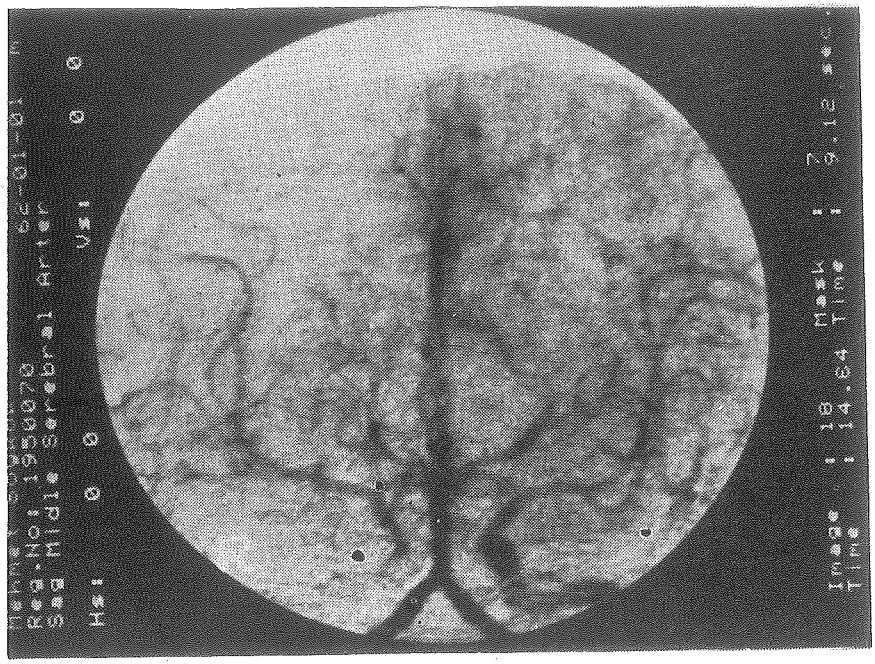

Figure 1
Occlusion of right a. carotis interna

Thoracal arteries: Thoracal region arteries of two patients were examined. Occlusion of right subclavian artery was determined in a patient whose both subclavian arteries were examined with arteriosclerosis diagnosis. During the angio on the same patient, stenosis of the left common carotid artery and occlusion of the right vertebral artery were determined. On the other hand, in an angio performed on a patient with an indication of pulsatile mass in the right axillar region due to travma, travmatic aneurysm of the right axillar artery was determined.

Abdomen and distal parts arteries: Since in the examination of the abdominal aorta, the examination of distal parts is also required, the results of extremity arteries were also reviewed together with abdominal vessels. The arteries from abdominal aorta to the popliteal arteries of twenty-one patients were examined. In all of the twenty-one patients who were examined with IV-DSA, arteriosclerotic changes in the abdominal aorta were determined. In addition to this result, in six patients abdominal aortic aneurysm (Figure 2); in one, advanced stenosis of abdominal aorta's distal parts; in three, advanced ateriosclerotic changes of arteries in abdominal aorta and distal parts; in two, occlusion of a.iliaca communis; in two, aneurysm of a.iliaca communis in one, steno-

sis of a.iliaca externa; in two complete occlusion of a.iliaca interna; in two stenosis of a.iliaca interna; (Figure 3) and in two, complete occlusion of femoral arteries were determined.

Renal arteries: Renal angiography with IV-DSA was carried out on twenty patients. Four of these patients were potential renal transplant donors and sixteen were examined with renal angio because of renovascular hypertansion indications. In one of the patients which was a potential renal donor for renal transplantation, arteriosclerotic stenosis of the polar artery going to the upper pole of the left kidney and arteriosclerotic stenosis of both renal arteries of one patient, were determined. The other renal angioes made because of this indication proved to be normal. With four patients on whom renal angio was performed because of renovascular hypertansion indication, stenosis of renal arteries and in twelve patients the renal arteries were normal Figure 4.

Upper extremity arteries: Arteria brachialis angiography was made in only two patients. In one of the patients, occlusion of a.brachialis and in the other renal patients with arterio-venous shunt in the elbow, aneurysm filling the cavity were determined.

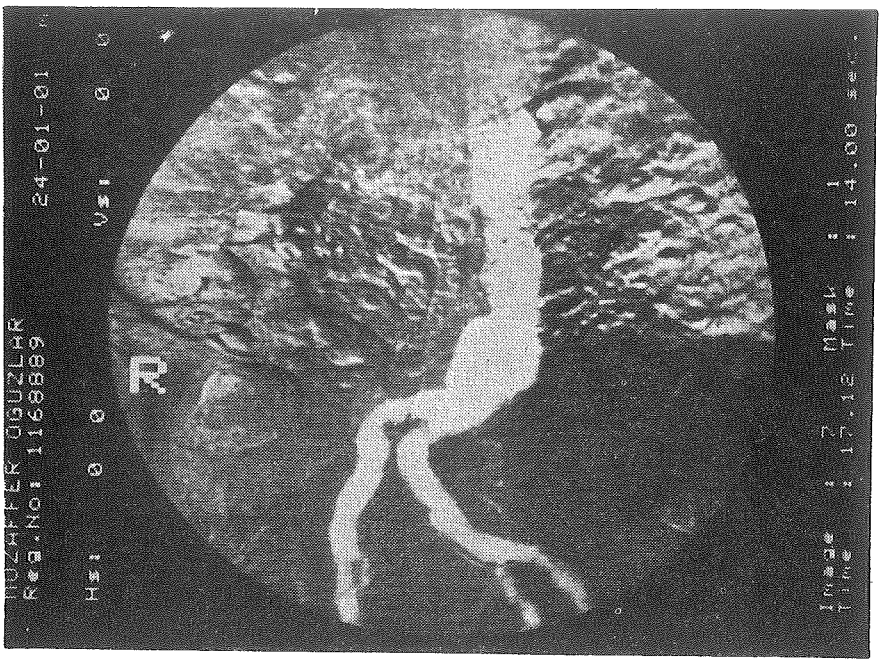

Figure 2
Abdominal aort aneurysm.

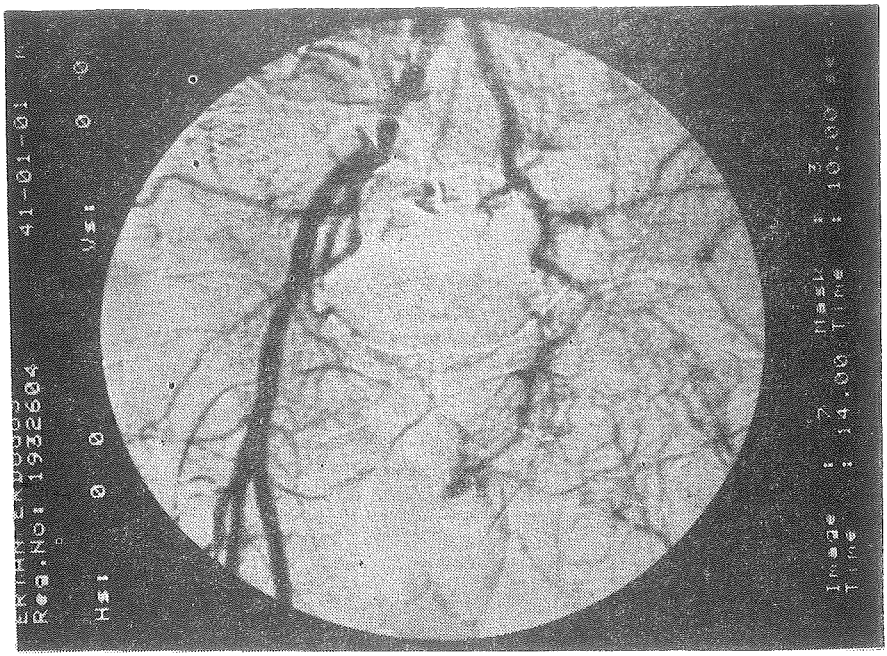

Figure 3
Complete occlusion of left external iliac and femoral arteries.

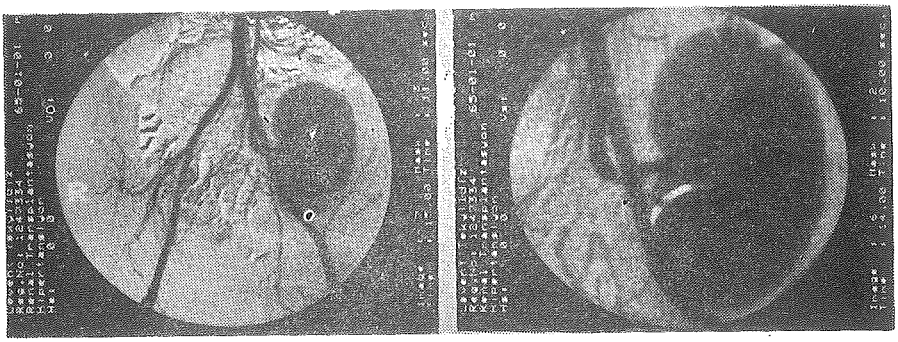

Figure 4
Renal artery stenosis of transplanted kidney.

Discussion

The most important characteristic of the digital subtraction angiography (DSA) technique, which is rapidly developing with its new clinical applications is the obtainment of arteriography by the infusion of intravenous contrast material. The advantage which this characteristic provides in clinical application is the practice of angiography on patients without hospitalization. In addition, the use of digital viewing

techniques provide some viewing processing facilities including remasking, pixel shift and filtration. This new technique obviates the need for an invasive process, such as selective catheterisation, and its complications.

In digital subtraction angiography, the faster the contrast material administrated intravenously enters the arterial circulation, the better the quality of the views will be.[3, 5] In heart diseases with low cardiac output or valvular pathology, the contrast material will be diluted centrally and the diagnostic value of the views of the arteries obtained by digital subtraction angiography will decrease. As a matter of fact, in an old patient on whom cerebral angio was performed, optimum quality views could not be obtained. In this patient who had congestive cardiac insufficiency, the contrast material was diluted centrally and the quality of the view became degraded.

In the literature on the comparative studies of the injection of the contrast material through a peripheric vein and a central vein, it has been reported that there was no difference in the quality of the views with the exception of two.[3, 5, 8] The first of these two exceptions, during the examination of the pulmonary arteries, the contrast material in the superior vena cava superposes on the right pulmonary artery and spoils the view. The second was the spoiler superposition of tha contrast material reflux, in the veins of the neck region where the blood could not flow well into the right atrium, on carotid bifurcation.

After the first 48 patients (in the angiographies excluding the cerebral angios) we started to use the peripheric method. With this modification we made in the tecnhique, the diagnosis value of the views was not affected even though there was reduction in the administration velocity of the contrast material. In the cerebral angios, on the other hand, this method was not used with the thought that the contrast material will be diluted peripherically and that the diagnostic value of the views will decrease.

A slight movement of the patient during the mask and following views will cause serious artefacts which spoil the quality of the subtracted image. Therefore, the patient must cooperate in order to obtain high quality image.[5, 6, 7] Intestinal peristaltic movements and the inability of the patient to hold his breath in abdominal aortic and renal angiogrphies, swallowing of the patients during artery carotis communis angiographies, the movement of the patients heads during cerebral angio were the causes of artefacts affecting the diagnosis value of the views. Diazepam was used to prevent this situation for children and patients

who could not cooperate. The breathing artefact can not be preventede even if the patient is sedated. However, the breathing artifact was reduced by remasking.

Considering the results of this study, when we compare digital subtraction angiography with conventional angiography, it can be seen that digital subtraction angiography is superior in some ways. The first is the speed at which the image is obtained and the obtainment of a clear view. Because the image is recorded to a computer disc and not to a film, the view can be observed immediately after the recording process is finished. Because the bones and soft tissues which superpose on the arteries are subtracted, only vascular structures are observed. Arteriography can be obtained after intravenous contrast material administration. This, not only provides the possibility of angio practice without hospitalizing the patients, but is also a less invasive method, because it reduces morbidity.

Consequently, the digital subtraction angiography (DSA) is a new technique which considerably reduces the morbidity and cost of angiographic processes and replaces conventional angiography.

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The Effects of Oral Prazosin on Total Plasma Digoxin Levels

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Summary

Prazosin and digoxin are frequently coadministered in clinical practice. In order to determine the effects of oral prazosin treatment on steady state digoxin levels, 20 patients receiving a constant maintenance dose of digoxin who had normal renal and liver functions, and were not receiving any other treatment, were selected and given 5 mg of prazosin for three days. Plasma digoxin levels were measured before, during the first and third days of treatment, and after prazosin was discontinued. It was found that prazosin significantly increased plasma digoxin levels. On discontinuation of prazosin, the digoxin levels returned to their previous values.

Key Words: Drug interaction, Prazosin, Digoxin, Heart failure.

Introduction

In recent years, it has been shown in increasing frequency that digoxin, a very well-known cardiac glycoside, has interactions with many other drugs.[1] In this respect quinidine, verapamil, nifedipine, spironolactone, amiodarone, and diltiazem were reported to increase plasma digoxin levels with different mechahisms.[2-11] The low therapeutic consentration range of digoxin has emphasized interactions in terms of both enhanced efficacy and toxicity.[2, 3]

On the other hand,prazosin is being used increasingly in current medical practice as a potent pereferal vasodilator, and in many clinical situations it is coadministered with digoxin.[12, 13]

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Although prazosin-digoxin interaction has been studied in a few experimental animal studies, to the best of our knowledge, it has not been investigated in humans.[14]

Therefore, the present study was undertaken to evaluate the effects of oral prazosin on plasma digoxin levels in 20 patients on maintenance digoxin therapy.

Materials and Methods

The study group comprised 20 patients (12 men 8 women) who consented to participate in the experiment. All of them had been followed by the outpatient section of the department of Cardiology of Hacettepe University Hospital. The mean age was 47 with a range of 26-76. The underlying cardiac diagnosis was primary hypertension in 17 patients and rheumatic valvular heart disease with hypertension 3. None of the patients had manifest congestive heart failure clinically. Their renal functions were normal with a mean creatinin clearence of 126 ± 27 ml/min. and all had normal liver functions, plasma proteins and hemoglobin values. None of them were taking any other drugs.

The patients were given oral digoxin tablets 0.25 mg/day at 8 a.m. for 8 days, after steady-state was achieved the first digoxin level was measured at 12 a.m. Then 5 mg/day of prazosin (2.5 b.i.d.) was added to this regimen taken at 10 a.m. and 10 p.m. On the first and third days of prazosin treatment, plasma digoxin levels were measured again at 12 a.m. for each patient considering the pharmacokinetic features of prazosin.[15] On the fourth day of trial prazosin was discontinued while digoxin was still administered. Although the metabolites of prazosin are known to be cleared from the body in 12 hours,[16] a safety period of 3 days, was allowed to pass[17] then the plasma digoxin level was measured again at 12 a.m.

The total plasma digoxin level was measured by radioimmunoassay (Abbott-TDx, 96-831-AZ).

A two tailed paired t test was performed for statistical analysis.

Results

The total serum digoxin levels of the 20 patients, before, during, and after prazosin trial, are shown in Table I. The mean value of the basal digoxin levels achieved after a week of digoxin administration was 0.94 ± 0.09 ng/ml. On the first day of the experiment mean plasma digoxin levels was found to be 1.34 ± 0.12 ng/ml ($P<0.01$), and on the third day of prazosin administration it was 1.51 ± 0.18 ng/ml($P<0.05$). On discontinuation of prazosin for three days, digoxin levels returned close to their previous values 0.99 ± 0.06 ng/ml, ($p>0.05$) (Table II).

TABLE I

SERUM DIGOXIN CONCENTRATIONS OF 20 PATIENTS WITH AND WITHOUT PRAZOSIN

Patient Number	Serum digoxin after 1 week of digoxin (ng/ml)	Serum digoxin after 1 day of prazosin (ng/ml)	Serum digoxin after 3 days of prazosin (ng/ml)	Serum digoxin on discontinuation of prazosin (ng/ml)
1.	0.35	1.73	1.03	1.02
2.	1.38	1.71	1.37	1.01
3.	1.47	1.46	1.62	0.80
4.	1.23	1.01	2.19	1.04
5.	1.76	1.01	0.90	0.62
6.	0.75	0.66	3.33	0.97
7.	0.32	0.91	1.09	0.91
8.	0.91	0.90	0.87	0.75
9.	0.55	0.70	1.66	0.60
10.	0.55	0.66	0.92	1.21
11.	1.41	2.90	1.73	1.43
12.	0.89	1.22	0.82	0.94
13.	0.91	2.62	2.01	0.81
14.	0.90	1.57	1.02	0.90
15.	1.07	1.74	1.54	1.19
16.	1.03	1.74	2.95	0.95
17.	1.35	1.71	0.72	0.92
18.	0.20	0.90	0.63	0.84
19.	0.72	1.20	3.00	1.61
20.	0.98	1.34	0.75	1.40
	mean ± Sx	mean ± Sx	mean ± Sx	mean ± Sx
	0.94 ± 0.09	1.34 ± 0.12	1.51 ± 0.18	0.99 ± 0.06

TABLE II

COMPARASION OF SERUM TOTAL DIGOXIN LEVELS BEFORE DURING AND AFTER PRAZOSIN ADMINISTRATION

Basal serum digoxin levels (ng/ml) mean ± Sx	Serum digoxin level after 1 day of prazosin (ng/ml) mean ± Sx	Serum digoxin level after 3 days of prazosin (ng/ml) mean ± Sx	Serum digoxin level on discontinuation of prazosin (ng/ml) mean ± Sx
0.94 ± 0.09	1.34 ± 0.12	1.51 ± 0.18	0.99 ± 0.06
The mean difference from the baseline values	0.405 ± 0.12	0.577 ± 0.202	0.051 ± 0.109
Statistical evaluation	$P < 0.01$	$P < 0.05$	$P > 0.01$

Discussion

Recently, the interactions of digoxin with many other drugs have been studied and different pharmacokinetic mechanisms were suggested. For example, quinidine has been shown to increase the oral absoption, and to decrease the volume of distribution and renal clearence of digoxin; while verapamil and spironolactone decreased its elimination by blocking the renal tubular secretion.[2, 3, 6-8]

On the other hand, in a recent study conducted on dogs, it was found that prazosin increased the free serum digoxin levels by reducing the plasma and nonspecific tissue binding and thus the pool of digoxin available for pharmacological activity.[14] They postulated that since digoxin is cleared by glomerular filtration and tubular secretion, tubular secretory pathways might become saturated with prazosin decreasing the digoxin clearence. As a result, the positive inotropic response to digoxin was increased twofold with the concemitant administration of these two drugs. Previous clinical studies also supported enhanced positive hemodynamic effects of digoxin when taken together with prazosin.[12, 13]

We studied the effects of oral prazosin on total serum digoxin levels in patients who were carefully chosen to have normal renal and liver functions, without heart failure or anemia and were not taking any other medication. We demonstrated that when oral digoxin and prazosin were used concomitantly, the serum digoxin levels significantly increased compared to the baseline level ($P < 0.01$). After prazosin was discontinued, the digoxin level returned to initial values ($P > 0.01$).

The design of our study does not permit us to explain the mechanism by which serum digoxin levels are elevated when prazosin is added. But, it would not be unreasonable to suggest that one of the following mechanisms may be operational: prazosin might increase oral absorption or reduce tubular secretion of digoxin. Since we did not measure the free levels of digoxin, we can not speculate about plasma protein and tissue bindings.

Because of the clinical and pharmacological importance of this probable interaction, well-organized, controlled studies are needed to elucidate the effects of the concomitant use of digoxin and prazosin and the possible mechanisms which are involved.

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1-Alpha Hydroxycholecalciferol in the Management of Renal Osteodystrophy

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Summary

1-alpha hydroxycholecalciferol, a synthetic but active analogue of 1,25 dihydroxycholecalciferol was administered in patients with chronic renal disease of varying etiology some of whom were receiving hemodialysis. After a follow up period of 6 to 70 months, it was concluded that this drug helped reestablish calcium, phosphorus and bone metabolism. The most consistent effect was the elevation of serum calcium levels although decrease in iPTH levels and some reduction in the serum alkaline phosphatase levels were also detected. No side effect other than transient hypercalcemia was recorded.

Key Words : 1-alpha hydroxycholecalciferol, renal osteodystrophy, parathyroid hormone.

Introduction

Renal failure is associated with many disturbances in the metabolism of divalent ions and these abnormalities have been collectively called "renal osteodystrophy". They may include hypocalcemia, hyperphosphatemia, hypermagnesemia, skeletal resistance to the calcemic action of parathyroid hormone (PTH), defective intestinal absorption of calcium, secondary hyperparathyroidism and elevated blood levels

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of PTH, soft tissue calcification, and bone disease. Many of these derangements may be the direct result or the consequences of vitamin D deficiency. Vitamin D deficiency is due to the kidney's inability to produce the active from of vitamin D, namely 1,25 dihidroxycholecalciferol (1,25 DHCC).[1, 2]

On the other hand, chronic renal failure (CRF) leads to increased blood levels of phosphate which is responsible for the high PTH levels seen in CRF-patients. Then, this elevated PTH, acting as an uremic toxin, results in at least some of the clinical signs of uremia (including anemia, skeletal abnormalities, peripheral neuropathy etc). Therefore, it can be said that high PTH levels are harmful for patients with CRF.[1, 2, 3]

In the last decade, many researchers tried to normalize elevated blood PTH levels. One of the major advances in this field is the use of active derivatives of vitamin D in order to reduce PTH levels.

1- alpha hydroxycholecalciferol (1-alpha (OH) D_3), an analoque of 1,25 DHCC, is one of these derivatives and it is rapidly converted to the 1,25 DHCC in the body. Published studies have encouraged us to apply this drug at a relatively earlier stage than before and have indicated that it might be a safe and reliable drug.[2, 4, 5]

Materials and Methods

The study includes uremic (CRF) patients treated medically and hemodialysis-patients (HD). Total number of the patients was thirty-five, 20 of them were receiving hemodialysis, the ages ranged between nineteen to sixtyfive; the average was 35.5 years.

The follow-up period was 6 to 70 months, an average of 38 months. The biochemical values were recorded at the pre-treatment phase and were repeated at monthly intervals. Serum iPTH was measured in our Nephrology Laboratory at Hacettepe Hospitals by radioimmunoassay based on the reaction of a labelled antigen (I^{125}-PTH) with the corresponding antibody. The principal stages of this assay are: preincubation of the hormone with the antihormone antibody, addition of the labelled hormone, agitation with the immunoadsorbent, centrifugation and, counting of the precipitate.

"One-alpha" in one microgram tablets from Leo Pharmaceutical Products Company, Copenhagen, were used. Therapy was begun with the dose of one microgram of 1-alpha (OH)D_3 every other day. Then the dose requirements were adjusted according to the serum calcium values. Three patients required an additional calcium-elevating medicine, $CaCO_3$ in a dosage of 2-4 gm. per os daily. All of the patients received

phosphorus-binding Al^{+++} preparations. Only in one patient, 1-alpha (OH)D_3 was elevated to two micrograms per day because of the inadequacy of the dose. Statistical analysis was performed using the Student's "t" test for paired data.

Results

The results of the 1-alpha (OH) D_3 treatment in nondialysed subjects and hemodialysis patients are presented in Table I and II. In both groups, serum calcium as well as serum iPTH levels changed remarkably; serum calcium increased while iPTH decreased during the therapy. Moreover, the serum level of phosphorus was significantly reduced only in patients receiving hemodialysis. Other laboratory findings, namely, alkaline phospatase, TRP, creatinine clearance, albumin and hemoglobin did not display significant alterations.

TABLE I

RESULTS OF 1-ALPHA (OH) D_3 TREATMENT IN MEDICALLY TREATED PATIENTS*

	Pre-Treatment	Post-Treatment	Significance
Calcium, serum, mg/dl	7.42 ± 0.30	9.01 ± 0.05	$P < 0.01$
Phosphorus, serum, mg/dl	5.52 ± 0.44	4.65 ± 0.36	n.s.**
Alkaline Phosphatase, KA U.	24.00 ± 4.21	18.05 ± 2.65	n.s.**
TRP %	30.86 ± 4.45	30.62 ± 6.34	n.s.**
Creatinine Clearance ml/min	16.40 ± 4.02	12.82 ± 4.02	n.s.**
iPTH, mU/ml	18.50 ± 3.50	11.01 ± 5.28	$P < 0.01$
Albumin, serum, g/dl	3.35 ± 0.12	3.44 ± 0.08	n.s.**
Hemoglobin, g/dl	9.34 ± 0.62	9.43 ± 0.61	n.s.**

* Results are expressed as means ± SD
** n.s.: not significant

TABLE II

RESULTS OF 1-ALPHA (OH) D_3 TREATMENT IN THE HEMODIALYSIS PATIENTS*

	Pre-Treatment	Post-Treatment	Significance
Calcium, serum, mg/dl	7.69 ± 0.26	8.66 ± 0.87	$P < 010.$
Phosphorus, serum, mg/dl	6.12 ± 0.37	4.70 ± 0.21	$P < 0.01$
Alkaline Phospatase, KA unit	22.16 ± 2.90	19.20 ± 2.68	n.s.**
iPTH mU/ml	19.61 ± 2.08	12.21 ± 2.03	$P < 0.01$
Albumin, serum, g/dl	3.12 ± 0.13	3.11 ± 0.07	n.s.**
Hemoglobin, g/dl	8.04 ± 0.31	8.23 ± 0.62	n.s.**

* Results are expressed as means ± SD
** n.s.: not significant

Discussion

Unlike the majority of the investigations in this regard, we applied 1-alpha (OH) D_3 to: 1) symptomatic hemodialysis patients and 2) also to our nondialysed CRF subjects which had residual function of their kidneys but with few symptoms. From Table I in the nondialysed group, the mean pretreatment value of creatinine clearance (C_{cr}) was as high as 16.4 ml/min and none of these subjects underwent hemodialysis programme during the therapy period. Sixteen of the adult patients treated with 1-alpha (OH) D_3 by Madsen and Olgaard[6] had a residual renal function defined by a mean C_{cr} of 7.2 ml/min before initiation of therapy; and seven of their patients were started on hemodialysis during the treatment period. On the other hand, Healey *et al*, began to treat three patients - with 1,25 $(OH)_2$ D_3 for 6 months-when their creatinine clearances were 32, 49, and 51 ml/min; they found that this therapy raised the serum calcium, normalized the serum levels of PTH, and healed the bone disease.[7]

The most consistent effect of 1-alpha (OH) D_3 in the uremic and dialysis groups was the early elevation of serum calcium levels. Moreover, this increase was found to be statisticaly significant. Normocalcemia was re-established and maintained. In our patients, normalization of serum calcium levels occurred within one week to six weeks. The data of other investigators[8, 9, 10] confirm this finding and indicate that most subjects will exhibit a rise in serum calcium after treatment with this drug within five days to four weeks.[9, 11]

Hypercalcemia, a frequent complication, may appear at any time during therapy with 1-alpha (OH) D_3. It usually occurs after 2 to 3 months of therapy, but has been recorded to be as early as 5 days and as late as 6 to 18 months after treatment.[12] In 2 of our patients, asymtomatic hypercalcemia was detected within the second week of therapy, but it disappeared gradually a few days after the dose was reduced. This latter finding was in accordance with most of the previous reports.[8, 9, 12, 13]

The high beginning dose may have been the cause for the early appearance of hypercalcemia. It is advisable, therefore, to institute therapy with very small doses of 1-alpha (OH) D_3. Our policy is the same as this.[12, 13] However, frequent measurements of serum calcium were mandatory to correct 1-alpha (OH)D_3 dose in order to avoid hypercalcemia.[5, 6, 13]

Another important finding was the decrease in the serum iPTH levels in both groups. PTH levels decreased by 37 % and 40 % in average in

uremic and dialysis patients, respectively. The reduction in the serum levels of PTH during therapy with 1-alpha (OH) D_3 was probably due to the rise in the concentration of serum calcium. It was also possible that 1-alpha (OH) D_3 itself, directly suppresses the parathyroid gland activity.[14, 15] In the literature, the effect of 1-alpha (OH) D_3 on serum levels of PTH has been reported in approximately 120 patients treated with this drug for periods of 3 weeks to 18 months.[8, 9, 13] The serum levels of PTH decreased by 13 % to 90 % of the control values in most of these patients, and in few (30 patients) the serum levels returned to normal. No change or an increase in serum PTH concentration was also reported.[9, 13, 16] The variability in these results may be related to differences in basal serum levels of PTH (reflecting the degree of hyperplasia of the parathyroid glands), daily fluctuations of serum PTH level, the type of PTH assay, the magnitude of rise in serum calcium, the dosage used, and the duration of therapy.

We thought the decrease in serum PTH and the increase in serum calcium reflected the action of 1-alpha (OH) D_3; and these were the evidences of re-correction of calcium, phosphorus and bone metabolism.[17, 18, 19]

Theoretically, treatment with 1-alpha (OH) D_3 will tend to increase serum phosphate. But in our study, especially in HD patients, the serum P levels apparently decreased. From published studies, the effect of 1-alpha (OH) D_3 on the concentration of serum phosphorus in uremics or dialysis patients is not consistent, and, an increase, a decrease, or no change in phosphorus levels have been reported.[5, 8, 10, 20] The decrease in serum phosphorus levels in our patients may result from the effects of diet deprived of phosphorus and also P-binding Al^{+++} preparations taken simultaneously together with "one-alpha". It is of utmost importance to monitor serum P concentrations during therapy with 1-alpha (OH) D_3 because the development of hyperphosphatemia, especially in the face of rising serum Ca^{++} concentration, would result in elevation of the serum CaXP product and augment the hazards of soft tissue calcification. If the value of this product approaches 55, every effort should be made to control serum P with P-binding antacids. If this procedure proves unsuccessful, the dose of 1-alpha (OH) D_3 should be reduced or temporary cessation of therapy should be considered.[7, 20]

Serum alkaline phosphatase (AP) activity usually decreases during therapy with 1-alpha (OH) D_3, but several months may elapse before its level returns to normal.[10, 11]

Although some reduction in serum alkaline phosphatase levels (AP) was detected in the late period (after 2 months), no statistically

significant difference between pre-treatment values and the values during therapy appeared. But, monitoring of AP could provide an additional guide for the adjustment of the dosage of 1-alpha (OH) D_3 for 2 reasons: first, reduction or near-normalization of AP reflects an improvement in bone disease, and, second, the occurence of hypercalcemia increases as AP returns to normal.

Serum albumin and Hb levels did not differ significantly, but the reduction in the need for transfusions seemed to be decreased.

In the nondialysed group, the mean creatinine clearance decreased approximately 22 percent during therapy with 1-alpha (OH) D_3. Despite its being statistically insignificant, serious questions could be raised regarding the deleterious effects of 1-alpha (OH) D_3 on renal function. Some studies which were done previously also support this finding.[6, 9, 14] Theoretically, 1-alpha (OH) D_3 could deteriorate renal function by a direct action on the kidneys, by producing hypercalcemia and metastatic renal parenchymal calcification. But, as some authors[1, 6, 14] did, we supposed that the decrease in glomerular filtration rates represented the natural progression of the existing renal disease. Administration of lower doses of 1-alpha (OH) D_3 might have caused less hypercalcemia. Hence, the probability of a decrease in renal function (GFR) is reduced.

The mild reductions in TRP percentages in the uremics were not significant.

Radiologic evaluation is not worth discussing since our x-ray data were inadequate and in addition, X-rays are generally considered unvaluable tools for the assessment of the effectiveness of 1-alpha (OH) D_3 therapy.[14, 16]

In our patients, principal clinical symptoms related to disordered calcium-phosphorus metabolism were bone pain, myopathy, pruritus and impotence. During therapy with 1-alpha (OH) D_3, some patients have shown some improvements in these symptoms. Four of the ten sexually impotent patients said that they became somewhat potent during therapy with 1-alpha (OH)D_3. But, clinical results were indefinitive because these are subjective symptoms. During the long term treatment of our patient with 1-alpha (OH) D_3 soft tissue calcification was not observed.

In conclusion;

1- One-alpha, via restoring Ca-P metabolism. may cause laboratory and clinical improvement in renal osteodystrophy. Its harmful effect on renal function is still controversial. Our results support the previous

reports regarding the beneficial effects of 1-alpha (OH) D_3 on the disturbed divalent ion metabolism in CRF. Our findings should be reliable because of the relatively long follow-up period. Individialization of the dose, rather than applying an rigid standard dose, is recommended to prevent any adverse effects.

2- All near-normo-and hypocalcemic patients who are dialysed in dialysis units should receive 1-alpha (OH) D_3 or 1,25 DHCC treatment.

3- Outpatients in the pre-dialysis state, however, should receive 1-alpha (OH) D_3 only if clinical symptoms are present.

4- Frequent clinical control and close monitoring of serum Ca and P should be established in all cases.

5- Because of the risk of calcium deposition, 1-alpha (OH) D_3 should not be given to the hyperphosphatemic patients unless serum P was reduced to normal by Al^{+++} gels or by diet.

6- No other important side effect than hypercalcemia has been encountered.

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Serum Prolactin Levels and Recurrence of Amenorrhea in Hyperprolactinemic Women After Bromocriptine Induced Pregnancy

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Summary

Thirty-two hyperprolactinemic women were followed up for 18 to 65 months after at least one bromocriptine induced pregnancy for investigation of whether the pregnancy had any adverse long-term effects on their hyperprolactinemic state. Eight women had two term pregnancies. The prolactin level decreased more than 50 % in 9 women after the pregnancy. Only one woman showed a prolactin level increase. Spontaneous uterine bleedings returned in 9 women, while 23 women remained amenorrheic. Thus it was determined that bromocriptine induced pregnancy in women with hyperprolactinemia has no negative long term effect on prolactin secretion.

Key Words: Bromocriptine induced pregnancy, Recurrence of amenorrhea, Prolactin.

Introduction

The development of pharmacologic agents with dopamine agonistic effect offers a new dimension in the treatment of the patients with

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hyperprolactinemia.[1] It is generally accepted today that dopamine agonist therapy is the treatment of choice for most infertile women with hyperprolactinemia and amenorrhea.[2,3] Several previous studies have shown that hyperprolactinemic women run a very small risk of developing serious pituitary tumor complications during pregnancy induced by bromocriptine. In a recent review Nillius *et.al*, showed that clinical complications, because of prolactinoma growth, occurred only in 2 % of the 488 pregnancies in women with hyperprolactinemia.[4] It has been reported that bromocriptine may reduce the size of the prolactinoma. This drug could be the primary treatment, irrespective of tumor size.[5]

There are few reports on the long-term effects of pregnancy on prolactine (PRL) hypersecretion or effect of multiple pregnancies on hyperprolactinemic women treated with bromocriptine. In this paper the results of a long-term follow-up of a group of women with hyperprolactinemia who had at least a one-term pregnancy after bromocriptine therapy are presented.

Materials and Methods

Thirty-two women, 20 to 35 years of age (median, 24.1±1.2), with long-lasting amenorrhea and hyperprolactinemia were followed up after having 40 total bromocriptine induced term pregnancies. Nine women had 2 term pregnancies: 8 of these were induced by bromocriptine, and 1 occurred spontaneously. The observation time after the delivery ranged from 18 to 65 months. All of the patients had secondary amenorrhea of 6 to 76 months duration (median, 20.2±5.3 months).

Radiologic examination, including polytomography and conned view of sella turcica, showed small radiologic changes, with cellar classification B1-B3 according to Thorner *et.al.*[6] None of the women had evidence of extrasellar extension.

PRL in serum was measured radioimmunologically by the use of IRE (Institut National Des Radioelements 6220 Flerus-Belgium) kit. The serum PRL concentration before treatment ranged between 660 to 1840 mIu/ml (median, 1245.6±21.3 mIu/ml). The upper limit for women at the time of study was 500 mIu/ml. After pregnancy and a period of lactation, PRL was determined within the first 2 months after weaning. None of the women were taking any drug known to stimulate PRL secretion. The routine endocrinologic evaluation gave no evidence of any abnormality in thyroid or adrenal function.

Treatment with bromocriptine was given in daily doses of 5 to 7.5 mg for 1 to 15 months (median, 3.6±0.4 months) before conception occured. The treatment was stopped as soon as pregnancy was suspected.

During pregnancy, all women were examined monthly with visual field tests. Pituitary tumor complications occurred in two women who developed continuous severe headaches and visual field defects. These complications improved when bromocriptine therapy was reinstituted, and their pregnancy could continue until term. All women were allowed to breast-feed. No complications occurred during the lactation period.

In the statistical calculations, the hormone values were converted to logarithms. Student's t-test was used for calculations of differences between mean values.

Results

Return of menstruation occured after a period of breast-feeding in 9 of the 32 women, with 40 total term pregnancies. Five of the women had regular uterine bleedings, 4 had oligomenorrhea and the remaining 23 had persisting amenorrhea.

The PRL values before bromocriptine treatment and after pregnancy, lactation, and weaning in the 32 women are shown in Table I. A decrease of 50 % or more pretreatment value, was found in 9 women. Before treatment, these 9 women had PRL concentration between 660 to 1480 mIu/ml (mean, 1096.2±192.4 mIu/ml), and after weaning 260 to 768 mIu/ml (mean, 556.5±64.1 mIu/ml). Six of them regained regular menstrual bleedings. Three women with pretreatment PRL levels of 660,840 and 960 mIu/ml had normal PRL levels (<500 mIu/ml) after completing breast-feeding. All of them exprienced return of regular uterine bleedings. The PRL concentrations in the women with amenorrhea and in those who regained uterine bleedings returned are shown in Table I. Both groups had a significant decrease in the mean serum PRL level after pregnancy and lactation ($P<0.01$). In most of the women with amenorrhea bromocriptine therapy was reinstutited.

TABLE I

MEAN PRL LEVELS IN 32 HYPERPROLACTINEMIC WOMEN BEFORE AND AFTER BROMOCRIPTINE INDUCED PREGNANCY*

	Prolactin (mIu/ml)		P
	Before treatment	After weaning	0.01
Women with persisting amenorrhea (n: 23)	1320.2 ± 180.2 (660-1840)	984.6 ± 91.1 (625-1290)	< 0.01
Women with return of menturation (n: 9)	916.5 ± 92.1 (680-1315)	625.3 ± 68.1 (260-990)	< 0.01

* (Range is in Parentheses)

Higher PRL level after pregnancy was found in one woman. Her PRL value before treatment (1480 mIu/ml) was increased after pregnancy (1660 mIu/ml). In this woman, bromocriptine induced pregnancy had been clinically uneventful, with no radiologic change of the pituitary fossa at sellar x-ray.

Eight women had two term pregnancies. One of the second pregnancies occurred without therapy, but the other 7 women required treatment with bromocriptine to conceive again. The PRL levels in the 8 women after the first and second pregnancies are shown in Figure 1. The mean PRL level before treatment (1423.3 $\pm$ 190.3 mIu/l; range 925 to 1860 mIu/ml) and after the first pregnancy (1246.8 $\pm$ 120.2 mIu/ml; range, 870 to 1600 mIu/ml), the mean PRL level after first and after second pregnancy (1080.6 $\pm$ 96.2 mIu/ml; range 760 to 1460 mIu/ml) did not differ significantly, but after the second pregnancy the mean PRL level had significantly decreased ($P<0.05$) when compared with the level before the first pregnancy.

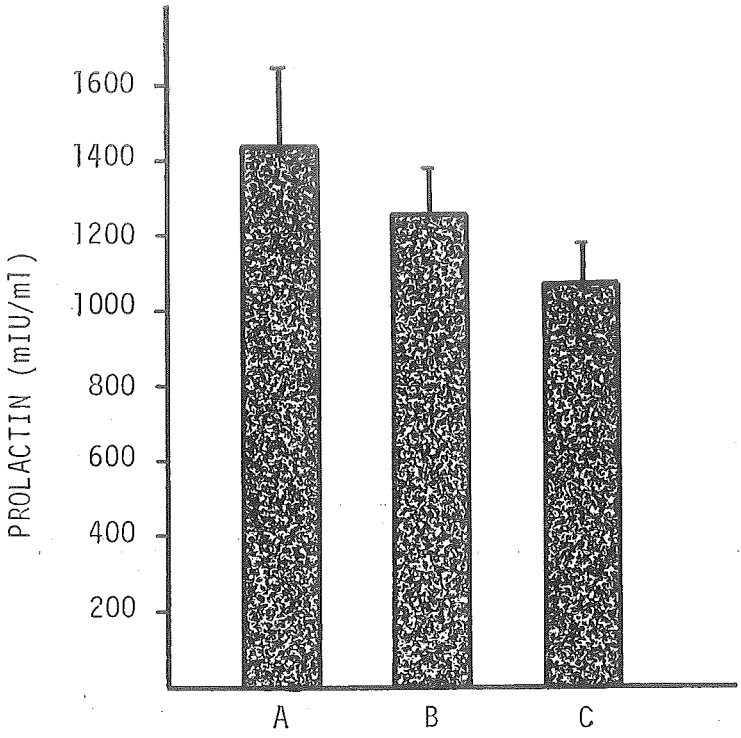

Figure 1

Mean PRL levels in 8 women with two bromocriptine induced pregnancies before treatment and after the first and second pregnancies. (A: Before treatment, B: After first pregnancy, C: After second pregnancy)

Discussion

In this study, 32 hyperprolactinemic women were followed up for 1 to 6 years after at least one bromocriptine induced term pregnancy. The results, like those of previous studies, show that patients with hyperprolactinemia run a very small risk of complication during pregnancy.[7-9] However, there are few reports on the long-term effects of pregnancy in women with hyperprolactinemia. Jewelewicz and Vande Wiele reported that 2 of 25 hyperprolactinemic women regained spontaneous menstruation after bromocriptine induced pregnancies.[10]

The PRL levels in serum decreased markedly in 28 % of the women after pregnancy, lactation and weaning. Only one woman had a significant increase of PRL secretion after pregnancy, but she had no symptoms or signs of complication. Thus, there are no indications that pregnancy and lactation make PRL secretion worse.

Spontaneous uterine bleedings returned in 9 of the 32 women after pregnancy (28 %). It was found that the mean pretreatment and post-pregnancy PRL levels of the women with return of spontaneous uterine bleedings were significantly lower than the women with persisting amenorrhea ($P < 0.01$). The former group of women were followed up for 3 years without any evidence of increased PRL levels again.

8 of the women who experienced two term pregnancies had lower PRL levels after the second pregnancy than pretreatment PRL levels, confirming the observation that PRL secretion seems to decrease after pregnancy. In a previous study of a similar group of women, it was found that multiparity had no adverse effects on the hyperprolactinemia.[11]

Discontinuation of long-term bromocriptine therapy results in return of amenorrhea in most hyperprolactinemic women.[12, 13] Few women continue to have regular bleedings and persistently lower PRL levels than before treatment. It may, therefore, be questioned whether the bromocriptine treatment before pregnancy is at least in part responsible for the postpartum decrease of PRL secretion. Furthermore, knowledge of natural history of prolactinoma is incomplete. In some patients, the decreased PRL secretion may, therefore, reflect a natural course.

In conclusion, this long-term follow-up study shows that bromocriptine induction of ovulation and pregnancy followed by lactation does not worsen the condition. On the contrary, a considerable number of hyperprolactinemic women have decreased PRL hypersecretion and experience the return of spontaneous uterine bleedings.

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Fetal Electronic Monitoring: A Diagnostic Tool in Determining the Mode of Delivery

A Prospective Study

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Summary

Management of complicated pregnancies with meconium stained amniotic fluid still remains controversial. To identify fetuses at risk and to determine the mode of delivery, fetal electronic monitoring is used as a diagnostic tool. Hence, in the current report, we observed the cesarean rate to be lowered from 65 % to 40 %. Intrapartum and postpartum analysis of neonatal complications were not different and were even better in the group where fetal electronic monitoring was used as a primary screening method. False positive values are still troublesome in fetal electronic monitoring, but even when used alone, continuous monitoring may decrease the high incidence of operative delivery to a reasonable degree.

Key Words: Fetal Electronic Monitoring (FEM), Meconium.

Introduction

Several studies emphasize the association between meconium and poor obstetrical and neonatal outcome.

Management of complicated pregnancies with meconium stained amniotic fluid still remains controversial. One must weigh the risks of delivery by cesarean section aginst the problems associated with complications that might be observed during labor. The present report summarizes the authors' experience with meconium stained amniotic fluid

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during labor in which antepartum fetal electronic monitoring was used as a primary screening tool for choosing the mode of delivery.

Materials and Methods

The study group consisted of 78 patients: 38 were in the test group and 40 were in the control group. All patients were evaluated according to the mode of delivery and the neonatal outcome in 1983-1984 at the Department of Obstetrics and Gynecology, Hacettepe University Schcol of Medicine

Among the patients with meconium stained amniotic fluid, only those pregnancies between 40 and 42 weeks were chosen. The following criteria were used during selection; a history of reguler menses, certainty of the last menstrual period (LMP), early prenatal visits, consistency of uterine size and gestationel dates at the time of delivery.

Contraction stress test (CST) and continuous intrapartum fetal electronic heart monitoring (FEM) were used. However, sampling of fetal scalp blood was not performed. The tracings were defined as negative if no late decelerations were seen during the 20 minute observation period during which there were, at least 3 contractions in 10 minutes with a duration of at least 40 seconds. A test was considered as positive when the tracing had late decelerations in more than at least 30 % of the contractions.[1]

The control group was chosen randomly and was followed up by the attending staff with manual auscultation.

Cesarean section (C/S) was the choice of intervention in the presence of meconium especially in those patients with unfavorable cervix. Operative delivery was performed unless prompt vaginal delivery was possible. The time when meconium was first observed, the consistency and the presence of staining of placental tissues were noted on the fetal code sheet and only the patients in which meconium was present at the rupture of the membranes were included. Patients were omitted when meconium was observed during labor when the amniotic fluid had previously been clear. Only pregnancies with a singleton fetus in vertex presentation were selected. Excluded were twin gestations, infants with congenital anomalies and obstetric conditions related with placental insufficiency syndrome, cephalopelvic disproportion (CPD), vaginal bleeding and other indications of primary C/S.[2, 3]

The end points of perinatal mortality and morbidity used for the analysis were; one and five minute Apgar scores of 6 or less, presence of meconium below the vocal cords at birth and consequent aspiration pneumonia.[4, 5]

The criteria for intervention in the study group were "positive CST", presence of severe cord compression signs and decreased "beat to beat variability" in fetal heart rate tracings.[6]

A pediatrician was present at delivery and the infants' mouth and pharynx were suctioned with a catheter upon delivery to observe the vocal cords of the infant.

Distribution of the control and test groups are given in Table I. Statistical analysis was accomplished by means of Students' t test or chi square as appropriate.

Primary indications for C/S were abnormal fetal electronic monitoring results in the test group, meconium and unfavorable cervix in the control group, respectively. All other indications were excluded.

Results

Complete data from 78 pregnancies were evaluated for this report. There was no statistically significant difference between the two groups (Table I).

TABLE I
AVERAGE VALUES AND STANDARD DEVIATIONS IN THE CONTROL AND TEST GROUPS

	Control Group (40)	Test Group (38)
Maternal Age	22,4 ± 4,6	23,6 ± 5,1
Parity	1,6 ± 2,4	1,3 ± 1,8
Gestationel Age	40,7 ± 0,9	40,5 ± 0,6
Birth Weight	3241 ± 454	3310 ± 514

The consistency of meconium, thick or thin meconium may alter the results.[7] The consistency of meconium in the test and control groups are given in Table II. Statistical analysis revealed no difference.

TABLE II
CONSISTENCY OF MECONIUM IN THE CONTROL AND TEST GROUPS

Meconium	Control Group	Test Group
Thick	26	28
Thin	14	10
Total	40	38

Fetal electronic monitoring results and mode of delivery are given in Table III and IV respectively.

TABLE III
TEST GROUP FETAL ELECTRONIC MONITORING RESULTS

Consistency of Meconium	Normal Fem Results	CST (+)	Severe Variable Deceleration	Beat to Beat Variability < 5
Thick	19	3	5	1
Thin	5	–	3	2
Total	24	3	8	3

TABLE IV
MODE OF DELIVERY IN THE CONTROL AND TEST GROUPS

Mode of Delivery	Control Group	Test Group
C/S	26 (65 %)	15 (39,5 %)
Vaginal Route	14 (35 %)	23 (60,5 %)

Total of 41 C/S were done (52,6 %); 15 in the test group (39,5 %) and 26 in the control group (65 %). Statistically significant difference was found between the two groups.

Although meconium was present below the vocal cords in 15 cases (19,2 %), only 1 clinically evident aspiration of pneumonia was observed (0,01 %), (Table V).

TABLE V
PRESENCE OF MECONIUM BELOW THE VOCAL CORDS

Consistency of Meconium		C/S	Vaginal Delivery
Test Group	Thick	3	1
	Thin	1	–
Control Group	Thick	4	3
	Thin	2	1

One infant who was delivered by C/S because of fetal distress, had an Apgar score of 8, but later developed aspiration pneumonia and died on the 8th postnatal day.

All patients in the test group were delivered by C/S if this test results were abnormal. One patient with normal fetal electronic monitoring result developed fetal distress during labor and was delivered by C/S.

Patients in the control group were delivered by C/S in case of unfavorable cervix (19 cases with Bishop scores of 4 or less). Others were given the chance of "normal" delivery under close supervision. During labor 4 fetal distress had been observed and delivered by C/S in the control group. Indications and frequency of fetal distress are given in Table VI.

TABLE VI
INDICATIONS OF C/S AND FREQUENCY OF FETAL DISTRESS DURING LABOR

Test Group	14 cases	: Abnormal FEM results
	1 case	: Fetal distress during labor (4,2 %)
Control Group	19 cases	: Unfavorable cervix
	4 cases	: Fetal distress during labor (18 %)

Low Apgar scores were found in 7 cases; 1 delivered by C/S the remaining 2 by vaginal route in the test group. 1 delivered by C/S the remaining 3 by vaginal route in the control group.

Discussion

The high incidence of C/S may be the result of early or unnecessary intervention of an obstetrician facing with meconium stained amniotic fluid during labor. Especially if fetal scalp pH determination is not available, the obstetrician confranted with the risk of intrapartum or immediate postpartum death of a fetus could easily decide on an operative delivery.

In the control group the incidence of C/S was 26/40 (65 %). The high C/S rate might have been caused by the authors' reluctance to use oxytocin in the presence of thick meconium and unfavorable cervix without any diagnostic evidence at hand. By using fetal electronic monitoring, this ratio was lowered to 15/38 (39,5 %). Again, this ratio is considerably high if compared with pregnancies without meconium. As meconium is normally present in 17,7 % of normal pregnancies, an agressive approach in order to decrease fetal morbidity and mortality may result with a very high ratio of C/S as seen in the control group.[8] Eliminating nearly half of these cases may be considered adequate. However, the authors believe that this ratio still needs reduction. For this purpose, additional parameters such as fetal scalp pH determination is mandatory.[4]

It is obvious that C/S is not an answer for solving the problem, but one may prefer a high incidence of C/S to a high incidence of perinatal morbidity and mortality. Preventing or predicting acidosis using available techniques may result in nearly a 50 % decrease in unnecessary C/S as mentioned in literature.

In this study, by lowering C/S rate from 65 % to 40 %, the authors added a reasonably reliable parameter in delivery management. By adding fetal scalp pH determination to FEM, better values may be reached.

Prediction and prevention of fetal distress antenatally may be possible only in half of these cases by physical profile scoring systems. Patients having abnormal FEM tracings display nearly a four-fold increase in fetal distress ratio in labor when compared to the group with normal findings (28,95 % versus 8,9 %).

In this study, by using fetal electronic monitoring alone, the ratio of fetal distress was lowered from 18,0 % to 4,2 % which is in accordance with the literature. Fetal electronic monitoring, Nonstress test (NST), Contraction stress test (CST), beat to beat variability (BBV), when used in combination, may predict 40 to 60 % of fetal distress antenatally. However, false positive and negative values are still troublesome in FEM, but continuous monitoring may be the only possible approach in combination with scalp pH determination for sometime come.[9]

Fetal distress is the cornerstone of prognosis in the management of meconium stained amniotic fluid in labor. The fetus is not likely to aspirate unless there is some element of central nervous system depression.[4]

The higher C/S rate for fetal distress in pregnancies with meconium and for induced labor may be explained by the authors' policy of intervention in the control group.[1, 8, 10] In spite of 15 cases of meconium found below the vocal cords, postnatally, only one clinically evident aspiration pneumonia was observed. This was the infant of the patient that had been delivered by C/S because of fetal distress and died 8 days after birth. This again confirms that fetal distress determines the prognosis and eluciates the necessity of establishing new methods of fetal distress in labor before distress occurs.

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Colloid Cysts of the Third Ventricle of the CNS

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Summary

In this paper, 8 third ventricle colloid cysts in the CNS which were operated on by microsurgical techniques in our clinic between 1978-1985 are presented. History of the patients, symptoms, diagnosis and results are discussed by reviewing the literature.

Key Words : Colloid cyst, CT, Third ventricle, CNS.

Introduction

Cysts of the third ventricle which are benign tumours of CNS are rare. They consist 0.5 % of all CNS tumours.[1,2] Though benign in nature, misdiagnosis can lead, to mortality, especially after lumbal puncture.[1] Preoperative diagnosis depends totally upon neuroradiologic examination because the symptoms and signs are not characteristic.[1,3,4]

In this paper, we present 8 third ventricle colloid cysts treated between 1978-1985 in the Department of Neurosurgery of Hacettepe University.

Material and Method

Eight third ventricle colloid cysts were treated in our clinic between 1978-1985. Ages, symptoms, clinical findings neuroradiologic examinations and postoperative results of the cases are listed in Table I. All of the patients were operated by the transcortical, transventricular approach using the surgical microscope.

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Results

There were 6 male and 2 female patients with an avarage age of 31.5 years (range 13 to 53 years). Headaches were the most common symptom which occurred in 6 patients (Table I). Personality changes and confusion developed in one patient and loss of vision developed in another. The duration of symptoms varied between 3 days to 3 years (avarage duration of 9 months).

In neurological examination, papilloedema was found in 5 patients. One patient had papilloedema with neck stiffness. Organic brain syndrome was found in one patient and one patient showed normal neurological findings.

Plain skull roentgenograms, cerebral angiography, ventriculography and CT scan were used in diagnostic evaluation. Four patients presented with findings of increased intracranial pressure in plain skull radiograms. Angiography was carried out in 6 patients and findings of hydrocephalus were established in all of these patients. Ventriculography showed tumoral mass in the third ventricle in 2 patients and the third ventricle could not be filled by contrast material in 2 other patients. CT was performed in all cases and the classical appearance of colloid cyst was seen only in 2 patients (Figure 1). Enlargement of lateral ventricles was established in the remaining 5 patients. In 2 of these patients, the third ventricle was not seen in CT sections.

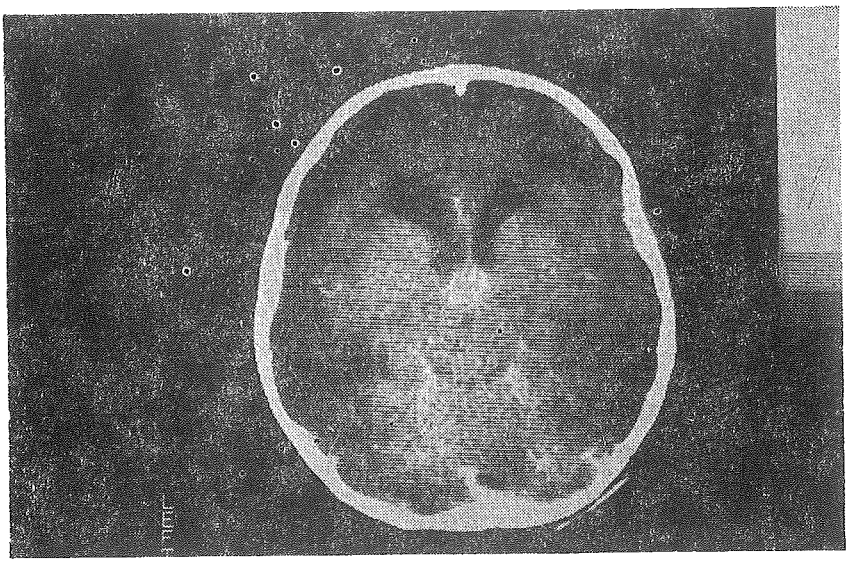

Figure 1

Classial appearance of colloid cyst were detected only in two patients. Atypical CT appearances may lead to misdiagnosis.

TABLE I

AGE AND SEX DISTRIBUTION, SYMPTOMS, CLINICAL FINDINGS, DIAGNOSIS AND RESULTS

	Age/Sex	Symptoms	Duration	Signs	Skull Radiography	Angiography	Ventriculography	CT	Prognosis
1	25/M	Headache	1 y	Papilledema	11 CP signs	Hidrocephaly	—	Colloid cyst	Well at 5 ys
2	48/M	Headache	1 y	Normal	Normal	Hidrocephaly	Mass in the III V	—	Well at 2 m
3	17/F	Headache	1 m	Papilledema	11 CP signs	—	Mass in the III V	Mas in the III V	Well at 1 y
4	38/F	Headache	y y	Papilledema	11 CP signs	Hidrocephaly + downward displacement of ICV	—	Mass in the III V	Well at 4 m
5	19/M	Headache + decreased vision	15 d	Papilledema	11 CP signs	Hidrocephaly	Univisible III V	Mass in the III V	Well at 2 y
6	53/M	Confusion + personality change	3 d	Organic mental syndrome	Normal	Hidrocephaly	Unvisible III V	Unvisible III V	Well at 8 m
7	39/M	Headache	1 m	Papilledema + stiff neck	Normal	Hidrocephaly + upward displacement of ICV	Mass in the III V	Mass in the III V	Well at 2 m
8	13/M	Headache	3 y	Papilledema	Normal	—	—	Colloid cyst	Well at 1 y

All the patients were operated on by the transcortical, transventricular approach under the surgical microscope. Colloid cyts were totally removed in all patients with a 100 % survival rate. Follow up examinations revealed normal neurological findings.

Discussion

The origin of to colloid cysts[2, 5-7] in the third ventricle of the CNS are still controversial. They can develop from neuroepithelial cells, ependirmal cells or diencephalic reess. According to Shoanqshoti the cysts are frequently confirmed in outopsy; however, occasionally they may be symptomatic in the elderly.[6] Witgand Symon noted predominance in men (M:W 2:1).[7, 8] In our series male patients constituted 75 % of all cases.

Kelly described three different clinical types of third ventricle colloids cysts.[3] (1) nonspesific increased intracranial pressure without localized neurological findings, (2) Dementia with or without headache, (3) Paroxismal drop attacks. Paroxismal drop attacks are rare. According to Dandy, these attacks occur by ball valve mechanism and may cause sudden death due to acute hydrocephalus.[9]

The diagnosis of a colloid cysts of the third ventricle depends mostly upon the neuroradiological examinations. Ventriculography was a most important diagnostic method before the invention of CT.[10] The cyst can be seen as a round shaped formation in the third ventricle. Cerebral angiography can reveal deplacement of ICV in addition to hydrocephalus. CT is the most reliable method in diagnosis.[10, 11] The colloid cyst is seen as an hyperdense round shaped lesion and it rarely becomes enhanced after the injection of contrast media. Isodense lesions are occassionally reported. In our series, we were able to find only two cases who had classical appearance of colloid cyst (Figure 1). Isodense lesions were found in 5 patients (71 %) which is much higher than the literature findings. For this reason, in order to reach a definite diagnosis ventriculography assisted CT should be performed more frequently.

In differential diagnosis other tumours of third ventricle such as ependymoma, astrocytoma, papilloma of choroid plexus and aneurysm of basillary artery must be excluded.

The proper treatment of colloid cyst is the total surgical removal of the lesion. Stereotactic aspiration is described by some authors. There are two different approaches in surgical intervention. (1) Transcortical approach, (2) Transcollosal approach. Although the first approach is the most acceptable method, Mc Kissock and Little presented that this

method has a high incidence of epileptic seizures as a result of cortical damage.[1, 7] All of our patients were operated on by the transcortical approach. No postoperative seizures and no mortality were recorded.

Occasionally, increased intracranial pressure can not be pulled down to normal levels even after successful surgical intervention. Such a condition is believed to be caused by compression and inflammation of the degenerated cells and the cyst fluid. In such cases, a shunting procedure is indicated.[12]

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Benign Lymphoid Hyperplasia of the Stomach

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Summary

A case of benign lymphoid hyperplasia of the stomach manifested by only one umblicated polypoid lesion is presented. The different radiological and endoscopical manifestations in the stomach are discussed, and the similarities in the clinical, roentgenologic and gastroscopic features to gastric malignancies such as lymphoma, leiomyoma and carcinoma, are presented.

Key Words : Benign lymphoid hyperplasia, umblicated polypoid lesion.

Introduction

Lymphoid hyperplasia of the gastroentestinal system is an uncommon, benign, nonspesific disorder characterized microscopically by an increase in the size and number of lymphoid follicles. The stomach,[1] the duodenum,[2] the terminal small bowel, colon and rectum[1, 3-7] may be affected by this benign process. Clinicaly, endoscopicaly and radiologicaly it closely simulates a malignant neoplasm.[2, 8] Hence, histological diagnosis is difficult.

Lymphoid hyperplasia is usually mistaken for malignant lymphoma or leiomyoma of the stomach. Prognosis of the gastric lymphoma is considered to be generally better than other gastric malignancies.[9, 10] This favorable prognosis may be partly due to the fact that many of the cases included under lymphoma of the stomach are cases of lymphoid hyperplasia.[2] It can be called benign lymphoid polyposis,[5] gastrointestinal pseudoleukemia[1, 11, 12] or localized hyperplasia of the lymphoid follicles.

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We recently observed a case of benign lymphoid hyperplasia of the stomach manifested endoscopically and radiologically as an umblicated polypoid lesion. To our knowledge, there has been no previous report of a single relatively big umblicated polypoid lesion.

Case Report

A 20 year-old man presented in March 1985 with recurrent epigastric pain associated with dyspepsia of three months duration. The pain was periodic and increased during fasting.

Physical examination was normal except for epigastric tenderness. C.B.C. and sedimentation rates were normal. An upper gastrointestinal series revealed a relatively small polypoid lesion in the antral area of the stomach (Figure 1). Endoscopic investigation with a fiber panendoscope disclosed a single protruding and centrally depressed lesion in the antrum (Figure 2), deformation of the duodenal bulbus and superficial mucosal ulceration. The biopsy specimen from the polypoid lesion showed lymphoid hyperplasia with normal mucosa (Figure 3). Gastric juice analysis showed a mild increase of free acid.

Figure 1
Upper gastrointestinal radiologic examination showing smooth-contoured, polypoid lesion in the antral region.

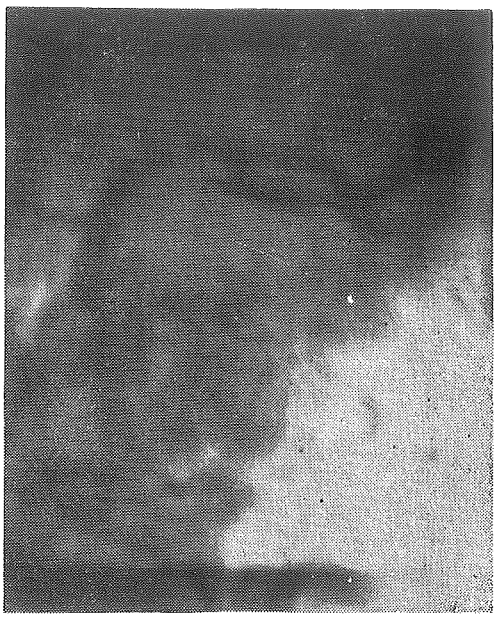

Figure 2
Endoscopic appearance of the polypoid lesion with central umbilication.

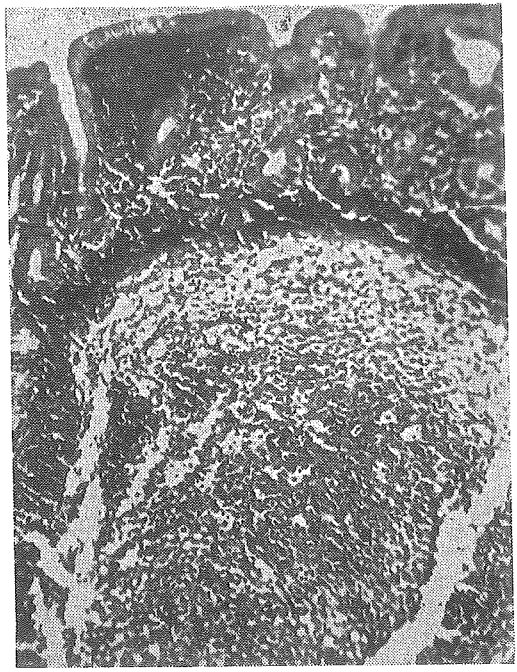

Figure 3
Biopsy from the lesion discloses hyperplastic lymphoid follicle immediately underneath the intact surface epithelium. H + E X 225.

Discussion

Lymphoid hyperplasia of the G.I.S. has been known since 1838.[13] Microscopic appearence is characterized by a simple increase in the size and/or number of lymphoid follicles in the submucosa. It is impossible to find the infiltrative and destructive features of malignant lymphoma and this disorder is never followed by true leukemia.[11, 14]

Radiologically, the appearance is polypoid in shape. Usually this looks like a typical cobblestone or polypoid lesion, but our patient had only one polypoid lesion which resembled a true polyp or leiomyoma (Figure 1). In seven of the patients with benign lymphoid hyperplasia reported by Perez and Dorfman,[15] the upper gastrointestinal X-ray studies were interpreted as polypoid, infiltrating or ulcerating carcinoma of the stomach. Two patients out of 15 had large rugae as the predominant finding. This different radiologic appearance may be due to an acidic environment in the stomach. Although Mainzer *et. al*,[16] suggested that low acidity plays an important role in the evaluation of a polypoid lesion in the stomach, our patient had hyperacidity. This condition may resemble gastric polyposis, polypoid thickening of the rugae, or multiple aberrant pancreatic tissue.[17]

Gastroscopic finding of an umblicated polypoid lesion on the pyloric antral wall, surrounded by a normal mucosa without decreased motility, suggested the possiblity of gastric leiomyoma or a true gastric polyp in our patient. Gross appearance of the lymphoid hypertrophy may be quite variable; eg. ulcerative, polypoid, diffuse giant rugal hypertrophy, cobblestoning, multiple umblicated lesions or a combination there of[18, 19] Therefore, some cases are also mistaken for malignant lymphoma.

In spite of the radiological, endoscopic and clinical findings closely simulating those of a gastric neoplasm, the pathologic picture was quite characteristic of the benign lymphoid hyperplasia as defined by Helwig.[13] Criteria of benign lymphoid hyperplasia are : 1)polymorphous cellular infiltration, 2)presence of active reaction center in the lymphoid follicles, and 3)ubiquitous scar tissue intimately mixed in lymphoreticular tissue.

Although numerous cases of benign lymphoid hyperplasia of the G.I.S. has been reported before, we were unable to find a case report of an umblicated, simple, polypoid and relatively large lesion in the literature.

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Vanishing Penis Syndrome

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Summary

A case of a twenty year old man with vanishing penis syndrome, which is a rare cause of mechanical impotence, is presented.

Key Words : Impotence, Vanishing penis syndrome, Male sexual dysfunction.

Introduction

Vanishing penis syndrome is a rare cause of mechanical impotence very often seen in patients with morbid obesity, large hydroceles or genital lymphoedema.[1] In this paper we present a patient with this syndrome caused by obesity.

Case Report

A twenty year old man was seen in the endocrinology ward with the complaint of a small penis on erection and unability to penetrate during intercourse. He was a healthy young man with normal sexual relations until a year ago when he started to put on weight.

His height was 1.69 m and he weighed 102 kg. On physical examination, his penis could not be seen due to the bulky fatty tissue of the mons pubis, and when this was retracted he had a normal penis (Figures 1, 2). During erection, glans was just visible between the crests of the fatty tissue.

His endocrinological investigations revealed no hormonal abnormality. He had negative Barr body and a normal spermogram. He was given a diet to lose weight; and after 6 months with a weight loss of 21 kg, he was able to have normal sexual intercourse despite the lack of change of his genital appearence.

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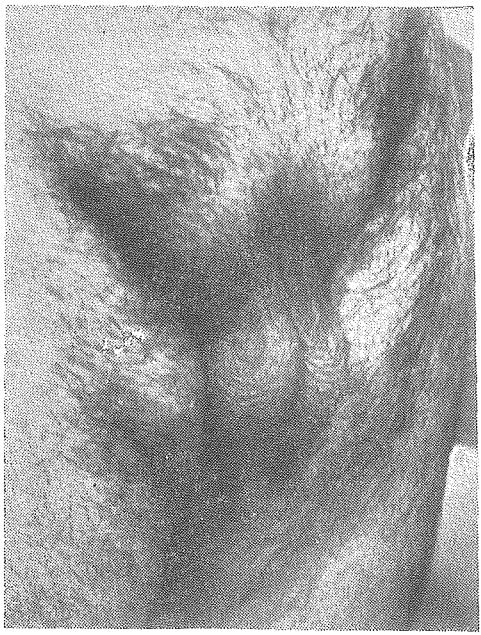

Figure 1
Genital appearence of the patient.

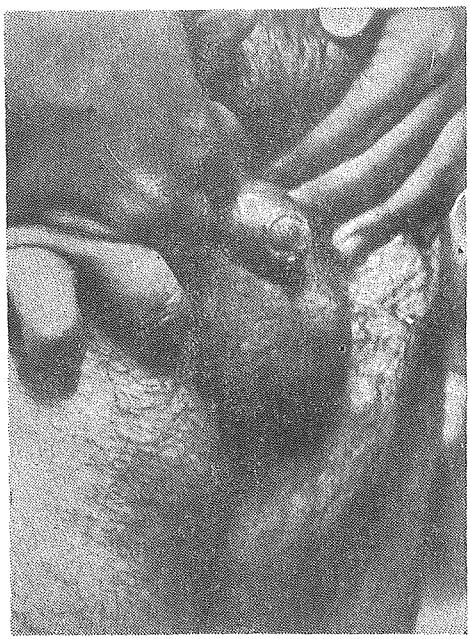

Figure 2
Normal penis after retraction of the fatty tissue.

Discussion

Masters and Johnson describe impotence as the state of unsuccessfull penetration of a woman in more than 75 percent of attempts.[2] Among many of its causes some are classified as mechanical causes which covers congenital deformities of the penis and Peyronie's disease. Rarely, vanishing penis syndrome is a mechanical cause in secondary impotence where the patient initiates and sustains an erection but is unable to conclude coitus satisfactorily. It is seen in patients with local causes such as morbid obesity, lymphoedema and giant hydroceles.

The treatment is aimed to relieve the cause such as: surgery for hydroceles, weight reduction, or treatment of lymphoedema. No other treatment is recommended because of the intact neural, hormonal and vascular mechanisms.

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Historicity of Disease-Acquired Immunodeficiency As a Case in Point

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Summary

Unlike science, whose subject matter is brought about by phenomena that occur again and again, history deals with events which happen once and only once and are as a rule unrepeatable. The past, the present and the future form a temporal continuum, and so far as the methods of science and history are applicable, there are no differences, in principle, between the phenomena and events in the three divisions of the time scale.

Individual cases of disease which are comparable to events and to disease phenomena on the whole that occured in the course of the evolutionary continuum of life (comprising historical times) will evidently occur in the future. The recent epidemics of AIDS may be compared, from a historical point of view, to the scourges of earlier times. As a cross-over infection with the responsible virus having crossed the (monkey-human) species barrier, potentially it seems to be even more devastating. However, consideration of the ever-present "surprise" element in human history might make us less pessimistic as to the future of the current spread of the acquired immunodeficiency disease.

Key Words: History and science, Temporal continuity and history, Empirical principles and temporal continuity, Acquired immunodeficiency disease in future history.

It is generally accepted that one of the reasons why the name of Hippocrates has been almost totally identified with the rise of "scientific" medicine in antiquity is his rational explanation of disease pheno-

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mena, with no recourse to supernatural factors. Diseases were, in his view, attributable to natural causes. The proposed theoretical framework to explain these phenomena, namely the theory of four humours, was inescapably metaphysical and, like others of its kind, had almost no correspondence with the world of facts - it offered a system of pseudo-explanations[1] as have been most of the philosophical speculations about the structure and functioning of the world. But, it was rational. In the opinion of Hippocrates, epilepsy for instance, was not "any more divine or more sacred than other diseases," its supposed divine origin being "due to man's inexperience".[2]

The recent epidemics of AIDS, the so-called Acquired Immunodeficiency Syndrome, is certainly not different basically from, say, common cold or influenza. Its comparatively frequent occurence in male homosexuals and other characteristics can be scientifically explained.

From the viewpoint of the evolution of medical science, we may well remember the case of leprosy in this context, although the biological properties of this entity are quite far from being similar to those of AIDS. Even today, at a time when the infection is rare and is accompanied by a certain degree of success in therapy, lepers are still kept in isolation in several parts of the world. In the present context, however, we shall be concerned with the cognitive and not socioethical aspects of the AIDS phenomenon, particularly so far as these would be relevant to the natural history of disease in general.

The Temporal Dimension of Medical History

The temporality of history seems to be taken so much for granted that we see no reason why we should discuss the matter at all. Being in principle "a detailed study of the past," history, that is human history, is delimited retrospectively by the discovery of writing, the classical means of human communication which lasts.

From a methodological point of view, and unlike science whose subject matter is brought about by *phenomena*, history deals with *events*. A phenomenon is a fact that occurs or is repeated again and again in the course of time, being as a rule infinite in number. An event, on the other hand, happens once and only once and is in principle unrepeatable; it is something special or specific, which tends to be seen as unique, actually the result of an encounter in space-time of several or many characteristics or attributes.[3] We can say that the more complicated a thing (or event) is, the more individual and the more "historical" it becomes,[4] whereas a phenomenon is to be seen as a general and sometimes universal aspect of reality.[3] From a philosophical standpoint,

an event is a particular, while *a phenomenon seems to be comparable to a universal*, and as concepts they have the sort of relationship to each other that exists between two philosophical entities - the concrete and the general (or the abstract). (In medical terms, a certain disease, say tuberculosis or gastric ulcer, is a phenomenon whereas a definite case of either pathology, a concrete human being, must be regarded as an event.) Phenomena have only family or group names, so to say, but an event has an additional first or given name.[3]

As we go back in time, events of written or "classical" history merge, as it were, with phenomena, and the concept of history goes far beyond its more or less usual boundaries. History, and medical history for that matter, is then superseded or supplanted by truly scientific fields such as geology, palaeontology, palaeopathology, palaeoanthropology, palaeoecology and others oriented to the study of past phenomena;[3] these disciplines are called "historical" natural sciences.[4]

Medical History as a discipline is more concerned with events and a field which we could call Medical Evolution is concerned rather with phenomena.[3] In the present article, however, we intend to use the term "history" in relevant contexts as it is so firmyl entrenched, and leave a deeper analysis of the concept of medical evolution to a later work.

The Present as Evolving History

The topic of the past or distant temporal limit of medical history and history in general seems to bring in its wake the question of their recent or near boundaries. If events and phenomena can be conceived of and studied as far back as possible in time, what will constitute their aspect facing us?

It may be interesting to quote the remarks of a medical historian of our time related to this question. One of the difficulties of presenting history being "that of deciding at what point the narrative should cease", he points out, "deeds and discoveries of our time will be found in works on history, not yet written". Thus, the full story of medical discoveries "must be told in the future, when their integration into the general concept of medical science is complete".[2]

Certainly there is truth in such a claim. From a basic standpoint indeed, it should be remembered that the future is contingent, that is possible but not certain, and that the same must apply to the past-what happened at a definite point in time was not, at least not necessarily, what should presumably have happened. But as events (and phenomena) take place in a continuum, *the "present" becomes the past* in the course of

time, just as the "future" is going to be our present, as a rule imperceptibly, and is incorporated into the flow to contribute, so to say, to the extending continuum. This is evidently in conformity with what has been mentioned earlier as to the main characteristics of history-that it is its event-orientedness which makes it different from science in the first place and not its retrospective temporality.

We may make the assumption that every event may be"historical" potentially, and that it would require somebody to render it "significant" or "meaningful" within a context worth investigating.

An important point at this stage of our discussion appears to be the determinism or indeterminism of happenings and occurences. In the case of phenomena, there seems to be no difference in principle between the earlier and present instances of the same phenomenon so far as scientific research is concerned, provided that it is scientifically accessible.[3] Seen particularly in the light of what has so far been discussed, it must be clear that the principle of determinism, that under the same conditions the same determinants will always produce the same effects, should apply to the past as well as to future phenomena. Otherwise, there would be no similarity, hence no link, between the past and the future (via the "present"), no uninterrupted flow (In like manner, other empirical principles operating in the world such as causality or indeterminacy too should be regarded as valid within the whole range of temporality).

As for events, as a rule and at the same level of organization (atomic, molecular, organic...), they seem to obey the principle of determinism less strictly than phenomena which are appreciably simpler in structure. As the "specific" events of history loose their identity by becoming incorporated into the "general" phenomena of science, they become more clearly expressible in statistical terms.

The consideration of phenomena and events in an evolutionary setting and space-time continuum would comprise everything that has existed and leave nothing behind.

AIDS: SYNDROME OR DISEASE?

The title of an excellent editorial would be illuminating for an analysis of the term, "acquired immunodeficiency (or immune deficiency syndrome".[5] Full use of this article is made here, which would serve as an infrastructure for the consideration of the condition.

The virus responsible for this condition is a retrovirus belonging to the subfamily Lentivirinae, variously known as human T-cell lympho-

tropic virus type III (HTLV-III), lymphadenopathy AIDS virus (LAV), AIDS-associated retro-virus (ARV), or simply the AIDS virus, (and more recently human immunodeficiency virus (HIV)). The condition is, then, an infection. It has a chronic course, but the overall prognosis can only be speculative for many years to come.[5]

The term AIDS has first stood for a group of disease processes secondary to a defect of cell-mediated immunity. Before it was definitely shown to be due to a virus, the immune deficiency was assumed to have multiple causation[5]-hence, apparently, its consideration as a "syndrome", a set of symptoms occuring together. What is interesting from an evolutionary standpoint is that the related virus is new to man. Epidemiologically speaking, infection with the virus is potentially lethal to all men, women and children irrespective of whether or not they are homosexuals, promiscuous people or are drug users.[5]

The virus persistently infects a small minority of mature T-helper lymphocytes in the peripheral blood, lymph nodes and spleen, and also the nerve cells throughout the brain; in both cases it is slowly replicative and can be detected as integrated and unintegrated pro-viral DNA. It is cytopathic to both kinds of cells, the slow cytopathic effect on brain cells being irreversible and cumulative and taking at least two years to become manifest. The infection can thus kill as a result of brain damage and without immune suppression and opportunistic infection.[5]

According to the majority of research workers the virus is present, and thus transmissible through, semen, saliva, tears and urine as well as blood. The infection can also be transmitted, as has been observed, to the newborn or unborn baby through the diseased mother. A different view is that transmission of the virus is blood-borne, like hepatitis B virus. Both are transmitted during anal intercourse, because this regularly causes minor or major injuries of and bleeding from the rectal mucosa, similar lesions rarely occuring in vaginal intercourse.[5] In both the homosexual and heterosexual practices, however, and in keeping with this view, the penis too must reveal abrasions and bleeding so that the transport systems of the two partners could come into contact to make the transmission possible. This would be possible, furthermore, not only by means of the contact between external genitals but by a contact of any bodily parts during the intercourse. As a final point, it would be important to keep in mind that the AIDS virus infection does not bring about any lesion in the genital organs and thus should not be regarded as a characteristically[5] venereal or sexually transmitted disease such as gonorrhoea or syphilis, at least from a theoretical or nosological point of view.

Particularly interesting for us would be to consider another subfamily of retroviruses, Oncornavirinae, known to be significant in cancer causation. In both the phenomena of malignancy and AIDS, what appears to be in the foreground at the biological level is the reduced, impaired capacity of the immune system of the organism to cope with the invader. Another common characteristic concerns the level of molecular biology, namely the integration of the virus genome in the cellular genome in cases of viral carcinogenesis[6] and that of the pro-viral DNA in the genome of brain cells in AIDS.[5]

Being the outcome of a primary infection with a definite, "known" virus and of the accompanying cellular immunological deficiency, one may wonder why we should still call this phenomenon a syndrome and not a disease. The general problem of drawing a demarcation line between diseases and syndromes may need further clarification from a conceptual viewpoint. In the case of AIDS, however, the quality of being a *biological entity* with its etiological, pathological and clinical aspects fairly clearly forming a whole is much more satisfactorily expressed by the term "disease" than by "syndrome". (The term "disease" could readily be omitted in this context as in the case of so many other conditions.)

The Natural History of Disease

The term AIDS seems to have been coined by contrasting the related condition with "congenital" immune deficiency syndromes. The well-established viral origin of the "acquired" form could have certain implications for us. But to regard the AIDS virus as "a necessary and sufficient cause[5] of the phenomenon is empirically unjustified, because although it is the main, constant or ever-present etiological agent in the process, it is not its "cause" in a strict sense. Stated otherwise, the virus is not the sole determinant of the phenomenon in question. In the absence of what we may call contributory or nonspecific factors or conditions, a microorganism would seldom if ever lead to an infection.

To state the issue in a logically satisfactory manner, we can point out that the *necessary cause* must be complemented by *sufficient determinants*. (The term "sufficient" should evidently mean "complementary" and not "sweeping" or "overall").

It may be interesting, though not surprising, to know that skin-test anergy is common in AIDS patients.[7] As is generally known from clinical practice, viruses do cause nonspecific immune depression, with resultant secondary and sometimes lethal infections. Among renal transplant patients, decreases in the ratio of helper to suppressor cells are

associated with secondary or opportunistic infections and Kaposi's sarcoma just as in acquired immunodeficiency disease. In the latter case the tumour is often generalized and more aggressive, with variable responses to chemotherapy.[7] All these are in conformity with the fact that immune disfunction leads to tumour growth; and the degree of biological malignancy must be linked with the severity of this disfunction.

From a natural historical and ecological point of view, a significant matter is that the AIDS virus has crossed the species barrier[5] and thus become a "new" pathogenic microorganism for man. The disease is thus a *crossover infection*, which is to be seen as a human extension of the simian acquired immune deficiency. This may perhaps be linked to a significant aspect of evolutionary biology and pathology, namely that viruses may be transmitted to the offspring and later generations through heredity by their incorporation into the structure of genes and that mutation and the formation of a new species are due to a viral infection of the genetic structure in the former species.[8]

Implications

From an evolutionary point of view, diseases as natural phenomena may be of significance in the development of a species, because some of them can endanger its existence.[9] Evidently, however, the majority of diseases do not pose such a threat. Historically speaking, we might perhaps remember certain epidemics with which we could establish medical and social parallelisms and see a possible pandemics of AIDS in this light. Although it has been no more than six years since the first cases were reported in the West, the characteristics of the disease so far observed may give us certain clues as to its epidemiological future.

The earlier scourges such as plague or smallpox, no longer world-wide problems, are among those that would readily come to mind. The preventive (hygienic and/or immunological) measures above all have rendered such sweeping epidemics controllable and even almost extinct. But the present epidemic, the causative agent being new to or unkown by human organism, and together with its insidious progress, seems to be potentially even more devastating. Its chronicity would lead to the preservation of the responsible virus in the human organism hence in the community, for a relatively long period of time. Are we going to witness, in the face of complex interterritorial relationships of modern human societies, a pandemic at a historically unknown scale?

Until a little more than a century ago, pathogenic microorganisms were indeed in the foreground of social life so far as health issues were

concerned, and it seems that we have been living a similar transitional period in the case of malignancy in that our scientific knowledge and therapeutic possibilities as related to cancer have been steadily developing in the past few decades. Although the AIDS virus is a "new" microorganism attacking directly the immune system and the brain, we should not lose sight of the fact that the future is, as discussed above, contingent, and that there is evidently always room for "surprises" in human history. The achievements of modern scientific medicine since the middle of the last century could not be predicted, except perhaps in bare outline, by a great majority of those who were observing the developments at the time.

The names of Pasteur, Koch, Lister, Fleming may come first to our mind among so many others in the struggle against infection. The successful researcher or research team involved in the prevention and/or therapy of acquired immunodeficiency disease would apprently be the foremost candidate for the Nobel Prize in medicine. And the competition between the Institut Pasteur of Paris and the National Cancer Institute at Bethesda, which has obviously not come to an end following the isolation of the virus, becomes readily understandable.

However, for those prospective readers who would be interested in an overall evolutionary setting of human affairs rather than in such details as prize lists, history, whether medical, social or other, would probably mean something deeper.

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