HACETTEPE BULLETİN of MEDİCİNE SURGERY

12

1979

HACETTEPE BULLETIN OF
MEDICINE/SURGERY
VOLUME 12 / Nos. 1-2 / JAN.-APRIL 1979

Natriuresis and Prostaglandin E-Like Activity During Elevated Renal Arterial Pressure in Isolated Rabbit Kidney

Nimet Ü. Gündoğan, M.D.*

Introduction

It has been reported that the renal venous blood of hypertensive patients contain various types of prostaglandins.[1] Further, it has been shown that injection of know vasoconstrictor agents such as angiotensin II,[2] noradrenalin,[3] into renal artery and stimulation of renal nerve[4] causes intrarenal vasoconstriction which leads to the increase in the level of PGE_2-like material in the renal venous blood. These observations suggest that prostaglandins might act intrarenally as a mediating homoral agent in the regulation of renal blood flow.[5] On the other hand, a number of works published, state that, it is possible for renal prostaglandins to serve as the natriuretic hormone responsible for sodium excretion in the pressure natriuresis.[6, 7, 8, 9]

In an attempt to resolve the question of whether PGs mediate inpressure natriuresis we have investigated the phenomenon using an isolated perfused rabbit kidney preparation. In this preparation venous effluent superfused assay organ was used for detecting PGE_2-like activity as urine volume, sodium and potassium excretion were determinated simultaneously.

Materials and Methods

Isolated Perfused Organ

Rabbits of either sex, 2-3 kg. b.w., were anesthetized with sodium pentobarbital, 30 mg/kg, i.v. The kidney was isolated according to the

* Associate Professor of Physiology, Faculty of Medicine, Hacettepe University, Ankara/Turkey.

method described previously and perfused through the renal artery by a peristaltic pump delivering a constant flow of 10-12 ml/min. at the beginning when perfusion pressure PP was 60-80 mmHg.[10]

Perfusion was provided by a technique depicted in Figure 1. Perfusion pump (Havvard model 505-1200) has two channels, one of which is used for perfusion of the kidney and the other for superfusing rat stomach strip (RSS). Venous effluent of the kidney was collected in a 15 ml volume reservoir connected to a three way stopcock. A by-pass circuit was prepared between the stopcock A and the stopcock Bfixed on the channel before the pump and used for superfusion RSS without changing the volume. Perfusion pressure was measured by a pressure transducer (Statham P 23 AA 11151) connected to the channel which was used for perfusion of kidney proximal to the cannulated renal artery. Isometric contraction of the bioassay organ was measured by force displacement transducer (Grass FT 10 C) and urine flow was measured by a magnetic tipper. Kidney PP, RSS isometric contractions and urine drops were recorded simultaneously on the Grass polygraph (Model 7 D 53IV3). The perfusion fluid was Krebs' solution composed as follows: (mM): NaCl: 112; $NaHCO_3$: 25; KCl 5; NaH_2PO_4: I; $MgCl_2$: 0.5; $CaCl_2$: 2.5; and glucose: 11.5 Constant temperature at 37°C was provided by the thermoregulator pump (Haake type F V 115) in two connected jackets. Krebs' solution gassed with 5 % CO_2 in O_2.

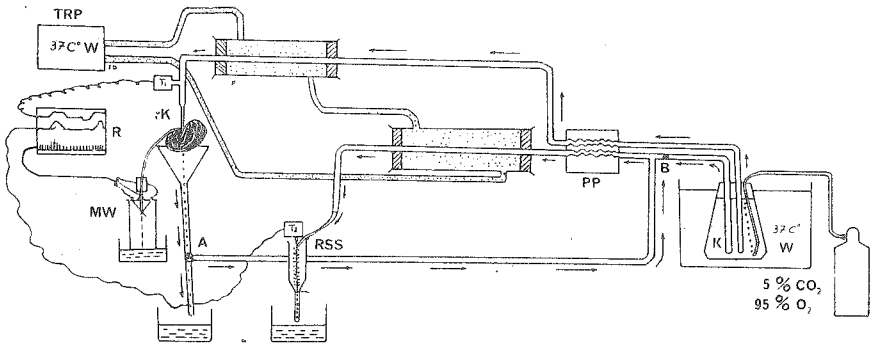

Figure 1

Schematic diagram of the experimental set up used for the isolated perfused kidney and isolated superfused rat stomach strip. Water bath (W), Krebs' solution container (K), aerated by 95 % O_2 and 5 % CO_2, two channel perfusion pump (PP), thermoregulator pump for two connected jackets (TRP), isolated perfused rabbit kidney (rK), recording urine drops by magnetic writer (MW), three-way stopcock (A,B), rat stomach strip (RSS), pressure transducer (T_1) recording of test organ contraction by force displacement transducer (T_2), and recorder (R).

Detection of Prostaglandin E_2-Like Material in Isolated Kidney

Venous Effluent

Two methods were used:

a. In continuous method, the stream of venous effluent from the kidney superfused assay organ RSS which was prepared according to the method used by Vane[11]. RSS was made insensitive to acetylcholine, 5-hydroxytryptamine, catecholamine by infusing a mixture of antagonists to give the stated final concentration of the active bases atropin 10^{-7} g/ml (n=2), dihydroergotamine $5X10^{-7}$ g/ml (n=2), phenoxybenzamine hdyrochloride 10^{-7} g/ml (n=2), mepiramine 10^{-7} g/ml (n=1), and for PG synthetase inhibition lysine-aspirin was added to Krebs' solution at the concentration of $1.6x10^{-5}$ (n=6) RSS were subjected to 1-2 g. tension and superfused with constant flow (10 ml/min.) by a peristaltic pump throughout the experiments. Test organ was allowed to equilibrate for one hour until a steady base line at resting tension was established.

b. In the intermittent method (by extraction procedure) renal venous effluent which had superfused the RSS was collected during normal and high perfusion pressure for ten minutes (n=6) and centrifuged to deposit any blood cells. Samples were extracted according to the method of Gilmore and his associates.[12] The acidic lipids were further purified by thin-layer-chromatograpy on 0.3 mm thick silica gel layers with ethylacetate-methanol water (8:2:5) as the solvent system.[13]

At the beginning of the experiment, the isolated kidney and assay organ were perfused and superfused with Krebs' solution. The renal venous effluent was run off till equilibrium was reached. The standard PGE_2 was given directly to the assay organ in logarithmically increasing doses (10, 20, 40, 80 ng) and responses were recorded (Figure 2). When the kidney venous effluent was completely clear, the assay organ was superfused by renal venous effluent without increasing the renal perfusion pressure, using a 15 ml. reservoir via the three way stopcocks A and B (Figure 1). Perfusion pressure was kept constant at the starting level during the superfusing of the assay organ and PGE_2 was once again given directly in increasing doses with the renal venous effluent. Both superfusion of Krebs' solution and venous effluent in logarithmically increasing doses of PGE_2 failed to change the responses. The maximum response of the assay organ was ascertained with high doses of PGE_2 and other responses were calculated as percentages of the maximum. Figure 3 illustrates the log, dose-response relationship. The recorded results of

both conditions in normal and high perfusion pressure were represented in log, dose-response graphic on semilogarithmic paper and the PGE_2-like activity of renal venous effluent was calculated.

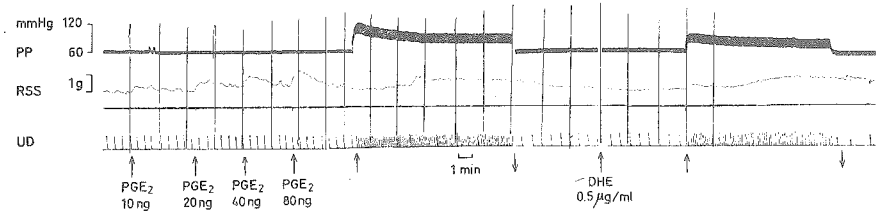

Figure 2

Parameters recorded simultaneously by the polygraph from isolated, Krebs' perfused rabbit kidney and the test tissue. The upper tracing shows the perfusion pressure of the kidney (PP), the middle one shows the contractions of rat stomach strip (RSS), and the lower tracing shows the urine drops (UD) recorded from the ureter. Each vertical line in the lower tracing shows the urine drops recorded by magnetic writer. First portion of the figure shows the responses recorded after giving PGE_2 directly into the channel which superfuses only the RSS. After the first section, the single arrow (↑) indicates the rise in the PP due to the increase in the volume of Krebs' solution passed through the kidney. As can be seen clearly, the urine drops have increased in number during this period. In spite of attempting to keep renal PP constant at high perfusion level this has decreased, gradually, and parallel to this, contraction has taken place in the RSS. When the perfusion volume was decreased (→), both PP and number of urine drops fell to approximately normal levels and contracted RSS relaxed. The experiment was repeated after 0.5 µg/ml dihydroergotamine (DHE) was added to the Krebs' solution and the same result was observed.

The first ten minutes represent the normal control period. This period was followed by a second period during which the perfusion pressure was acutely increased for ten minutes. During these two periods venous effluent samples were collected for thin-layer-chromatography. Perfusion pressure decreased in following control periods at the starting level. Kidney and assay organ were allowed to rest for twenty minutes. During the last ten minutes of this resting period, control urine samples were taken as RSS lossing were observed simultaneously, and the perfusion pressure again was increased acutely for ten minutes. The acute pressure increase was repeated thrice.

The change in urine volume was estimated ml/min. Sodium and potassium were determinated in Baird otomic flame photometre model KY3[14] and values were calculated as U_{Na}. V µEq/lt/min., U_K.V µEq/lt/min.

The results were evaluated statistically using Student's "t" test.

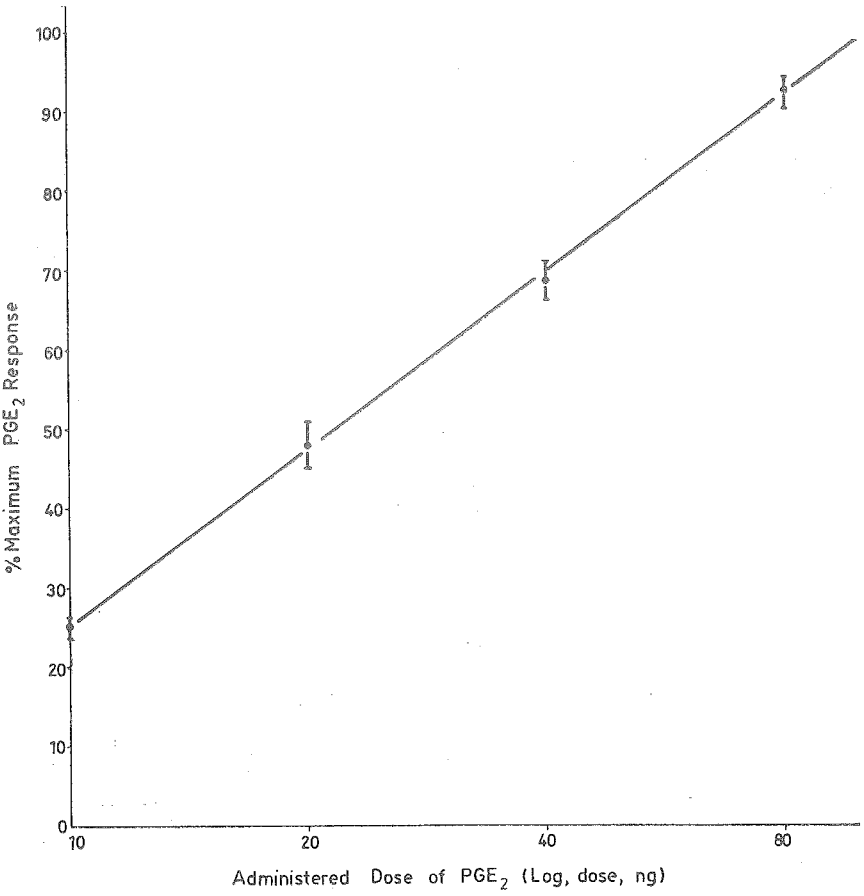

Figure 3

Logarithmic dose-response curve of PGE_2 found in the fundus muscle strip of rat stomach. Each point corresponding to each dose indicates the mean value of the separate experiments while the vertical bars on these points show the standard errors.

Results

Experiments on Assay Organ

Dose-dependent maximal percent responses of the venous effluent perfused assay organ to standart PGE_2 means are seen in Table I.

Logarithmic dose-response curve was obtained with the maximum percent PGE_2 responses in assay organ. Assay organ contractions in normal

TABLE I

RSS MAXIMAL PERCENT RESPONSES TO STANDART PGE_2

PGE_2	10 ng	20 ng	40 ng	80 ng
PGE_2 maximal Percent response mean	25.30	47.60	69.49	92.11
SEM	± 1.524	± 2.733	± 2.108	± 2.046
Number of experiments	(10)	(10)	(10)	(10)

and high perfusion pressure as a response to PGE_2-like activity in renal venous effluent can be estimated by the help of logarithmic dose-response curve (Figure 3). Table II illustrates test organ responses as maximal percent response and PGE_2-like activity as a nanogram (ng) in normal and high perfusion pressure.

TABLE II

MAXIMAL PERCENT RESPONSE IN RSS AND PGE_2-LIKE ACTIVITY OF VENOUS EFFLUENT

	Initial level of Perfusion Pressure (PP→60-80 mmHg)		Acute elevation of Perfusion Pressure (PP↑90-120 mmHg)	
	Maximal Percent response to PGE_2	PGE_2-like activity (ng)	Maximal Percent response to PGE_2	PGE_2-like activity (ng)
Mean	24.32	9.10	54.42	22.39
SEM	± 2.27	± 1.01	± 4.09	± 1.65
Number of experiments	(10)	(10)	(10)	(10)

When perfusion pressure was increased, assay organ simultaneously contracted (Figure 4). The difference in the percent of maximal contraction of the assay organ is 30.10 ± 3.20 ($P < 0.001$) and difference of PGE_2-like activity is 13.21 ± 1.36 ng ($P < 0.001$). There is a positive correlation between high perfusion pressure and increasing PGE_2-like activity.

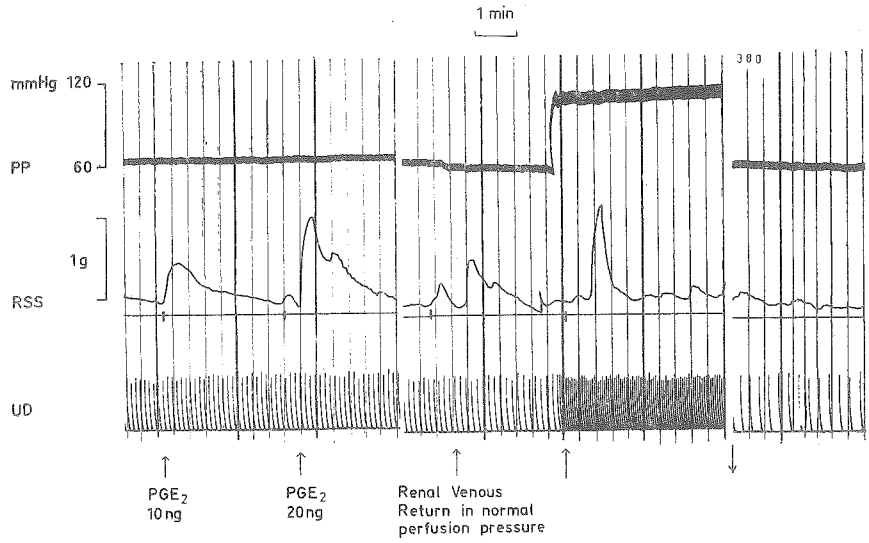

Figure 4

This tracing shows in particular the contractile response obtained after normal venous return passed over RSS. The important point in the tracing is that the same contractile response was observed when the PP was increased. However, the response obtained this time is twice the normal venous return response. Perfusion Pressure (PP), rat stomach strip (RSS), urine drops (UD).

The degree of this correlation is ($r=0.97$; $P < 0.001$) significant. Addition of lysine-aspirin to the Krebs' solution at the concentration of $1.6X10^{-5}$ mol/Lt prevented the release of PGE_2-like material.[15] The estimated amount was found to be 7.0 ± 0.5 ng ($n = 6$).

During both initial and the first period of acute elevation of perfusion pressure, venous effluent was collected for thin-layer-chromatography. In normal perfusion pressure, venous effluent contains a spot having an Rf value of 0.859 which is similar to that of standard PGA_2. However, when the perfusion pressure was acutely increased by 30-40 mmHg, another spot was obtained having an Rf value of 0.485 which was near the standard PGE_2 value (Rf: 0.5). The chromatographic identification of the released PGE_2-like materials due to increase perfusion pressure, was illustrated in Figure 5 as a sample of one chromatographic work.

Immediately after perfusion pressure was increased, a notable contraction was observed in assay organ during at high perfusion pressure,

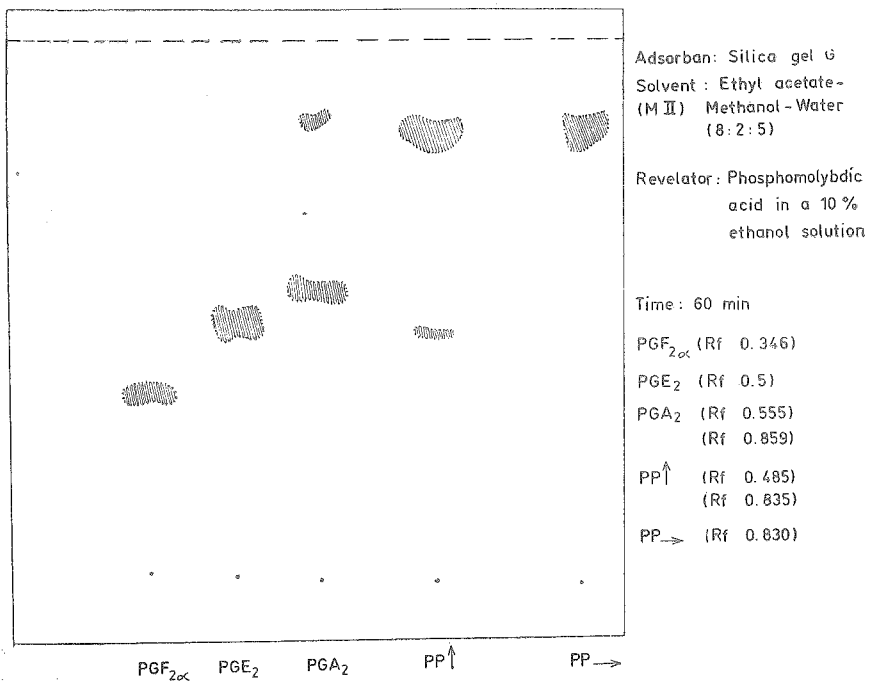

Figure 5

Illustrates some typical findings in one experiment of thin-layer-chromatography. Standard ($PGF_{2\alpha}$, PGE_2, PGA_2 spots were on the left and both in normal (PP→) and at high perfusion (PP↑), venous effluent findings were on the right. A spot was obtained when per usion pressure at the initial level (PP→), the Rf value of this spot was 0.830, which is near to the second spot of standard PGA_2 (Rf 0.859). In high perfusion pressure, venous effluent gave two spots. Their Rf values were 0.485 and 0.835 The first value was near the standard PGE_2 (Rf 0.5) and the second was near the second spot of standard PGA_2 (Rf 0.859) value.

assay organ contraction increased progressively (Figure 6). In spite of keeping renal perfusion pressure constant at high perfusion level, renal perfusion pressure decreased spontaneously and progressively as the assay organ contracted. It is seen in Figure 6. Number of urine drops per unit time increased as the perfusion pressure was increased (up to 120 mmHg), However successive increases in perfusion pressure did not cause a corresponding rise in the number of urine drops, (Figure 6). When perfusion pressure decreased to the normal control value, assay organ contractions returned to the initial level as renal venous effluent superfused it (Figure 7).

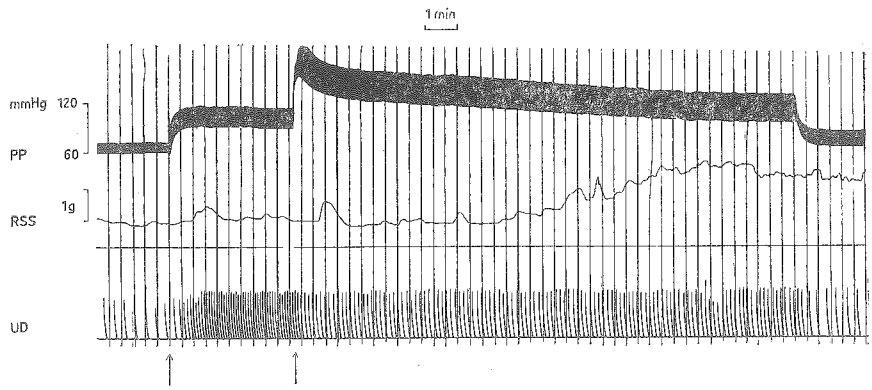

Figure 6

Immediately after PP was increased, a notable contraction was observed in RSS, and during this high PP period assay tissue contraction increased progressively and the number of urine drops per unit time increased also, but successive increases in PP were not followed by a corresponding rise in the number of urine drops. Arrows (↑–↑) indicate two successive increases in PP.

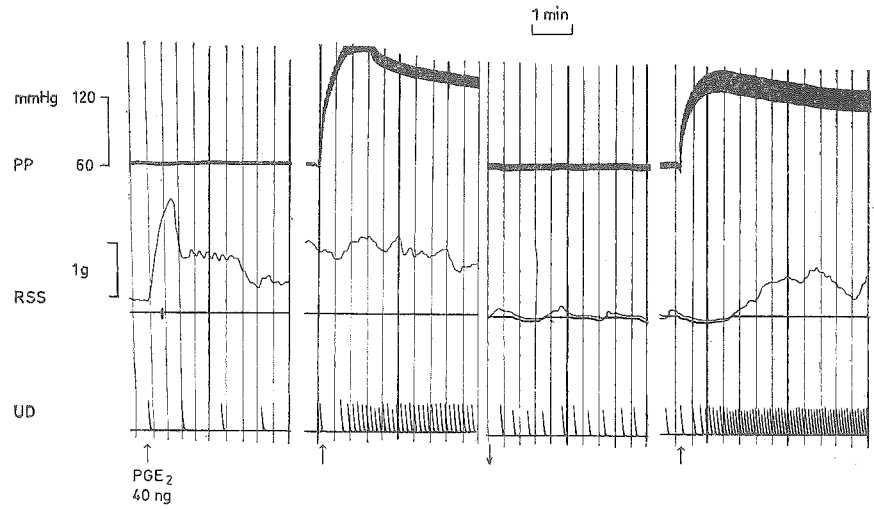

Figure 7

Isolated rabbit kidney perfused with Krebs' solution containing atropine 0.5 μg/ml. The arrow (↑) indicates the rise in PP due to the increase in the volume of Krebs' solution passed through the kidney and the arrow (→) indicates the control level of PP, but the number of urine drops were still more than at starting level as RSS relaxed. perfusion pressure (PP), rat stomach strip (RSS), urine drops (UD)

In each of the eleven experiments the perfusion pressure was increased three times. Control samples were obtained after a ten minute resting period (between two high perfusion pressure levels). Urine drops were collected for ten minute intervals during normal and high perfusion pressure. Urine flow values in both conditions are seen in Table III.

TABLE III

THE EFFECT OF ACUTELY INCREASED PERFUSION PRESSURE ON URINE VOLUME IN ISOLATED PERFUSED RABBIT KIDNEY

Perfusion Pressure 60-80 mmHg (PPX) 90-120 mmHg (PP?)	PP→	PP↑	PP→	PP↑	PP→	PP↑
Time (minute)	1-10	10-20	30-40	40-50	60-70	70-80
Urine volume mean values (ml/min)	0.92	7.30	1.58	7.77	0.96	6.35
SEM	±0.16	± 0.72	± 0.56	± 1.20	± 0.15	± 1.00
Mean difference between the members of each pair of experiments	6.31 ± 0.71 (P < 0.001)		6.19 ± 0.91 (P < 0.001)		5.38 ± 1.00 (P < 0.001)	
Number of experiments	(11)	(11)	(11)	(11)	(11)	(11)

Urinary sodium excretion (U_{Na}. VμEq/min) values are shown in Table IV.

Urinary potassium excretion (U_K. VμEq/min) values are indicated in Table V.

As seen in Tables III, IV and V, PP was acutely elevated on three occasions. Each of these three acute elevation periods caused significant rises in urine volume ml/min ($P < 0.001$, $P < 0.001$, $P < 0.001$); sodium excretion μEq/min ($P < 0.001$, $P < 0.001$, $P < 0.001$); and potassium excretion μEq/min ($P < 0.05$, $P < 0.01$, $P < 0.01$). These findings are illustrated in Figure 8.

TABLE IV

THE EFFECTS OF ACUTELY INCREASED PERFUSION PRESSURE ON URINARY SODIUM EXCRETION IN ISOLATED PERFUSED RABBIT KIDNEY

Perfusion Pressure 60-80 mmHg (PP→) 90-120 mmHg (PP↑)	PP→	PP↑	PP→	PP↑	PP→	PP↑
Time (minute)	1-10	10-20	30-40	40-50	60-70	70-80
Urinary sodium excretion mean values (μEq/min)	14.05	86.89	15.99	97.27	16.55	69.76
SEM	± 2.55	±11.53	± 2.78	±15.62	± 2.44	±10.01
Mean difference between the members of each pair of experiments	72.83 ± 11.74		83.72 ± 15.56		73.20 ± 15.62	
	(P < 0.001)		(P < 0.001)		(P < 0.001)	
Number of experiments	(11)	(11)	(11)	(11)	(11)	(11)

TABLE V

THE EFFECTS OF ACUTELY INCREASED PERFUSION PRESSURE ON URINARY POTASSIUM EXCRETION IN ISOLATED PERFUSED RABBIT KIDNEY

Perfusion Pressure 60-80 mmHg (PP→) 90-120 mmHg (PP↑)	PP→	PP↑	PP→	PP↑	PP→	PP↑
Time (minute)	1-10	10-20	30-40	40-50	60-70	70-80
Urinary potassium excretion mean values (μEq/min)	0.56	3.30	1.50	3.99	0.64	2.66
SEM	±0.16	±1.02	±0.60	±1.27	±0.19	±0.78
Mean difference between the members of each pair of experiments	2.73 ± 0.91		2.84 ± 0.88		2.02 ± 0.63	
	(P < 0.001)		(P < 0.001)		(P < 0.001)	
Number of experminets	(11)	(11)	(11)	(11)	(11)	(11)

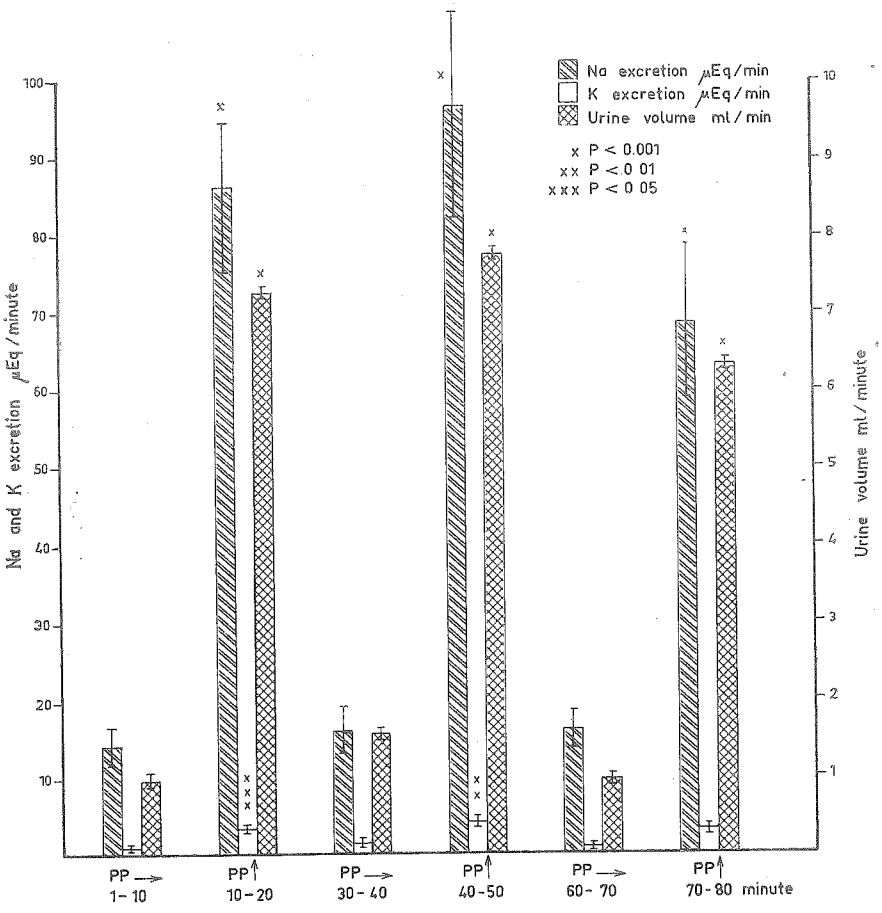

Figure 8

Urine volume, sodium and potassium excretion per minute are seen, both during normal and high perfusion pressures. 60-80 mmHg perfusion pressure (PP→); 90-120 mmHg perfusion pressure (PP↑)

Discussion

Along with other intrarenal physical factors, it has been postulated that renal prostaglandins have a function as "natriuretic hormone" in regulating sodium excretion.[6, 7, 9] It has been demonstrated that PGE_2, PGA_2, $PGE_{2\alpha}$ and PGD_2 are synthetized in the renal medulla.[16, 17] It has also been reported that when some of these group E, A, D prostaglandins were infused into the renal artery, marked diuresis as well as an increase in sodium excretion occured as a result of their effect on intrarenal blood flow.[5, 17, 18, 19]

During the first acute elevation of PP by 30-40 mmHg, a notable contraction were observed in the assay tissue as urine volume, sodium and potassium excretions were significantly and simultaneously increased ($P < 0.001$, $P < 0.001$, $P < 0.05$). Contraction of the assay organ was the evidence for the release of an active substance from the rabbit kidney. This was neither acethylcholine, 5-hydroxytryptamine, catecholamine nor histamine because addition of antagonists to these substances failed to prevent RSS contractions. So the active substance which caused isometric contractions of assay organ was most probably PGE_2 like material because it was particularly sensitive to it. On the other hand, in thin-layer chromatographic studies at high PP, a spot was obtained which had a Rf value very close to the Rf value of standard PGE_2. So we can assume that the contraction of assay organ at high PP was due to increase of PGE_2-like material in venous effluent. It is known that the principal PG of the renal medulla is PGE_2,[20]; therefore it seems interesting that the venous effluent from the kidney contains increased amounts of PGE_2-like material whenever the PP is increased (Figure 6, 7). When aspirin was added into the perfusion fluid, a decrease in the amount of PGE_2-like material was observed. It has been concluded that elevation of the PP acted as a stimulus in activating synthesis of PGE_2 (Figure 6).

Here, two questions come to mind (a) why should an elevation in the renal PP result in an increase in PGE_2-like material release and b) are PG's responsible for the increase in sodium excretion observed during renal PP elevation? To answer the first question, it is necessary to study the structural properties of the kidney. In the inner medulla the blood flow changes markedly with variations in renal arterial pressure, whereas renal cortical flow does not.[21] There are interstitial cells lying in the inner medulla of the kidney in man and rodents which link some of the foregoing facts together. The cells have extensive cytoplasmic processes which terminate in close proximity to the loops of Henle and the blood capillaries. These cells also have abundant osmophilic granules in their cytoplasms. When hypertension is present, these papillary granules become sparse.[22] The hypertensive rats not only and significantly fewer papillary granules but also had significantly lower concentration gradients for sodium and urea when PP was raised. It has been established that the material contained in osmophilic granules of the innermedulla are PG's[22, 23] The blood flow of the renal papilla is not autoregulated with changing in blood pressures. It may be assumed that the elevated PP is the stimulus causing release of these PG's from the granules of the inner medullary cells like the other stimuli causing intrarenal vascular stretch.[2, 3, 4]

Furthermore, the inner medulla is usually exposed to low oxygen and is dependent upon glycolysis for the production of ATP. Therefore, the inner medulla is well adapted to a low oxygen medium metabolically. The finding that increasing oxygen from 5 percent to 95 percent had the capacity to increase the synthesis of PG's in inner medullary tissue slice is considered to be of particular interest because the PO_2 in the inner medulla is low, and is potentially dependent upon the rate of inner medullary blood flow.[24] Therefore, in the inner medulla of the kidney a potential mechanism exists by which the rate of blood flow could modulate PG production by measuring the amount of oxygen delivered to this area of synthesis. In our study, the increase in PG release observed upon raising PP may be due to these renal properties.

To answer the second question, we should remind ourselves the results of some of the investigations previously done. Infusion of PGE_2 and PGA into renal artery resulted in marked diuresis and natriuresis accompanied by an increase in renal blood flow with intra renal redistribution from medulla to cortex.[5, 18, 19] These hemodynamic changes are similar to those accompanying the natriuresis after saline loading which results in an increase in urinary excretion of PGE and $PGF_{2\alpha}$ in rats,[25] as well as insignificant changes in intrarenal concentration of PGA_2 in rabbits.[26] Following the inhibition of PG synthesis by indomethacine (INDO), sodium excretion in chronically saline loaded rabbits markedly decreased with a concomitant fall of PGA_2 concentration in papillae and outer medulla.[26] In dogs, administration of INDO is followed by a reduced renal cortical blood flow.[27] Furthermore, removal of PG precursors by a diet deficient in essential fatty acids, is accompanied by reduced sodium excretion.[28] In contrast, precursor substances like arachidonic acid when given to dog, caused a significant increase in PG synthesis while diuresis and natriuresis were observed. But when a nonsteroidal anti-inflammatory drug (NSAID) was administered to the same dogs, diuresis and natriuresis were abolished.[29] These results thus clearly demonstrated the involvement of PG in renal handling of sodium. Chronic studies on-rabbits however showed that the large changes in sodium excretion do not necessarily lead to changes in renal PGE excretion and that chronic inhibition of PGE_2 synthesis does not by itself, lead to large alterations in sodium or potassium excretion.[30] This also pointed to the importance of acute stimulus.

On the other hand, Dusing and his co-workers demonstrated that the renal sodium excretion in rats (the extracellular volume (ECV) of which was nonexpanded) is unaffected by inhibition of PG synthetase.

However, in acutely salt-loaded animals PG appears to play an important role in the regulation of renal sodium excretion, and therefore, must be considered as an additional mediator of the natriuresis following acute expansion of the extracellular fluid volume.[31] According to these findings PG deficiency signs appeared among acutely ECV expanded salt-loaded animals which were pretreated with INDO. Mechanisms determining the natriuresis in ECV expansion are not yet completely understood. But the findings suggest that PG may inhibit the intrinsic capacity for Na-absorption in more proximal parts of the nephron, possibly via intra renal physical factors.[31] Accordingly, the most effective medium for PG's in regulating renal functions is an increased blood volume which is coming to the kidney. Studies of Tobian[32] and Papanicalou[33] also point out to the importance of increasing the renal blood flow and volume expansion in releasing renal prostaglandins.

A third question may arise regarding reliability of the responses of isolated perfused rabbit kidney. Throughout the experiment the isolated Krebs' perfused rabbit kidney had a physiological response to normal and elevated perfusion pressure as shown by the constancy of the values in the control and experimental periods respectively. It is evident in Tables I, II and III, that urine volume (ml/min), sodium and potassium excretion (μEq/min) values in three control periods were very close and no significant differences existed between first and last mean values in controls. It was the same for urine samples which were taken in elevated PP. This means that the functions of the isolated perfused kidney did not change during the course of experiments.

In summary the present experiments demonstrated that an acute elevation in the PP causes increases in the volume of the urine; and sodium, potassium excretions as PGE_2-like substance activities are also simultaneously increased in the renal venous effluent. Thus PGE_2-like substance gains importance in answering the main question; whether or not the PG's are responsible for the pressure natriuresis.

Summary

The effect of elevating renal arterial pressure on urine volume, sodium and potassium excretions were studied using an isolated Krebs' perfused rabbit kidney. Increasing perfusion pressure (PP) by 30-40 mmHg caused significant increases in urine volume, sodium and potassium excretions respectively ($P < 0.001$, $P < 0.001$, $P < 0.05$) while prostaglandin E_2 (PGE_2)-like activity was followed by a corresponding rise ($P < 0.001$) in renal venous effluent. The difference in the percent of maximum contraction

of test organ was 30.10 ± 3.20 ($P < 0.001$) and the difference in PGE_2-like activities 13.21 ± 1.36 ng ($P < 0.001$). There was a positive correlation between high PP and increasing PGE_2-like activity. The degree of this correlation was ($r = 0.96$; $P < 0.001$) significant.

The data are compatible with the concept that pressure natriuresis is mediated by increase in peritubular capillary pressure which decreases proximal tubular sodium reabsorption. However there is speculation on the possibility of a hormonal mechanism accounting for the natriuresis which is activated by increased PP. In our investigation, an acute increase in PP caused diuresis, natriuresis and kaliuresis while PGE_2-like activity showed an increase in renal venous effluent. Therefore, our findings suggest that PGE_2, may modulate pressure natriuresis.

Acknowledgements

The author gratefully acknowledges the invaluable help of Dr. R. K. Türker, Professor of Pharmacology, Medical Faculty, University of Ankara who criticized and supplied lysine-aspirin (Bayer, Leverkusen, Germany), and that of Dr. J. E. Pike Upjohn, Kalamazoo, Michigan U.S.A. who generously provided of PGE_2, PGA_2, $PGF_{2\alpha}$ for us, and thanks to M.D.'s Ü. Bayazıt, Mr. R. Coşkun, and Mr. I. Köseoğlu for their excellent technical assistance.

REFERENCES

1. Edward, W. G., Strang, G. G. and Hunt, J. C.: A vasodepressor lipid resembling Prostaglandin E_2 (PGE_2) in the renal venous blood of hypertensive patients. J. Lab. Clin. Med. **74**: 383, 1969.
2. Mc Giff, J. C., Crowshaw, k., Terragus, N. A. and Loigro, A. J.: Release of a prostaplandin like substance into renal venous blood in the response to anpiotensin II. Cir. Res. **26**: 121, 1970.
3. Fujimato, S. and Lockett, M. F.: Intrarenal of prostaglandin E by noradrenalin, Int. Congr. Pharmacol. **4th Basel**, 122, 1969.
4. Dunham, W. E. and Zimmerman, G. B.: Release of prostaglandin like material from the dog kidney during renal nerve stimulation. Am. J. Physiol. **219**: 1279 1970.
5. Johnston, H. H., Herzog, J. P., Lauler, D. P.: Effect of Prostaglandin E_1 on renal hemodynamics sodium and water excretion. Amer. J. Physiol. **213**: 939, 1967.
6. Selkurt, E. E., Womark, I., and Dailey, W. N.: Mechanism of natriuresis during elevated renal arteriol pressure. Amer. J. Physiol. **209**: 95, 1965.
7. Kaloyanides, G. J.: Effect of perfusion pressure on sodium and calcium excretion in the isolated dog kidney, Amer. J. Physiol. **138**: 462, 1971.

8. Kaloyanides, G. J., Di Bone G. F., and Baskın, P.: Pressure natriuresis in the isolated kidney. Amer. J. Physiol. 220: 1663, 1973.
9. Lee, J. B.: Natriuretic "hormone" and renal prostaglandins. Prostaglandins. 1: 1, 1976.
10. Gündoğan, Ü. N., and Türker, R. K.: Influence of pulmonary circulation on diuretic action of Prostaglandin E_2 Pharmacology, II: 273, 1974.
11. Vane, J. R.: A sensitive method for the assay of 5-hydroxytriptamine. Br. J. Pharmac. 12: 344, 1957.
12. Gilmore, N., Vane, J. R. and Wyllie, S. H.: Prostaglandin release by the Spleen, Nature (London). 218: 1135, 1968.
13. Green, K. and Samuelson, B.: Prostaglandins and related factors: XIX thin-layer chromatography of prostaglandins. J. Lipid. Res. 5: 117, 1964.
14. Meites, S. and Faulkner, W. R. In: Manual of practical micro and general procedures in clinical chemistry. (Thomas C. C.) Springfield, Illinois, U.S.A, 1962, p. 30.
15. Vane, J. R.: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. Nature New. Biol. 231: 232, 1971.
16. Lee, J. B., Crowshow, K., Takman, B. H.: The identification of Prostaglandin E_2, $F_{2\alpha}$ and A_2 from rabbit kidney medulla. Biochem. J. 105: 1251, 1967.
17. Friesinger, G. C., Olez, O., Sweetman, B. J., Nies, A. S., Data, J. L.: Prostaglandin D_2 another renal prostaglandin. Prostaglandins. 15: 969, 1978.
18. Fulgraff, G., and Meinforth, A.: Effects of prostaglandin E_2 on excretion and reabsorption of sodium and fluid in rat kidney (micro-puncture studies). Pflugers Arch. 330: 243, 1971.
19. Gross, J. B., and Barter, F. J.: Effects of prostaglandins E, A and $F_{2\alpha}$ on renal handling of salt and water. Amer. J. Physiol. 225: 218, 1973.
20. Whorton, A. R., Smigel, M., Oxates, J. A., and Frolich, J. C.: Regional differences in prostaglandin formation by the kidney. Prostacyelin is a major Prostaglandin of renal cortex. Biochemia et Biophysica Acta. 529: 176, 1978.
21. Thuran, K.: Renal hemodynamics. Am. J. Med. 36: 698, 1964.
22. Tobian, L., Ishii, M., and Duke, M.: Relationship of cytoplasmic granules in renal papillary interstitial cells to "post salt" hypertension, J. Lab. Clin. Med. 73: 309, 1969.
23. Dunn, M. J., Staley, R. S., and Harrison, M.: Characterization of prostaglandin production in tissue culture of rat medullary cell. Prostaglandins. 12: 37, 1976.
24. Zenger, T. V., Levitt, M. J., and Davis, B. B.: Effect oxygen and Solute on PGE and PGF production by rat kidney slices, Prostaglandins, 13: 143, 1977.
25. Dusing, R., Opitz, W. D., and Kramer, H. J.: The role of prostaglandins in the natriuresis of acutely salt loaded rats. Nephron. 18: 212, 1977.
26. Lee, J. B., and Attallah, A. A.: Response of intra-renal PGA_2 to salt loading in rabbit. Circ. Res. Suppl. I. 24: 75, 1974.
27. Kirschenbaum, M. A., While, N., Stein, J. N., and Ferris, T.: Redistribution of renal cortical blood flow during inhibition of Prostaglandin Synthesis. Am. J. Physiol. 227: 801, 1974.
28. Rosenthal, J., Simone, P. G., and Silbertgleit, A.: Effects of a prostaglandin deficiency on natriuresis and blood pressure. Prostaglandins. 5: 435, 1974.

29. Tannenbaum, J., Splawinski, T. A., Oates, J. A., and Neis, A. S.: Enhance renal prostaglandin production in the dog. I. Effects on renal function. Circulation Res. 36: 197, 1975.

30. Lifschitz, M. D., Patak, R. V., Fadem S. Z., and Stein, J. H.: Urinary Prostaglandin E excretion: Effect of chronic alterations in sodium intake and inhibition of Prostaglandin Synthesis in rabbit. Prostaglandins, 16: 607, 1978.

31. Dusing, R., Melder, B., and Kramer, H. J.: Prostaglandins and renal function in acute extracellular volume expansion. Prostaglandins, 12: 3, 1976.

32. Tobian, L., Schaning, S., Seefeldt, C.: The influence of arterial pressure on the antihypertensive action of a normal kidney, a biological servomechanism. Ann. Int. Med. 60: 378, 1964.

33. Papanicolaou, N.: Investigation of mechanism of prostaglandin release. Experientia, 28: 275, 1972.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 1-2 / January-April 1979 / pp. 19 – 24

Heparin Secreted from Marrow Mast Cells and Osteoporosis*

O. Şahap Atik, M.D. / Naci M. Bor, M.D.*** / Turan Kutkam, M.D.**** / M. Talat Göğüş, M.D.*****

The number of the mast cells in the bone marrow of the persons with senile osteoporosis is more than in normal individuals.[2] The mast cells synthesize, reserve and release heparin.[7] On the other hand, it has been observed that long term heparin therapy causes osteoporosis and multiple fractures in many patients.[4, 5, 6, 8]

We carried out this research project to find out whether heparin secreted from the marrow mast cells can lead to osteoporosis and to clarify its role in the pathogenesis of senile osteoporosis.

Material and Methods

Twenty adult female Swiss Albino rats, each weighing approximately 140 grams were utilized in this series of experiments. A dosage of 1.15 IU per g of body weight of sodium heparin per day was subcutaneously given to ten rats. Then animals in the control group received an equivalent volume of steril saline.

At the end of the eight week, the radiograms of the right knees of all rats were taken and developed at the same time. Step wedge was used to minimize the standart error.[12] Diffuse transmission density of all radiograms were then determined by using Macbeth Quanto Log^R Densitometer (Model No. TD-102).

* From the Medical and Surgical Research Center, Hacettepe University, Faculty of Medicine, Ankara.
** Specialist in the Department of Orthopaedics and Traumatology.
*** Chief and Professor in the Medical and Surgical Research Center.
**** Chief and Professor in the Department of Pathology.
***** Chief and Professor in the Department of Orthopaedics and Traumatology.

After the animals were killed, each right femur were cleaned of soft tissue, decalcified, sectioned, and stained with hematoxylineosin and toluidine blue. Serum alkaline phosphatase level was determined in the blood of the animals.

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

Results

Serum Alkaline Phosphatase Level: Mean value of the serum alkaline phosphatase was 26.13 $\pm$ 4.02 King-Armstrong Unite in the control group, and was 24.81 $\pm$ 3.82 King-Armstrong Unite in the heparin-treated group (Figure 1). There was no significant difference between the groups ($P > 0.200$).

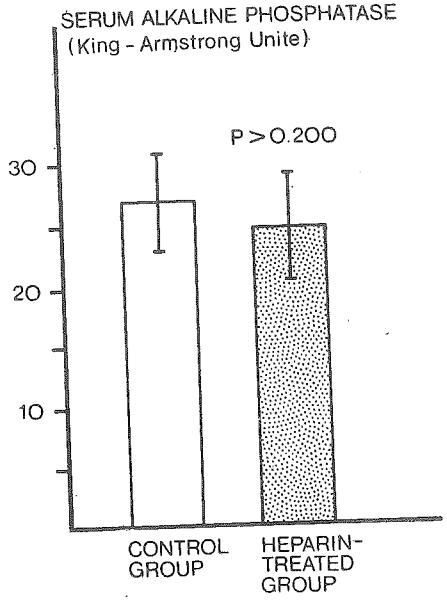

Figure 1

Serum Alkaline Phosphatase levels in the control and heparin-treated groups.

Bone Density: Bone density of the heparin-treated group was lower than that of control group. Mean value of the density (Quanto Log) was 1.24 $\pm$ 0.14 in the heparin-treated group, and was 0.96 $\pm$ 0.05 in the control group (Figure 2). The difference between the groups were highly significant ($P < 0.001$).

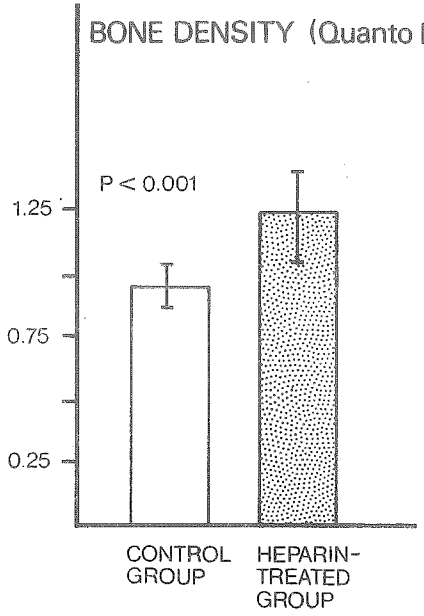

Figure 2
Bone Density in the control and heparin-treated groups.

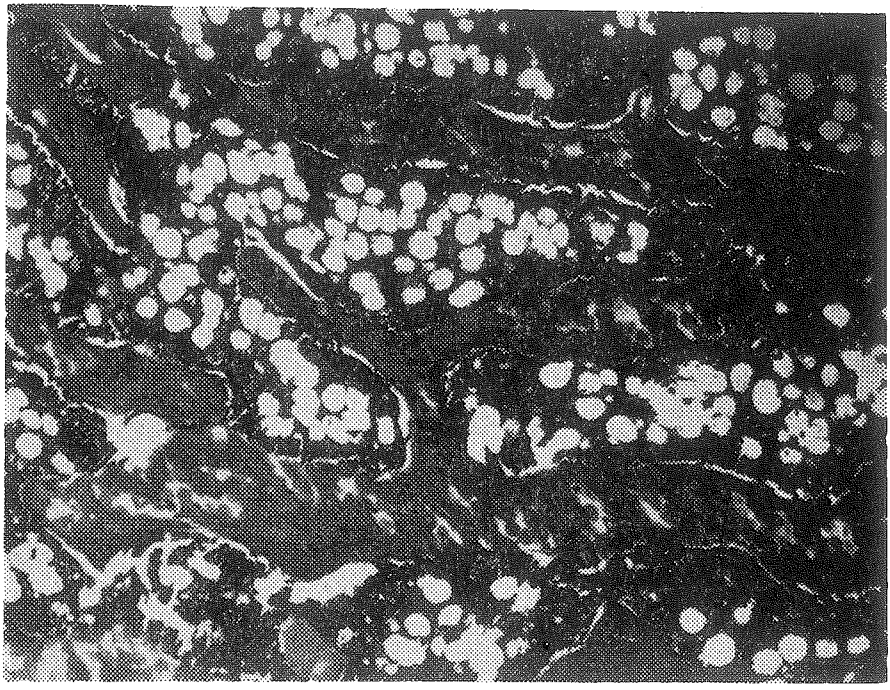

Figure 3
Bony spicules and marrow in the medullary area of the control group. H and E,X 100.

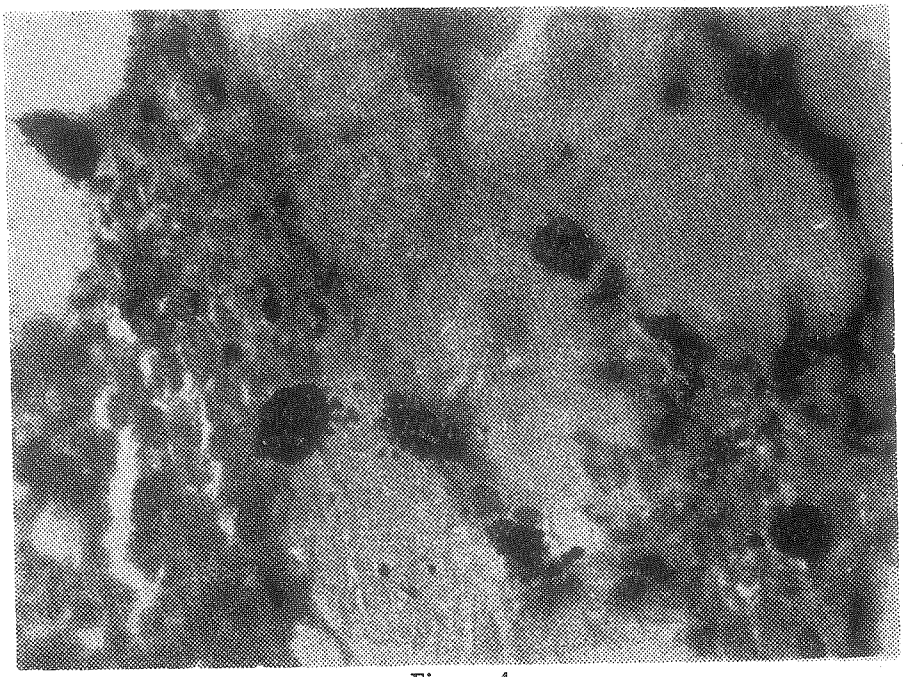

Figure 4

The significant decrease in the size and number of the bony spicules in medullary area of the heparin-treated group. H and E, X 100.

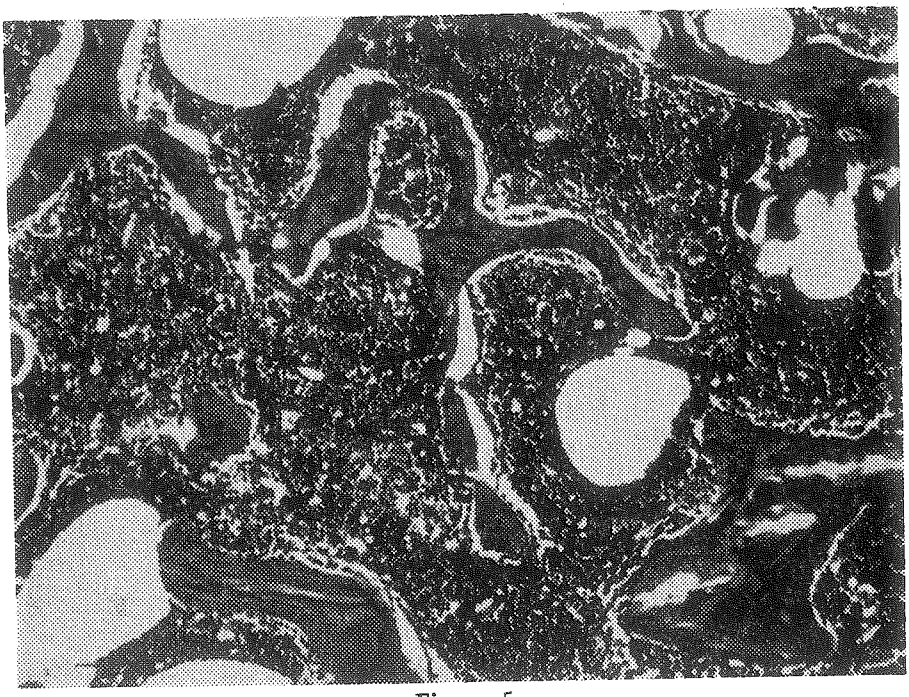

Figure 5

The mast cells in the bone marrow of the control group. Toluidin blue, X 1000.

Histological Examination: Bony spicules were less in number thin and the distance between them were decreased in the heparin-treated group compared to that of the control group (Figures 3 and 4). On the other hand, the mast cells were identified by their highly metachromatic staining with toluidine blue in the control group (Figure 5). But they were completely disappeared in the heparin-treated group.

Discussion

We have determined by using radiodensitometric and histologic examination that exogenously administered heparin produces osteo-, porosis in animals. This result supports the findings of the other clinical and experimental studies.[1, 3, 4, 5, 6, 8, 9, 10]

It is however unclear how heparin produces osteoporosis. The following theories have been proposed to explain this phenomenon:

1. Heparin is mucopolysaccharide in structure. There is probably competitive inhibition between heparin and the other mucopolysaccharides which are normal constituents of the bone tissue and take part in osteogenesis.[9]

2. The addition of small amounts of a commercial heparin solution to the medium in which bone tissue is cultured markedly enhances suboptimal concentrations of parathyroid extract which stimulate bone resorption. It is suggested that heparin be considered a cofactor.[3]

3. Heparin may decrease lysosomal stability and, asa result, stored proteolytic enzymes (probably collagenase) are released and lead to bone resorption.[2, 11]

After producing octeoporosis in the rats by exogenously administering heparin, the examination of the mast cells in the bone marrow is the original part of our research. There was no mast cell in the bone marrow of the heparin-treated group, that is, they were degranulated. Exogenous heparin probably caused the release of endogenous heparin-containing granules from the mast cells. Osteoporosis was mainly produced by exogenous heparin, but the contribution of endogenous heparin is also probable.

On the basis of the avaliable experimental and clinical evidences, there are substantial grounds for concluding that heparin secreted from marrow mast cells may have a role in the pathogenesis of senile osteoporosis.

Summary

Osteoporosis was produced in the rats with exogenous heparin and the mast cells were counted in the bone marrow. On the basis of the available clinical and experimental evidences, the effects of exogenous heparin on the bone tissue and the mast cell, and the role of heparin secreted from marrow mast cells in the pathogenesis of senile osteoporosis were discussed.

REFERENCES

1. Beljan, J. R. and Hellewell, A. B.: The effect of chronic heparin administration on the mineralization of an experimental osseous defect. Clin. Orthop., 125: 205, 1977.
2. Frame, B. and Nixon, R.: Bone marrow mast cells in osteoporosis of aging. N. Engl. J. Med., 279: 626, 1968.
3. Goldhaber, P.: Heparin enhancement of factors stimulating bone resorption in in tissue culture. Science, 147: 407, 1965.
4. Griffith, G. C., Nichols, G., Asher, J., Flanagan, B.: Heparin osteoporosis. JAMA, 193: 85, 1965.
5. Sackler, J. P. and Liu, L.: Heparin-induced osteoporosis. Br. Radiol., 46: 548, 1973.
6. Schuster, J., Meier-Ruge, W., Egli, F.: Zur pathologie der Osteopathie nach Heparin behandlung. Dtsch. med. Wschr., 45: 2334, 1969.
7. Selye, H.: The Mast Cells. Butterworths, Washington, 1965.
8. Squires, J. W. and Pinch, L. W.: Heparin-induced spinal fractures. JAMA, 241: 2417, 1979.
9. Stinchfield, F. E., Bankaran, B. and Samilson, R.: The effect of anticoagulants on bone repair. J. Bone Joint Surg.. 38-A: 270, 1956.
10. Thompson, R. J.: Heparin osteoporosis. J. Bone Joint Surg., 55-A: 606, 1973.
11. Vaughan, J. M.: The Physiology of Bone. 69, Oxford, London, 1970.
12. Wuehrmann, A. H. and Manson-Hing, L.: Dental Radiology. 3 rd Ed., Mosby Comp., Saint Louis, 1973, p. 28.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 1-2 / January-April 1979 / pp. 25 - 34

Niacin/Pantothenic Acid/ Protein Interrelationships Affecting the Nutrivite Values of Winter Wheat for Weanling Rats

Bahtiyar Ünver, Ph.D.* / Constance Kies, Ph.D.**

The quantity of protein in wheat varies from 7 to 22 percent, theaverage value is higher than for rice and corn, the other major cereals.[1] Whole wheat is a good source of isoleucine, leucine, threonine and valine but only a fair source of lysine, methionine, phenylalaline and tryptophan.[2] Lysine is generally considered to be the first limiting amino acid. Hence, a greater guantity is needed to maintain nitrogen balance equilibrium when wheat is the sole dietary food protein source.[1] Theoreti cally, the ingestion of 228 g of white flour or 150 g of whole ground wheat per day sould satisfy the human protein need according to the estimate made by Hegsted.[3]

Wheat seems to satisfy human protein requirements except for periods of stress such as fast growth, pregnancy and lactation.[1] Studies in humans showed that supplementation of wheat with lysine and nonspecific nitrogen improved significantly nitrogen retention[4] or reduced nitrogen lost from the body.[5] Another approach was the addition of other sources of protein to wheat diets to achieve a better balanced amino acid pattern. Studies showed that wheat based mixed diets provided generous amounts of essential amino acids to healthy adult human when subjects consumed 5.0 to 8.0 g nitrogen per day.[6, 7, 8]

* Asistant Professor of Department of Nutrition and Dietetics, Hacettepe University, Ankara, Turkey.

** Professor of Department of Food and Nutrition, University of Nebraska, Lincoln. 68503 U.S.A.

Much interest has been generated in improvement in protein quality/quantity of wheat by genetics. Use of new varieties through breeding processes increased significantly the protein content in the grain without reduction in the yield and not depressing balance of essential amino acids.[9, 10, 11]

The apparent ability of wheat to supply sufficient quantity and quality of protein is dependent upon intake of nutritients other than protein as well. These nutrient interactions are many and varied. Obviously, protein adequacy is well known to be dependent upon dietary energy adequacy. Early evidence indicated that vitamins are involved in protein metabolism.[12, 13, 14]

The objective of the current project was to examine some interrelationships among protein, niacin and pantothenic acid content of high a protein wheat flour produced by the Department of Agronomy, University of Nebraska in Lincoln. Since wheat is used as human food, the metabolic performance of a high protein wheat flour was studied both in adult human subjects and in growing rats. In this paper only rat study was reported.

Experimental Procedure

Experimental Design:

Male weanling rats of the Spraque-Dawley strain were used. Each treatment group consisted of 4 rats. Assignment to groups was made so that the mean weight of each group was identical. Rats were individually housed in metabolic cages arranged randomly on racks. Whole wheat flour was prepared as breads at 3 protein levels (6.6, 8.4 and 9.5 %). They were crumbled and slightly toashed in an oven at 250°F (121°C) for 10-15 minutes. Composition of the rations were given in Table I.

At each level of protein intake, whole wheat bread crumbs were fed with 2 percent full vitamin, 2 percent full vitamin minus niacin, 2 percent full vitamin minus pantothenic acid and no vitamin supplement Thus, 12 treatments were used. As a positive control, a 10 percent vitamin free case in (8.2 percent protein) diet supplemented with a full vitamin mix was also employed.

Collection of Data:

During the 28-day feeding trails, rat were allowed to consume food and water ad libitum. Rats were weighed twice a week. Amount of ration consumed by each rat was determined by weight on a weekly basis. Urine and feces were collected in 1 week period lots. Urine samples were composited for the total study period for each rat and were frozen until use (1 composite per rat per 28 days). The weekly feces collections

were also composited for the full 28-day feeding period. These were digested in concentrated hydrochloric acid and stored at room temperature until the time of analysis. Following sacrifice of rats by carbon dioxide suffocation, tails were removed from rats and carcasses were wrapped in aluminum foil and frozen for later analyses. In preparation for analysis the frozen rat carcasses were autoclaved at[15] Ib pressure for 30 minutes and were ground and blended. Nitrogen in urine, feces and carcasses was determined by Kjeldahl method.[15] Niacin, pantothenic acid in urine were measured by microbiological method.[16, 17] The data were subjected to statistical analysis, including analysis of variance and Duncan's Multiple Range tests.

TABLE I

COMPOSITION OF WHOLE WHEAT BREAD CRUMB RATIONS

Ration Code	Wheat Crums[a] %	Casein[b] %	Protein[c] %	Vitamin Supplement			
				Full %	Minus Niacin %	Minus Pantothenic acid %	Crumbs (No. supp.) %
A	98.0	0.0	6.6	2.0	—	—	—
B	98.0	0.0	6.6	—	2.0	—	—
C	98.0	0.0	6.6	—	—	—	—
D	98.0	0.0	6.6	—	—	—	2.0
E	98.0	0.0	8.4	2.0	—	—	—
F	98.0	0.0	8.4	—	2.0	—	—
G	98.0	0.0	8.4	—	—	2.0	—
H	98.0	0.0	8.4	—	—	—	2.0
I	98.0	0.0	9.5	2.0	—	—	—
J	98.0	0.0	9.5	—	2.0	—	—
K	98.0	0.0	9.5	—	—	2.0	—
L	98.0	0.0	9.4	—	—	—	2.0
M	0.0	10.0	8.2	2.0	—	—	—

a Whole wheat bread contained varried amounts of whole wheat flour, wheat starch, yeast, sugar, salt, mineral mixture, oil. glycerol monostearate, glycerine and water. Whole wheat flour and starch were varried according to the nitrogen content of the breads. Whole wheat breads which were "crumbled" for ration formulation.

b Vitamin free casein. Casein ration also contained 65 % starch, 10 % corn oil, 8 % sucrose and 5 % salt mixture.

c Protein content for wheat crumb rations calculated by Nx5.7. Protein content or casein ration calculated by Nx 6.29.

Results

Growth bar graphs of rats fed 6.6, 8.4 and 9.5 percent protein rations from whole wheat bread crumbs with the test variables of several vitamin regimes are shown in Figure 1. Mean food intake, weight gain, nitrogen balance, urinary niacin and pantothenic acid excretions, protein efficiency ratio (PER) and carcass protein are given in Table II.

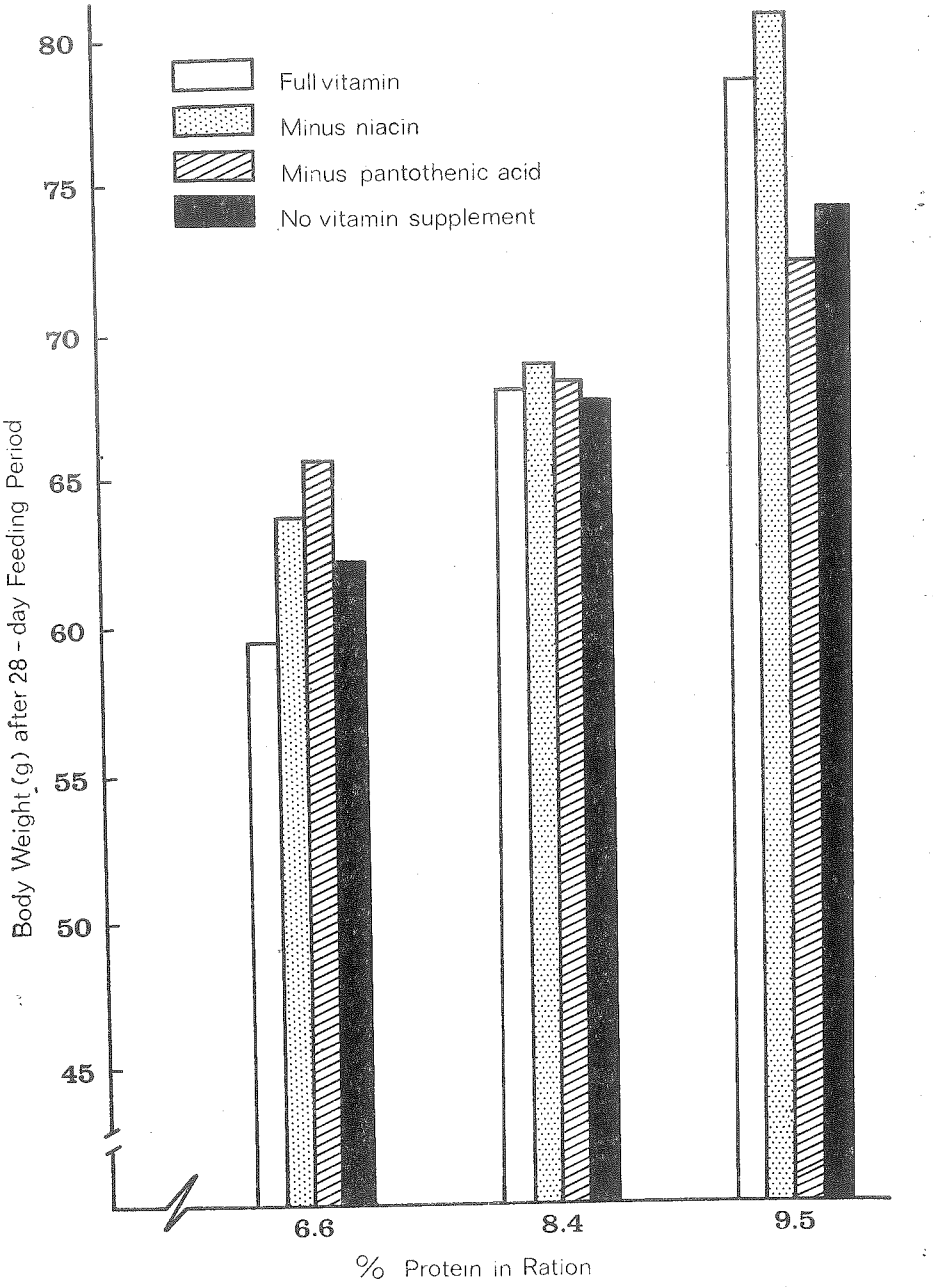

Figure 1

Ration protein level versus mean body weights of weanling rats as affected by niacin and pantothenic acid exclusion in ration.

TABLE II

MEAN FOOD INTAKES, WEIGHT GAIN, NITROGEN BALANCE, URINARY NIACIN AND PANTOTHENIC ACID EXCRETIONS, PROTEIN EFFICIENCY RATIO AND CARCASS PROTEIN OF RATS FED WHOLE WHEAT BREAD CRUMBS AND CASEIN RATIONS

Rations	No. of Rats	Average Food Intake (g/week)	Average Gain (g/week)	Nitrogen Balance (mg/N/24 hr)	Protein Efficiency Ratio	Carcass Protein (%)	Urinary Niacin[b] (μg/24 hr)	Urinary Pantothenic[b] Acid (μg/24 hr)
			6.6 % Protein from Whole Wheat Bread Crumbs					
Full vitamin	4	35.00	1.88	+ 22.76	0.82	15.99	13.13[1]	45.32[1]
Full vitamin minus niacin	4	36.25	2.88	+ 20.62	1.22	15.89	9.91[1]	65.96[1]
Full vitamin minus pantothenic acid minus pantothenic acid	4	38.50	3.13	+ 25.51	1.28	16.18	19.65[1]	50.78[1]
No vitamin	4	34.38	2.19	+ 17.86	1.05	17.13	9.69[1]	36.39[1]
Mean		36.03	2.52	+ 21.69	1.09	16.40	13.10	49.64
			84 % Protein from Whole Wheat Bread Crumbs					
Full vitamin	4	37.31	3.88	+ 35.66	1.25	16.04	17.59[2]	45.76[3]
Full vitamin minus niacin	4	41.44	3.94	+ 33.11	1.16	16.00	12.36[2]	90.52[2]
Full vitamin minus pantothenic acid	4	40.13	3.75	+ 33.88	1.11	15.72	23.75[2]	46.87[3]
No vitamin	4	40.75	3.63	+ 37.11	1.08	16.12	14.30[2]	46.88[3]
Mean		39.91	3.80	+ 34.94	1.15	15.97	17.00	57.51

TABLE II (Cont.)

Rations	No. of Rats	Average Food Intake (g/week)	Average Gain (g/week)	Nitrogen Balance (mg/N/24 hr)	Protein Efficiency Ratio	Carcass Protein (%)	Urinary Niacin[b] (μg/24 hr)	Urinary Pantothenic[b] Acid (μ2/24 hr)
			9.5 % Protein from Whole Wheat Bread Crumbs					
Full vitamin	4	46.13	6.56	+ 43.88	1.49	17.52	30.55[3]	70.98[4]
Full vitamin minus nicacin	4	48.38	7.13	+ 46.61	1.50	15.56	15.29[4]	90.96[4]
Full vitamin minus pantothenic acid	4	41.44	4.81	+ 33.10	1.25	15.30	30.71[3]	56.94[4]
No vitamin	4	43.75	5.56	+ 39.41	1.35	16.10	15.18[4]	45.86[4]
Mean		44.93	6.02	+ 40.75	1.40	16.12	22.93	66.19
			Mean of Three Protein Intakes (8.2 %)					
Full vitamin	4	39.48	4.11	+ 34.10	1.19	16.52	20.42[5]	54.02[6]
Full vitamin minus niacin	4	42.02	4.65	+ 33.45	1.29	15.82	12.52[5]	82.48[5]
Full vitamin minus pantothenic acid	4	40.02	3.90	+ 30.83	1.21	15.73	24.70[5]	51.56[6]
No vitamin	4	39.62	3.79	+ 31.46	1.16	16.45	13.06[5]	43.04[6]
Mean		40.29	4.11	+ 32.46	1.21	16.13	17.68-	57.87
			8.2 % Protein from Casein					
Full vitamin	4	66.81	18.94	—	3.37	17.04	—	—

a Growth cages were used, urine cannot be collected.

b Different number superscripts dentote values significantly different from one another at the 5 percent level of significance with each protein intake group.

As shown in Figure I, growth of rats was more influenced by level of wheat protein in the rations than by exclusion of niacin, pantothenic acid or the total vitamin supplement. Average weekly weight gains of rats while receiving 6.6, 8.4, and 9.5 percent protein rations were 2.52, 3.80 and 6.02 g, respectively. Variation in vitamin supplementation for groups did not result in marked changes in growth (Figure 1). Rats fed the casein control ration gained 18.95 g weekly.

Mean feed intake data shown in Table II did not vary markedly among groups of rats maintained on various wheat rations. However, rats receiving the casein control ration consumed nearly 70 percent more ration than did rats receiving wheat crumb rations. Nitrogen balances of rats indicated also that protein level in the ration was more important than vitamin treatments. Nitrogen balances of rats were increased from 21.69 to 34.94 to 40.75 mg nitrogen per 24 hours as the protein level increased from 6.6 to 8.4 to 9.5 percent in whole wheat bread crumb rations. Analysis of variance indicated a highly significant effect of protein level on nitrogen balances of the animals.

No statistically significant differences in nitrogen balances were found related to exclusion of niacin, pantothenic acid or total vitamin mix from the test rations. Also, PER and carcass protein percentages for rats fed the various test regimes indicated no significant changes due to level of ration protein or to vitamin treatment. However, the casein control ration gave a much higher PER value than did the wheat rations.

Statistically significant differences were found in urinary pantothenic acid excretion as a result of omission of niacin from the test ration. As shown in Table II, group mean urinary pantothenic acid excretion (all 3 protein intake levels) was 82.48 mg per 24 hours with niacin omitted while 54.02, 51.56 and 43.04 mg per 24 hours, respectively, when the ration contained full vitamin supplementation, vitamin supplementation minus pantothenic acid or no vitamin mix.

Urinary niacin excretion was higher when either the rations were fully supplemented with vitamins or the vitamin mix minus pantothenic acid was employed than when no vitamin supplement or when the vitamin supplement minus niacin was used. Niacin excretion at the 9.5 percent protein intake level was significantly higher when the rations were supplemented either with full vitamin or full vitamin minus pantothenic acid than with other diet treatments. Group mean urinary excretion of niacin (all 3 protein intake levels) with the full vitamin ration, vitamin mix minus pantothenic acid ration or unsupplemented ration were 20.42, 12.52, 24.70 and 13.06 mg per 24 hours, respectively.

Discussion

In general, results obtained from this study were similar to those of the human study.[18] Protein quantity and quality in the rations were important for growth. Increasing protein level from 6.6 to 9.5 percent in the ration resulted in increased total weight gain of about 2½ times. As expected, food intake was the main factor responsible for the differences. As food intake increased, growth was increased. Food intakes of rats were also increased with protein level of the rations.

The PER was unaffected by level of dietary protein in the ration. In theory, PER values do eliminate variables caused by differences in feed in take butonly at similar levels of protein intake. No difference in percent carcass protein was found as a result of increase in protein in the rations. At first glance, this seems to be controdictory, to the progressively more positive since rats receiving the higher protein rations were also heavier. These results could be due to accumulation of fat in the body.

Excluding either niacin or pantothenic acid from the rations did not cause any significant effect upon growth, nitrogen balance, PER or percentage of carcass protein of rats. These results are seemingly in disagreement with results or rat bioassy conducted in this laboratory.[19]

Urinary excretion of niacin and pantothenic acid was also increased gradually as the protein level of the the rations were increased. Similar results were found by Tao[25] and Chan[19] that higher urinary niacin and pantothenic acid excretion accompanier higher intake of protein. Since increased protein also resulted in greater growth, the need for pantothenic acid and niacin would theoretically be increased causing a lowering of excretion of these vitamins. However, feed intake also increased. Since the wheat flour itself as well as the yeast contributed niacin and pantothenic acid to the rations, increased in intake of these vitamins would occur with increased feed intake even with those rations in which niacin or pantothenic acid supplement had been excluded from the mix. Even at the highest protein level, dietary protein qualitatively and quantitatively was inadequate for maximal growth and protein synthesis. Hence, excess niacin and pantothenic acid would be excreted.

Interrelationships between niacin and pantothenic acid were also observed in this study. Pantothenic acid excretion was higher in niacin deficient rats than in other groups at all levels of protein in the ration. If niacin is limiting, then excess pantothenic acid would be excreted. A similar effect was noted on effect of exclusion of pantothenic acid on niacin excretion. Omission of niacin from the ration would make niacin a first limiting dietary factor; thus, reducing body need for pantothenic acid.

In general, more dramatic effects were observed in urinary niacin and pantothenic acid excretion under circumstances of exclusion of one vitamin from the ration than when the total vitamin supplement was omitted. This was particularly true in reference to interrelationships between excretion of these 2 vitamins. This situation varifies that there should be a balance among the vitamins in order to be utilized efficiently in the metabolism.

Summary

The effect of wheat bread rations of 3 levels of protein content to which full vitamin supplements, full vitamin supplement without niacin or full vitamin supplement without pantothenic acid on various paramete rs of growing rat metabolic performance were investigated. Growth and nitrogen balances of rats were more affected by level of protein and the quality of protein in the rations than by vitamin treatment. Nitrogen balances of rats were significantly improved with increased protein in the rations. No statisticaly significant effects on percentage carcass protein were found as a result of change in level of ration protein or vitamin treatment. Niacin excretion of rats was significantly increased when pantothenic acid was omitted from the supplementation mix and pantothenic acid excretion was increased when niacin was omited. In conclusion, wheat protein even in high levels is not adequate by itself for normal growth and development.

REFERENCES

1. Aykoyd, W. R. and Doughty, J.: Wheat in human nutrition. Food and Agriculture Organization of the United Nations, Rome, 1970.
2. Orr, M. L. and Watt, B. K.: Amino acids contents of foods. Home Economics Research No. 4, U. S. Dept of Agriculture. Washington, D. C. 1957.
3. Hegsted, D. M.: Wheat: Challenge to nutritionist. Cereal Sci. Tody **10**: 257, 1965.
4. Kies, C. and Fox, H. M.: Determination of the first-limiting amino acid of wheat and triticale grain for humans. Cereal Chem. **47**: 615, 1972.
5. Kies, C. Fox, H. M. and Chen, S. C. S.: Effect of quantitative variation in non-specific nitrogen supplementation of corn, wheat, rice and milk diets for adult men. Cereal Chem. **49**: 26, 1972.
6. Clark, H. E., Howe, J. M. and Magec, J. L., Malzer, J. L.: Nitrogen balances of adult human subjects who consumed four levels of nitrogen from a combination of rice, milk and wheat. J. Nutr. **102**: 1647, 1972.
7. Ashur, S. S., Clark, H. E., Moon, W. H. and Malzer, J. C.: Nitrogen retention of adult subjects who consumed wheat and rise supplemented with chick pea, sesame, milk or whey Am. J. Clin. Nutr. **26**: 1195, 1973.

8. Clark, H. E., Malzer, J. L., Onderka, H. M., Howe, J. M. and Moon, W. H.: Nitrogen balances of adult human subjtets fed combination of wheat, beans, corn, milk and rice. Am. J. Clin. Nutr, 26: 702, 1973.
9. Middleton, G. K., Bode, C. E. and Bayles, B. B.: A Comparision of the quantity and quality of protein in certain varieties of soft wheat. Agron. J. 46: 500, 1954.
10. Johnson, V. A., Schmidt, J. W., Mattern, P. J. and Haunold, A.: Agromic and quality cheracteristics of high protein F2-derived families from a soft red winter hard red winter wheat cross. Crop Sci. 3: 7, 1963.
11. Mattern, P. J., Salem, A., Johnson, V. A. and Schmidt, J. W.: Amino acid composition of gelected high-proten wheat. Cereal Chem. 45: 437, 1968.
12. Reader, V. and Drummond, J. W.: CLVII. Relation between vitamin B and protein in in the diet of grawing rats. Physiological role of vitamin B. Part II. Biochem. J. 20: 1256, 1926.
13. Sarett, H. P. and Perlzweing, W. A.: The effect of protein and B vitamin levels of the diet upon tissue content and balance of riboflavin and nicotinic acid in rats. J. Untr. 25: 173, 1943.
14. Wintrobe, M.H., Stein, H. J. and Foolis, R. H. Jr.: Nicotinic acid and the level of protein intake in the nutrition of the pig. J. Nutr. 30: 395, 1945.
15. Scales, F. M. and Herrison, A. P.: Boric acid modification of the Kjeldahl method for crop and soil analysis. J. Ind. Eng. Chem. 12: 350, 1920.
16. Freed, M.: Methods of Vitamin Assay. The Associacition of Vitamin Chemists, Inc. Interscience Publishers. 3rd ed. New York, N. Y. 1966.
17. Zook, E. G.: Pantothenic acid in foods, Agriculture Handbook No. 97. U. S. Dept. Of Agriculture, Washington, D. C., 1956.
18. Ünver, B.: Niacin/pantothenic acid/protein interrelationships effecting the nutritive values of winter wheat for humans. A dissertation. University of Nebraska. Lincoln, Neb., 1974.
19. Chan, T. H. A.: Niacin/protein andpantothenic acid/protein interrelationships influencing nutritional value of dry breakfast cereals, M. S. Thesis. University of Nebraska. Lincoln, Neb., 1972.
20. Tao, H. K.: Protein/pantothenic acid interrelationships in growing rats and pantothenic acid nutriture of alcoholic patients. A dissertation. University of Nebreska, Lincoln, Neb., 1970.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 1-2 / January-April 1979 / pp. 35 - 41

Dupuytren's Contracture in Two Families*

Metin Atasu, Ph.D. / Nasır Özdemir, M.D.*****

First, in 1834 Dupuytren reported the cases of contractures of fingers resulting from the retraction of aponeurosis palmaris among brewery workers. According to the author the formation of the condition was due to occupation and hand activity.[1]

Since then, additional cases of Dupuytren's contracture (DC) have been reported. The condition was sometimes associated with chronic alcoholism, diabetes mellitus, epilepsy and diseases in mesenchymal tissue origin or having autoimmunologic background in the majority of the cases.[2-6] Familial and hereditary conditions were also reported.[5, 7-16]

In the present report the phenotypic, genetic and dermatoglyphic findings in two families showing DC will be presented.

Case Reports

The pedigree of the family A is shown in Figure 1. I-4 and I-6 showed unsufficient evidence of the condition and the ages of death of them could not definitely described by their relatives.

II-4 has DC, but he has no affected children yet.

II-5, 67 year-old healthy female except for DC. She first noted the onset of the flexion deformities on her both hands at age 50 Since that time, the contractions have become so severe particularly of the fourth and fifth fingers. She could not shake hands and grasp objects.

III-1, 52 year-old male of DC in good health. He first noted the onset of deformity on his both hands when he was 48 years old. The contractions had become so severe at age 51 and corrected by operation. Now, he has no limitating activity and he is using his both hands.

* From Hacettepe University, Faculty of Medicine Department of Orthopaedics.
** Associate Professor of Genetics and Statistics.
*** Associate Professor of Orthopaedics.

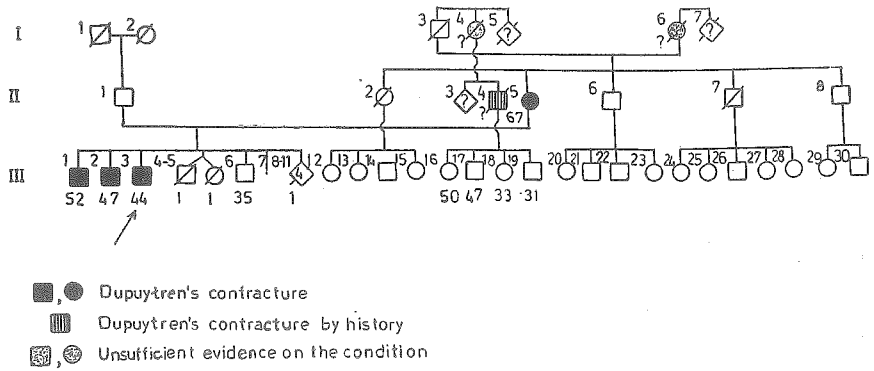

Figure 1
The pedigree of family A with DC

III-2, 47 year-old male of DC in normal health. He first noted the onset of deformity on his both hands at age 45. The contractions had become so severe when he was 46 years old.

III-3, 44 year-old healthy male of DC. He first noted the onset of deformity on his both hands at age 42. Since that time, the contractures have become severe.

The pedigree of the family B is shown in Figure 2.

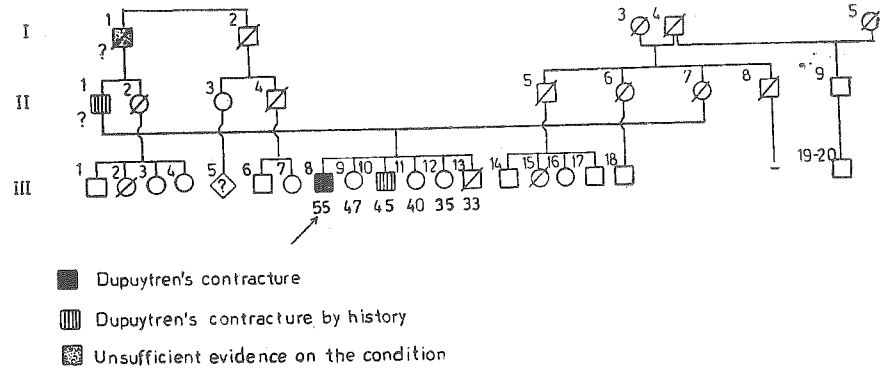

Figure 2
The pedigree of family B with DC

III-8, 55 year-old healthy male of DC. He first noted the onset of deformity on his both hands and aponeurosis plantaris on his both feet (Figures 3 and 4) at age 49. Then, the contractures had become so severe, particularly of the third and fourth fingers of the left hand and the fourth and fifth fingers on the right. The contractures were corrected by operation. He could not definitely described the age of death of II-1 showing unsufficient evidence of the malformation.

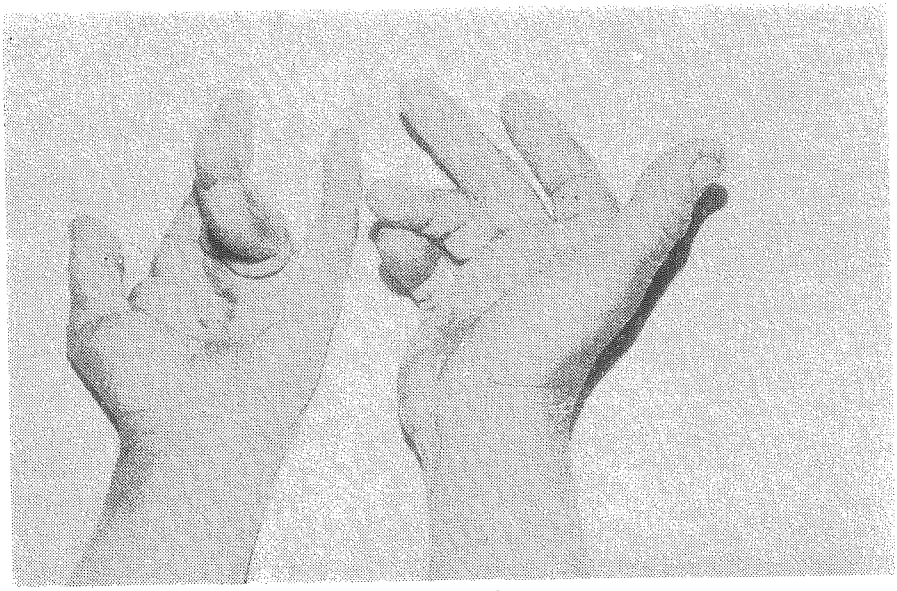

Figure 3
Photograph of the hands of III-8 from family B

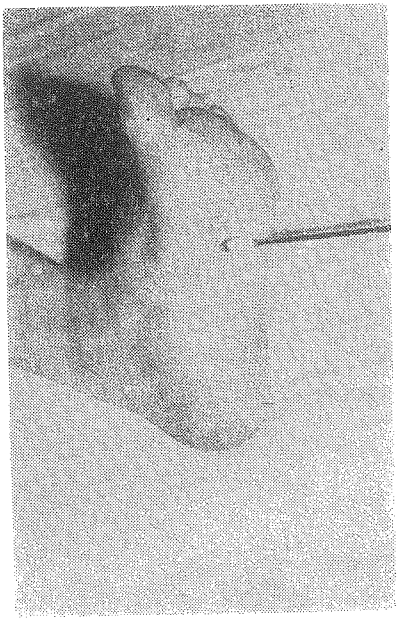

Figure 4
Photograph of the left foot of III-8 from family B

III-10, 45 year-old male of DC in normal health. According to the description of his brother (III-2) he has contractures on his both hands.

Genetic Findings

There are three sibshibs with affected cases in the second and third generations as seen in family A (Figure 1). For that reason, the penetrance could be complete. But, the proportion of affected and normal offspring of the affected parents from the same family between the fourth and the sixth decade differ significantly from the expected 1:1 ratio which is observed in dominant inheritance. There are two sibshibs with affected cases in the second and third generations and the proportion of affected and normal offspring of the affected parents from family B does not differ from the ratio in question. Therefore, the penetrance of DC is complete. The affected cases in both families showed the same degree deformity on their hands. In other words, the expressivity of the condition is full. So, the transmission of the condition in both families tends to be that of autosomal dominant with full expressivity.

But, if we consider that the mode of inheritance of DC is recessive, whether 1-6 from family A was homozygous for a gene or both 1-3 and I-6 were heterozygous, II-5 and her children (III-1, III-2 and III-3) are homozygous. Apparetly, the proportion of normal and affected offspring of affected parent in the third generation does not differ significantly from the ratio 3:1 expected in this shape of inheritance. 1-4 from the same family was heterozygous or homozygous and II-4 is homozygous (Figure 1). I-1 from family B was homozygous and half of the children of him was also homozygous for a recessive gene and the ratio in question is expected in this form inheritance. Two (III-8 and III-10) out of six children of II-1 have DC. The proportion the normal and affected offspring does also not differ from the expected 3:1 ratio as mentioned above (Figure 2).

The increase of males in DC can not be atributed to a genetic mechanism such as sex linkage, because, the males inherit the gene from fathers as well as mothers.

Dermatoglyphic Findings

The dermatoglphics of II-5, III-1 and III-2 from family A and III-8 from family B were studied. The dermatoglyphics of the cases from the families were normal except for II-5 from family A. She had a radial loop on her right fourth finger-tip (Table I). The percentage frequency of this type pattern on the same finger-tips of 250 female controls is 0.4.

TABLE I

FINGER-TIP PATTERNS, TOTAL FINGER RIDGE-COUNTS AND PLAMAR AND PLANTAR FORMULAE OF THE CASES OF DC FROM FAMILIES A AND B

Case	V	IV	III	II	I	TRC	Palmar fromula	Sole formula
Family A								
Lt II-5	U	U	U	R	U	25	IV H t t^b 4	III f 4
Rt	U	R	U	U	U		IV t 4	I III f p 4
Lt III-1	U	W	W	U	U	224	IV $\hat{H}$ t t^b 4	I III f p p' 4
Rt	U	W	U	U	U		IV $\hat{H}$ t t^b b4	I III f p 4
Lt III-2	U	U	U	R	U	142	IV IV t 5	I f 4
Rt	U	U	U	R	U		IV t 4	I $\hat{I}$ e f 4
Family B								
Lt III-8	U	U	W	W	U	154	IV $\hat{H}$ t t^b 4	I $\hat{I}$ III e f p' 4
Rt	U	W	W	R	U		III t 4	I $\hat{I}$ III e f p' 4

U- ulnar loop, R- radial loop, W- whorl, TRC- total finger ridge-count, Lt- left, Rt- right

Discussion

Manson reported a family showing DC. The father and his three sons were affected.[11] Crouch observed DC in two male monozygotic twins.[7] Skoog pointed out that the condition was due to a dominant gene which was responsible for predisposition.[14–15] Graubard found that all the cases of DC had Rh positive factor and he assumed that the mode of inheritance of DC fitted the pattern autosomal dominant.[8] Lygonis noted that a dominant gene was responsible from DC without causing other disorders.[13] Hueston and Maza and Goodman reported that DC showed a tendency to be that of an autosomal dominant trait.[4,12] Stein et al. noted that hypoplastic muscle fibers in palmar aponeurosis was inherited recessively.[16] Kipikasa reported an increasing familial and hereditary incidence of DC. However, the author could not definitely prove wrong in the opininons of the authors[7–16] on the transmission of DC whether it was dominant or recessive. Besides, according to the author heredity was not the only etiologic factor in the formation of DC. The condition was also due to the diseases affecting the autoimmunologic balance or the tissue in mesenchymal origin.[5] Jakubowski noted that

autosomal dominant and irregular inheritance with various expressivity were observed in DC. For that reason, according to the author the mode of inheritance of DC was not clear.[9]

First of all, we believe that DC is hereditary. Secondly, the shape of the transmission of the condition fits the pattern of autosomal dominant rather than that of autosomal recessive. The age of onset of the cases of DC affects the penetrance. Indeed, the age of initial onset varies from middle age to old age and many family members have died from other causes before developing the condition or they have no DC when the pedigrees are recorded. Besides, it should be noted that the cases previosly reported and presented here had no associated diseases excepting for Kipikasa's cases[5] and were otherwise healthy individuals. We also belive that the associated diseases do not affect the formation of DC.

Summary

The phenotypic, genetic and dermatoglyphic findings in two families showing Dupuytren's contracture are presented. The mode of inheri tance of the condition fits the pattern autosomal dominant rather thathat of autosomal recessive. The age of the onset affects the penetrance.

REFERENCES

1. Dupuytren, B.: Permanent retraction of the fingers, produced by an affection of the palmar fascia. Lancet. **2**: 222, 1934.
2. Arieff, A. J. and Bell, J.: Epilepsy and Dupuytren's contracture. Neurology. **6**: 115, 1956.
3. Herzog, E. G.: The etiology of Dupuytren's contracture. Lancet. **2**: 1305, 1951.
4. Hueston, J. T.: Dupuytren's contracture. Williams and Wilkins Co. Baltimore. pp. 1-120.
5. Kipikasa, A.: The share of heredity in the formation of Dupuytren's contracture. Acta Chir. Plast. **14** 1, 1972.
6. Larsen, R. D., Takagishi, N. and Posch, J. L.: The pathogenesis of Dupuytren's contracture, experimental and further clinical observations. J. Bone and Joint Surg. **42-A**: 993, 1960.
7. Crouch, H. cited in Kipikasa, A.: The share of heredity in the formation of Dupuytren's contracture. Acta Chir. Plast. **14**: 1, 1972.
8. Graubard, D. J. cited in Kipikasa, A.: The share of heredity in the formation of Dupuytren's contracture. Acta Chir. Plast. **14**: 1, 1972.
9. Jakubowski, J.: Rodzinne wystepowanie przykruozu Dupuytrena. Chir. Narz. Ruchu Ortop. Pol. **XII**: 3, 1976.
10. Kostia, J.: A Dupuytren's contracture family. Ann. Chir. Gneac. Fenn. **46**: 351, 1957.

11. Manson, J. S.: Heredity and Dupuytren's contracture. Brit. Med. Jour. 2: 11, 1931.
12. Maza and Goodman, R. M.: A family with Dupuytren's contracture. J. Hered 59: 155, 1968.
13. Lygonis, C.: Familiar Dupuytren's contracture. Hereditas. 56: 142, 1966.
14. Skoog, T.: Dupuytren's contraction with special reference to aetiology and improved surgical treatment; i!s occurence in epileptics; note on knuckle pads. Acta Chir. Scand. Suppl. 138: 1, 1948.
15. Skoog, T.: The transverse elements of the palmar aponeurosis in Dupuytren's contracture. Scand. J. Plast. Reconstr. Surg. 1: 51, 1967.
16. Stein, A. et al. cited in Kipikasa, A.: The share of heredity in the formation of Dupuytren's contracture. Acta Chir. Plast. 14: 1, 1972.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 1-2 / January-April 1979 / pp. 42 - 44

Posterior Dislocation of the Hip Joint Associated with the Avulsion Fracture of the Greater Trochanter*

A Case Report

O. Şahap Atik, M.D. / Şakir Memikoğlu, M.D.*** / Talat Göğüş, M.D.******

Femoral head or shaft fractures are not uncommonly associated with posterior hip dislocation.[2,3] In the literature no case has been described that corresponds to the one reported here. which representes an interesting complication of posterior dislocation of the hip joint associated fracture of the greater trochanter.

Case Report

A fourthy -three-year-old man was involved in a car accident and sustained posterior dislocation of the right hip associated with the fracture of the greater trochanter. There was no neurologic deficit on physical examination.

The posterior dislocation was reduced by closed method. For the avulsion fracture of the greater trochanter, open reduction was done. During the operation, no comminution was seen in the fracture fragment. It was located near the superior corner of the right acetabulum. Most of the m. gluteus medius was unruptured. The fixation was accomplished with a 80.0-milimeter cancellous screw. The patient was then placed in a spica cast. (Figures 1, 2, 3).

* From the Department of Orthopaedics and Traumatology, Hacettepe University, Faculty of Medicine, Ankara, Turkey.
** Specialist in the same Department.
*** Associate Professor in the same Department.
**** Professor and Chief of the same Department.

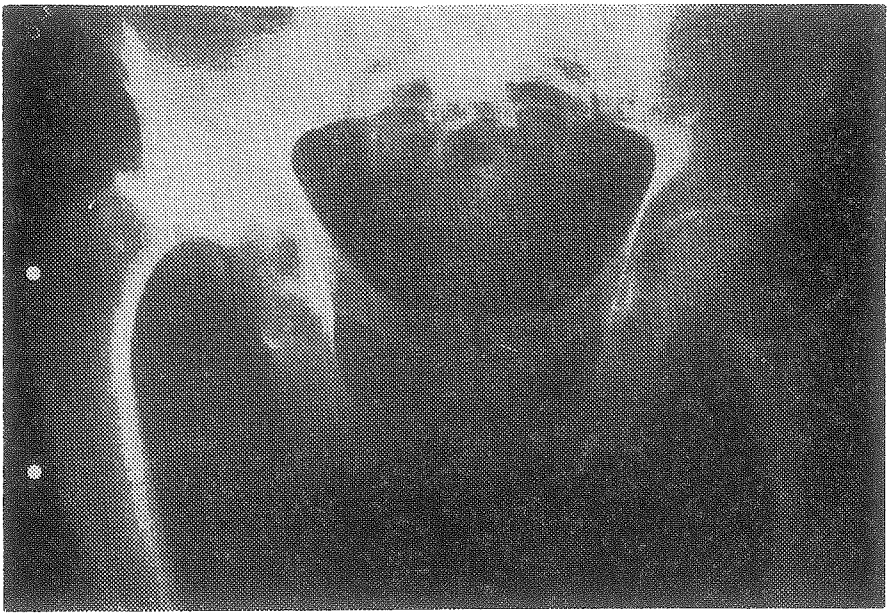

Figure 1

Anteroposterior roentgenogram of the fracture-dislocation.

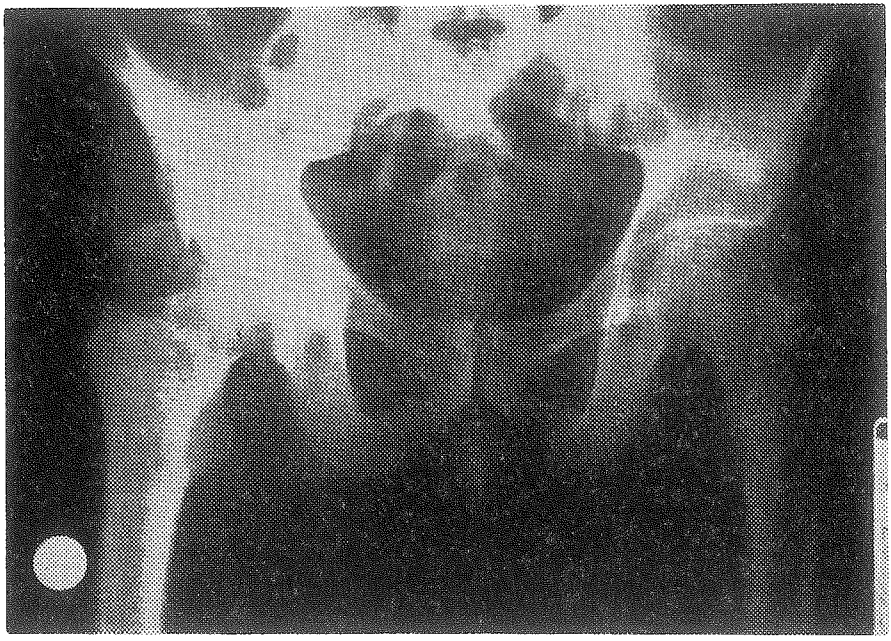

Figure 2

Anteroposterior roentgenogram made after reduction of the dislocation of the hip joint.

Figure 3
Anteroposterior roentgenogram of the hip after operation.

Comment

In previously reported cases of posterior dislocation of the hip joint, avulsion fracture of the greater trochanter was not part of the injury, and the present case derives its interest from that circumstance.

Some authors recommend to fix the fracture fragment internally when displacement is greater than one centimeter. With present knowledge of the case, the mechanism of abduction was almost normal even though displacement of the fragment is greater than one centimeter. We think that cases similar to ours may also be treated non-operatively.

REFERENCES

1. Clawson, D. Kay, and Melcher, J. Peter: Fractures and dislocations of the hip. In Fractures, Edited by Charles A. Rockwood, Jr.. and David P. Green, Philadelphia, J. B. Lippincott, 1975, pp. 1055.
2. Dehne, E., and Immerman, E. W.: Dislocation of the Hip Combined with Fracture of the Shaft of the Femur on the same side. J. Bone Joint Surg., 33-A: 731 **33-A:** 1951.
3. Fina, C. P., and Kelly, P. J.: Dislocations of the Hip with Fractures of the Proximal Femur. J. Trauma, **10:** 77, 1970.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 1-2 / January-April 1979 / pp. 45 - 48

Dermatoglyphic Findings in Congenital Dislocation of the Hip: Familial Study*

Metin Atasu, Ph.D. / Nasır Özdemir, M.D.*** / Yaser Muşdal, M.D.*****

The unusual dermatoglyphics is suggested in congenital malformations, particularly in congenital deformities of hands and feet whether the cause of the conditions in question is hereditary or not.[1,2]

The genetics of congenital dislocation of the hip (CDH) is considered to be complex. The role of polygenic acetabular configuration and monogenic joint laxity in the etiology of CDH was shown by Czeizel et al.[3]

In this communication the dermatoglyphic findings in CDH will be presented.

Materials and Methods

Finger-tip and palm prints and sole prints over the hallucal area were obtained from 14 male and 69 female in the total 83 individuals of CDH and 86 male and 83 female relatives of them without CDH.

The control group consisted of 250 male and 250 female, unrelated and healthy individuals who lived in Ankara.

Prints were taken using kleenprint ink and paper and statistical analyses were done by Chi-square test.

---

* From Hacettepe University, Faculty of Medicine, Department of Orthopaedics.

** Associate Professor of Genetics and Statistics, Deparment of Genetics and Medical Biology, Faculties of Medicine and Dentistry, İstanbul Academy of Economical and Commercial Sciences, İstanbul, Turkey.

*** Associate Professor of Orthopaedics.

Results

The comparisons of percentage frequencies of the pattern types for all fingers of the individuals of CDH, their relatives without CDH and control individuals showed no significant differences (Table I). The comparisons of percentage frequencies of palmar configurations of the individuals of CDH, their relatives without CDH and control individuals are shown in Table II. The percentage frequencies of I and $\hat{I}V$ loops and f and t′ triradii increased and IV, H and H^r loops and t and t″ triradii decreased on the palms of the individuals of CDH than those of control individuals. The relatives of individuals of CDH had also less IV and H^r loops on the palms than those of control individuals. Besides, the relatives without CDH of the individuals of CDH had more $\hat{I}V$ loops and less e triradii on their palms. The comparisons of the percentage frequencies of the configurational types in the hallucal areas of the individuals of CDH, their relatives without CDH and control individuals showed no significant differences (Table III).

TABLE I

COMPARISONS OF PERCENTAGE FREQUENCIES OF THE PATTERN TYPES FOR ALL FINGERS OF THE INDIVIDUALS OF CDH, THEIR RELATIVES WITHOUT CDH AND CONTROL INDIVIDUALS

Pattern types	Individuals of CDH	Relatives without CDH	Control individuals
Ulnar loops	53.3	54.0	56.2
Radial loops	3.1	4.3	3.4
Whorls	38.9	36.7	36.3
Arches	4.7	5.0	4.1

Discussion

All the parameters except for palmar I and H loops and f, t, t′ and t″ triradii revealed no significant differences between the individuals of CDH and control individuals. The significant differences connected with palmar IV, $\hat{I}V$ and H^r loops of the individuals of CDH from those of control individuals seem that are not in value. Because, the similar deviations were observed in both the individuals of CDH and their relatives without CDH.

TABLE II

COMPARISONS OF PERCENTAGE FREQUENCIES OF CONFIGURATIONAL TYPES ON THE PALMS OF INDIVIDUALS OF CDH, THEIR RELATIVES WITHOUT CDH AND CONTROL INDIVIDUALS

	Individuals of CDH	Relatives without CDH	Control Individuals
Loops			
I	4.2[1]	2.1	2.3
Ir	4.8	3.0	4.6
II	2.4	3.6	3.6
$\hat{I}I$	0.0	0.0	0.2
III	45.8	45.0	41.6
$\hat{I}II$	0.6	0.0	0.9
IIIT	11.4	9.5	9.7
IV	43.4[1]	41.7[1]	55.1
$\hat{I}V$	1.8[1]	2.7[1]	0.9
IVu	0.0	0.0	0.3
H	9.6[2]	13.6	14.9
$\hat{H}$	24.7	18.9	22.8
Hr	0.6[1]	0.9[1]	1.7
Triradii			
e	4.8	3.8[1]	6.6
f	3.03	1.2	1.2
t	59.6[1]	66.3	68.6
t'	41.0[1]	35.8	32.9
t''	7.2[1]	9.5	11.7
tb	24.1	21.6	24.3
tr	0.0	0.0	0.0
tu	2.7	3.3	3.0
z	0.0	0.0	0.0
z'	2.1	2.4	2.6
z''	1.6	1.8	2.0

1 Significant at the 5 % level
2 Significant at the 1 % level
3 Significant at the 0.1 level

TABLE III

COMPARISONS OF PERCENTAGE FREQUENCIES OF CONFIGURATIONAL TYPES IN THE HALLUCAL AREAS OF THE INDIVIDUALS OF CDH, THEIR RELATIVES WITHOUT CDH AND CONTROL INDIVIDUALS

	Individuals of CDH	Relatives without CDH	Control individuals
Loops			
I	84.1	79.0	84.3
Î	34.1	40.9	40.3
Triradii			
e	37.2	42.4	41.9
f	83.2	82.3	81.7

Summary

Finger-tip and palm prints and sole prints over the hallucal area of the individuals of CDH and the relatives of them without CDH were compared with those of control individuals. The percentage frequencies of I loops and t′ triradii increased and H loops f, t and t″ triradii decreased on the palms of the individuals of CDH.

REFERENCES

1. Holt, S. B.: The genetics of dermal ridges. Charles C Thomas. Publisher. Springfield. Illinois. U.S.A. 1968.
2. Schauman, B., Alter, M.: Dermatoglyphics in medical disorders. Springer-Verlag. New York, Heidelberg. Berlin. 1976.
3. Czeizel, A., Tusnady, G., Vaczo, G., and Vizkelety, T.: The mechanism of genetic predisposition in congenital dislocation of the hip. J. Med. Genet. 12: 121, 1975.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 1-2 / January-April 1979 / pp. 49 - 50

Sinding Larsen-Johansson Disease of the Patella*

(A Case Report)

O. Şahap Atik, M.D. / Şakir Memikoğlu, M.D.*** / M. Talat Göğüş, M.D.******

This traction lesion is rare and it occurs in adolescents, usually boys, between 10 and 14 years of age. Pain, soft tissue swelling and tenderness are noted over the lower less commonly the upper, pole of the patella. It causes limp, inability to kneel and run. The patella may be expected to be located at a higher level in maturity.[1,2]

Case Report

A thirteen year-old boy was admitted to the hospital for pain and inability to use his left knee. There was a history of falling onto the knee prior to admittance. Physical examination revealed minimal soft tissue swelling and tenderness over the lower pole of the patella. X-ray revealed a traction lesion on the patella (Figure 1, 2).

A cylinder cast was applied with the knee in extension. At the end of the six weeks, all of the symptoms and findings had subsided.

Comment

The etiology of this rare condition is unclear. But the physician must know and tell to the parents of the patient that it is not a fracture. When the patella is fractured, the soft tissue swelling is more and the borders of the fragments are seen as sharp in the roentgenogram. The healing is spontaneous within a matter of a few weeks. A cylinder cast applied with the knee in extension relieves symptoms and may expedite recovery.[1]

* From the Department of Orthopaedics and Traumatology, Hacettepe University, Faculty of Medicine, Ankara.
** Specialist in the same institution.
*** Associate Professor in the same institution.
**** Chief and Professor in the same institution.

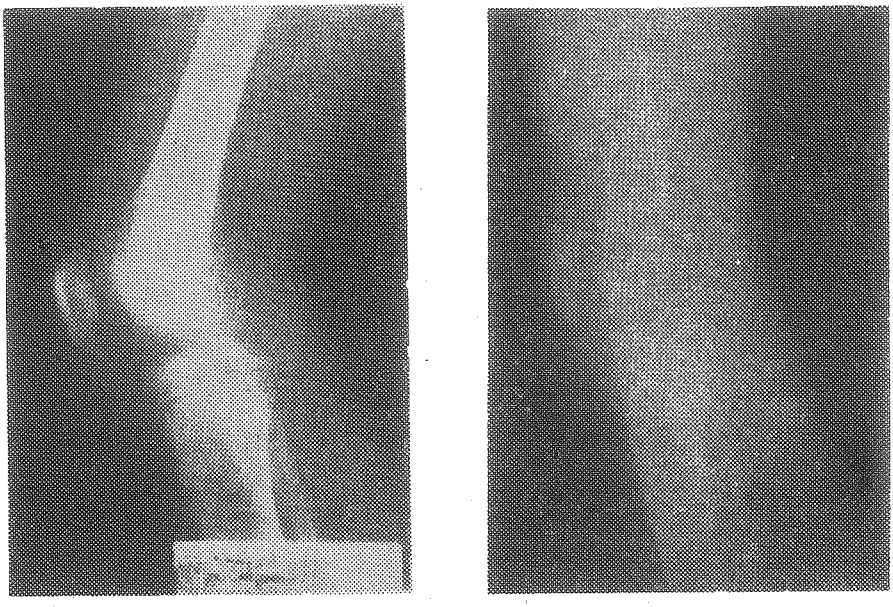

Figure 1, 2

Roentgenograms of the left knee showing a traction lesion on the lower pole of the patella (before and after treatment).

REFERENCES

1. Turek, L. S.: Orthopaedics. 3rd. Ed., J. B. Lippincott, Philadelphia, 1977, p. 1198.
2. Smillie, I. S.: Disease of the Knee Joint. Churchill Livingstone, 1974, p. 64.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 1-2 / January-April 1979 / pp. 51 – 57

Zinc Deficiency in Erythema Multiforme and Treatment With $ZnSO_4$*

Naci M. Bor, M.D. / Vedat Sezer, M.D.*** / Gülsen Öner, M.D.**** / Kemal Özkaragöz, M.D.*****

In a recent communication we reported that in a case of erythema multiforme serum zinc level was much reduced and that supplementation of this ion caused complete relief.[3] Since then nine subsequent cases have been studied and in all zinc deficiency was uniformly documented. The purpose of this communication is to present the data of this experience.

Material and Methods

All ten cases were from the outpatient department of Hacettepe University Hospital. Their ages varied from 8 months to 60 years. History of the disease ranged from one week to 50 years.

The patients came to our laboratory in post-absorptive state usually after a night's rest a 9-10 AM. A detailed history especially as related to allergic diseases was obtained. Care was particularly exercized to uncover focal infections, intestinal parasites and chronic diseases such as diabetes mellitus. Patients were later subjected to a complete physical examination. Laboratory studies included, hematocrit, determination of blood sugar, serum proteins, serum cholesterol and total lipids were measured and a complete urinalysis.

* From Hacettepe University Medical and Surgical Research Center and the Department of Dermatology, Ankara, Turkey.

** Professor of Physiology and Director of Surgical and Medical Research Center.

*** Professor of Dermatology.

**** Associate Professor of Physiology, Senior Researcher, Surgical and Medical Research Center.

***** Professor of Pediatrics, Allergist.

Blood samples for determination of trace element was taken into syringes washed with demineralized water. The same precaution was taken for other glassware as well.

Serum zinc levels were determined using Perkin Elmer Mod. 103 Atomic Absorption Spectrophotometer. If they were found below normal values, $ZnSO_4$ was given, 220 mg three times daily. The patients were invited for follow up studies on 3 rd, 6 th and 10 th days and monthly there after.

Results

Clinically all patients, were improved by the tenth day of treatment as the serum zinc levels revealed a significant rise. The response was usually prompt although in some instances it was delayed for few days. Upon attaining full effect of the drug patients first noticed reduction of itching and fading of redness of the active lesions. Gradually the oldest lesions disappeared and new ones failed to occur. The clinical course of the persisting lesions became milder and duration grew shorter. Upon exposure to specific allergens, or common effectors such as increased physical or psychological stress or ingestion of bitter food and alcohol recurrence or aggravation of the symptoms was observed. Manifestations were however not as heavy as before zinc therapy and their duration was shorter.

The mean serum zinc level was 63.70 ± 4.16 µg/dl on the first examination (Table I, Figure 1)). There was a marked rise on the third day of treatment reaching 80.00 ± 5.50 µg/dl ($P < 0.025$), while the patients were somewhat improved. On the tenth day it had arrived at 97.25 ± 4.95 µg/dl ($P < 0.005$) and according to the later examinations it was maintained near this level (96.8 ± 7.27 µg/dl, $P < 0.005$).

TABLE I

BLOOD ZINC LEVELS (µg/dl.)

	Before Treatment	After Treatment 3. Day	10. Day	Later
Zinc	63.70 ± 4.16	80.00 ± 5.5	97.25 ± 4.95	96.8 ± 7.27
	$n = 9$	$n = 4$	$n = 4$	$n = 5$
		$P < 0.025$	$P < 0.005$	$P < 0.005$

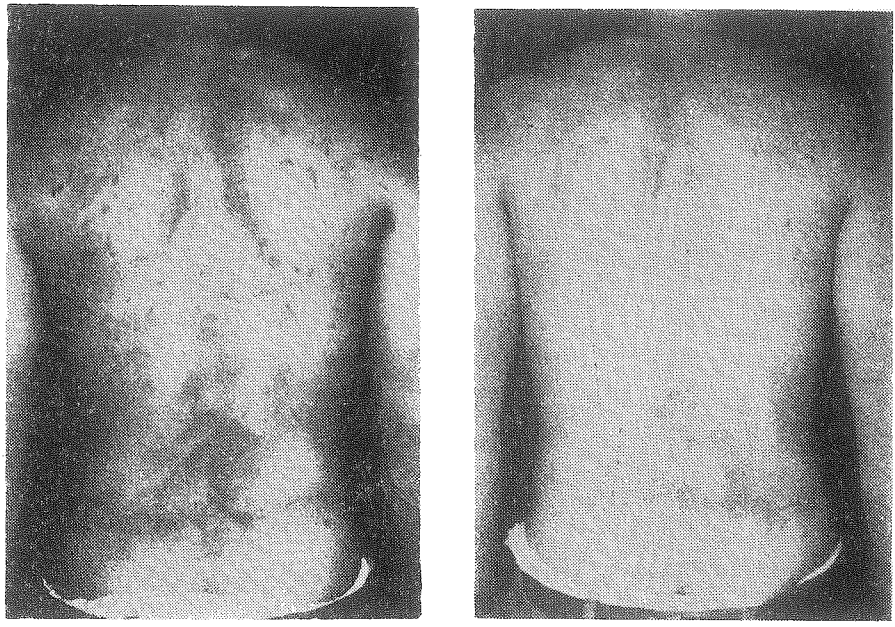

Figure 1

A) Before treatment

B) 3 days after treatment wint zinc Sulfate.

The mean fasting blood sugar was 96.78 ± 4.73 mg %. Hematocrit, cholesterol and total lipids and urinalysis were within normal limits (Table II).

TABLE II

Year	Length (cm)	Weight kg.	Fasting blood sugar (mg/dl	Choles-terol mg/dl	Total Lipids mg/dl	Albumin g/dl
35.6 ±	169.83 ±	69.66 ±	96.78 ±	171.26 ±	596.5 ±	5.26 ±
5.35	3.17	2.24	4.73	21.68	52.3	0.24

Case Histories

Case 1: S. K. a 55 year old white male reported with erythematous lesions starting at the age of 4 years.

He did not however enjoy a complete remission during his life time. A careful history failed to uncover allergens. Most of the episodes

e treated with antiallergic drugs, sedatives and when very severe with isone. The patient suffered with these complaints for over 50 years ch on occasion seemed to get aggravated with no apparent cause.

On admission he was a well developed well nourished male whose of legs and arms as well as back and chest revealed numerous mul-rm erythematous lesions. Many of these were bullous some were sted while others were infected. Between these lesions innumerable tch marks were present. Physical examination was otherwise unre-ling. He was 1.73m. tall and weighted 76.5 kg.

His blood cholesterol was 268.6 mg/dl, lipids 881.2 mg/dl, blood ar 75 mg %. His serum zinc was 60.0 µg/dl and Cu 70.0 µg/dl.

All the antiallergic and antibiotic medications were discontinued d $ZnSO_4$ $7H_2O$ 220 mg after each meal was started. After a transitory gravation his symptoms were alleviated and the lesions were clini-ly much improved on the tenth day. Serum zinc levels were 110 µg/dl d Cu 95 µg/dl. Serum histamin however was still very high 0.714 /ml.

On later follow up he continued to improve stating specifically at no new lesion occured.

Case 2: M. S. 34 year old while male was referred to us for ythematous lesions appearing after dinner. They were numerous and ostly located at his back, abdomen and legs. These complaints were esent since six years.

On physical examination he was a well developed well nourished hite male. Multiform erythematous lesions were present on his back, gs, and abdomen. Lesions had occasionally appeared on his face.

Fasting blood sugar was 106.2 mg %, serum cholesterol was 140 g/dl, total lipids 466.2 mg/dl, Hb: 15.5 gr. Serum zinc was 65 µg/dl, u 69.0 µg/dl and Mg. 2.0 µg/dl.

He was placed on zinc sulfate, 7 H_2O t.i.d. He returned for a re-check n the third day and stated that the lesions were still occuring however ss severe. Their duration was also much less. Serum zinc was 69 µg/dl nd serum copper 70 µg/dl. On the sixth day his complaints were greatly iminished while serum zinc level had climbed to 77.0 µg/dl and Cu o 83.0 µg/dl. On the tenth day the lesions had completely disappeared nd he felt no discomfort. Serum zinc and copper levels had both risen o 100.0 µg/dl.

were treated with antiallergic drugs, sedatives and when very severe with cortisone. The patient suffered with these complaints for over 50 years which on occasion seemed to get aggravated with no apparent cause.

On admission he was a well developed well nourished male whose skin of legs and arms as well as back and chest revealed numerous multiform erythematous lesions. Many of these were bullous some were crusted while others were infected. Between these lesions innumerable scratch marks were present. Physical examination was otherwise unrevealing. He was 1.73m. tall and weighted 76.5 kg.

His blood cholesterol was 268.6 mg/dl, lipids 881.2 mg/dl, blood sugar 75 mg %. His serum zinc was 60.0 µg/dl and Cu 70.0 µg/dl.

All the antiallergic and antibiotic medications were discontinued and $ZnSO_4$ $7H_2O$ 220 mg after each meal was started. After a transitory aggravation his symptoms were alleviated and the lesions were clinically much improved on the tenth day. Serum zinc levels were 110 µg/dl and Cu 95 µg/dl. Serum histamin however was still very high 0.714 µg/ml.

On later follow up he continued to improve stating specifically that no new lesion occured.

Case 2: M. S. 34 year old while male was referred to us for erythematous lesions appearing after dinner. They were numerous and mostly located at his back, abdomen and legs. These complaints were present since six years.

On physical examination he was a well developed well nourished white male. Multiform erythematous lesions were present on his back, legs, and abdomen. Lesions had occasionally appeared on his face.

Fasting blood sugar was 106.2 mg %, serum cholesterol was 140 mg/dl, total lipids 466.2 mg/dl, Hb: 15.5 gr. Serum zinc was 65 µg/dl, Cu 69.0 µg/dl and Mg. 2.0 µg/dl.

He was placed on zinc sulfate, 7 H_2O t.i.d. He returned for a re-check on the third day and stated that the lesions were still occuring however less severe. Their duration was also much less. Serum zinc was 69 µg/dl and serum copper 70 µg/dl. On the sixth day his complaints were greatly diminished while serum zinc level had climbed to 77.0 µg/dl and Cu to 83.0 µg/dl. On the tenth day the lesions had completely disappeared and he felt no discomfort. Serum zinc and copper levels had both risen to 100.0 µg/dl.

Table II shows the results of these cases.

Case 3: H.Y.: This was a 39 yr old white male whose complaints started four months ago. At the beginning his fingers and eyelids were swollen and itchy. Soon his entire body was covered with red multiforme lesions. He was given antihistamin drugs and was advised to refrain from common allergens. Despite these measures the patient continued to display the same lesions. He was therefore referred to our laboratory. His serum zinc level was 43 µg/dl on admission. After treatment with zinc sulfate a dramatic improvement occured on the third day as the serum zinc level reached 101 µg/dl. There was a transitory aggravation following ingestion of generous amount of alcohol.

Discussion

Halsted and Smith[12] reported reduction of serum zinc levels in indolent ulcers, cirrhosis and other liver diseases, pulmonary tuberculosis, myocardial infarction, uremia, and in pregnancy. Other investigators[8] added to this list malignant diseases and showed that in bronchial carcinoma serum zinc levels were significantly reduced. Davis confirmed this observation in 100 cases of pulmonary carcinoma and in majority of cases with metastases serum zinc level was significantly below normal levels.[9] Strain and associates[21] showed that serum cupper/zinc ratios increased in cases of pulmonary carcinoma due to an elevation of serum cupper. He reported serum zinc in these patients was within limits of normal. In lymphosarcoma serum zinc levels have been reported as markedly reduced and serum cupper increased resulting in altered zinc cupper ratio.[1]

Zinc deficiency has also been reported following intravenous alimentation for prolonged periods.[14] It appears that the list of diseases with zinc deficiency will extend as research in this field increases. These results however are in discordance with observations of Sinha and Gabrieli who found serum zinc levels within normal limits in liver cirrhosis, asthma, bronchitis, diabetes mellitus, chronic alcoholism and in pregnancy.[20]

There are very few reports however concerning serum zinc levels in allergic diseases.[3, 4, 7] Kampschmidt and Pulliam[13] were able to demonstrate reduction of serum zinc and iron levels in rabbits and rats in which anaphylaxis was precipitated. In this series we have demonstrated that in cases of erythema multiforme serum zinc levels are much reduced. In a parallel series wihch is presently in publication we have

observed that in many forms of allergic diseases serum zinc level as well as copper levels are almost uniformly reduced.

Relationship between reduction of serum zinc levels possibly involves permeability of the cellular membranes. Chvapil et al[5] demonstrated that zinc causes stability of lysosomal membranes. The same author later,[6] demonstrated inhibitory effect of zinc ions on platelet aggregation and serotonin release reaction. We, in 1976[2] demonstrated that a positive correlation existed between serum zinc levels and mast cell degranulation.

Kazimierczak[15] was able to prevent occurance of anaphylaxis in guinea pigs treated with intraperitoneal zinc. Thus he claimed that this ion had a preventive effect. Cho et al[4] used pulverization of zinc ion in cases of anaphylaxis and was successful in delaying occurence of bronchoconstriction. Considering this and the fact that zinc stabilizes the cellular membrane it seemed reasonable to us that this ion may cause some improvement in cases of erythema multiform. Upon observation of beneficial effects of the ion on the clinical course of the disease we were encouraged to try in other forms of allergic diseases. The results of this study which is presently being conducted introduces zinc as an addition to the armamentarium of many forms of allergic diseases. That it has a definite effect on erythema multiforme has been demonstrated in this communication. It is also possible that in some other diseases zinc ion may have a curative effect. Among these bleeding peptic ulcers have been beneficially effected by this drug.[17, 18, 19]

Summary

In 10 cases of erythema multiforme serum zinc levels were found much below normal values. Zinc Sulfate was given orally to these patients 220 mg. three times daily. Marked improvement of existing lesions and prevention of new episodes of allergic manifestations was observed.

REFERENCES

1. Babacan, E., Çavdar, A. O., Arcasoy, A.: Serum Zinc Levels, Lymphocyte counts and functions in pediatric hodgkin's disease. Estratto dal "Boll. Ist. Sieroter Milanese 56: 229, 1979.
2. Bor, N. M., Öner, G., Noyan, A., Arcasoy, A., Çavdar, A., Sözer, S.: Relation between mast cells and serum zinc levels. New Ist. Contr. Clin. Sci. 11: 136, 1976.
3. Bor, N. M., Öner, G., Babül, A.: Erythema Multiforme'de Çinko Tedavisi. Doğa, 3: 87, 1979.
4. Cho, C. H., Dai, S., Ogle, C. W.: The effect of zinc on anaphylaxis in vivo in the guinea-pig. Br. J. Pharmac. 60: 607, 1977.

5. Chvapil, M., Ryan, I. N., Zukoski, C. F.: The effect of zinc and other metals on the stability of lysosomes. Proc. Soc. Exp. Biol. Med. **140**: 642, 1972.
6. Chvapil, M., Weldy, P. L., Stankova, L., Clark, D. S., Zukovski, C. F.: Inhibitory effect of zinc ions on platelet agregation and serotonine release reaction. Life Sci. **16**: 561, 1975.
7. Chvapil, M., Stankova, L., Bartos, Z., Cox, T., Nichols, W.: Motility of peritoneal inflammatory cells after in vivo supplementation with zinc. J. Ret. Soc. **25(4)**: 345, 1979.
8. Davies, I. J. T.: Plasma zinc concentration in patients with bronchogenic carcinoma. Lancet **1**: 149, 1972.
9. Davies, I. J. T., Musa, M., Dormandy, J. L.: Measurements of plasma zinc. J. Clin. Path. **21**: 359, 1968.
10. Frommer, D. J.: The healing of gastric ulcers by zinc sulphate. Med. J. Austr. **2**: 793, 1975.
11. Greaves, M., and Boyde, T. R.: Plazma zinc concentrations in patients with psoriasis, other dermatoses and venous leg ulceration. Lancet **2**: 1019, 1967.
12. Halsted, J. A., Smith, Jr. J. C.: Plasma zinc in health and disease. Lkncet, **1**: 322, 1970.
13. Kampschmidt, R. F. and Pulliam, L. A.: Effect of delayed hypersensitivity on plasma iron and zinc concentration and blood leukocytes. Proc. Soc. Exp. Biol. Med. **147**: 242, 1974.
14. Kay, R. G., Jones, C. T., Pybus, J., Whitnig, R. and Black, A.: A syndrome of acute zinc deficiency during total parenteral alimentalion in man. Ann. Surg. **183**: 331, 1979.
15. Kazimierczak, W.: The influence of zinc ions administered in vivo on guinea-pig anaphylaxis. -Acta Physiol. Pol. **25(6)**: 609, 1974.
16. Keller, R. and Sorkin, E.: Selective incorporation of zinc into rat mast cells. Experientia **26**: 30, 1970.
17. Molokhia, M. M. and Portney, B.: Bleeding gastric erosion after oral zinc sulphate. Brit. Med. J. **1(6120)**: 1145, 1978.
18. Morgan, A. A.: Bleeding gastric erosion after oral zinc sulphate. Brit. Med. J. **1(6122)**: 1283, 1978.
19. Orr, K. B.: Healing of gastric ulcer by zinc sulphate. Med. J. Austr. **1(21)**: 244, 1976.
20. Sinha, N. S. and Gabrieli, R. E.: Serum copper and zinc levels in various pathologic conditions. Amer. J. Clin. Pathol. **54**: 570, 1970.
21. Strain, W. H., Mansour, E. G., Flynn, A., Pories, W. J., Tomoro, A. J., Hill, Jr. D. A.: Plasma zinc concentration in patient with bronchogenic cancer. Lancet **1**: 1021, 1972.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 1-2 / January-April 1979 / pp. 58 - 60

Congenital Absence of the Radius Associated with the Dislocation of the Hips and Cyanotic Heart Disease*

(A Case Report)

O. Şahap Atik, M.D. / M. Talat Göğüş, M.D.*****

Congenital absence of the radius is a skeletal limb deficiency that is also known as congenital aplasia or hypoplasia of the radius and congenital radial clubhand.

A number of other congenital deformities are associated with congenital absence of the radius; namely, harelip, cleft palate, clubfoot, hydrocephalus, absence or fusion of ribs, aplasia or collapse of the lung, hemivertebrae, and Fanconi's syndrome with severe anemia.[2]

In the present report we describe two new abnormalities which are simultaneously associated with the congenital absence of the radius.

Case Report

The four-year-old girl was the prudoction of a normal gestation and normal delivery. She was the seventh child, and both her father and brother had no thumb.

The patient had cyanosis and dyspne dating from infancy. The fingers and toes showed clubbing. There was bowing on tne forearms. The thumbs were absent, but the hands were surprisingly functional. The abduction of the hip joints were limited bilaterally and the rest of her physical examination was within normal limits.

* From the Department of Orthopaedics and Traumatology, Hacettepe University, Faculty of Medicine, Ankara, Turkey.
** Specialist in the Same Department.
*** Professor and Chief of the Same Department.

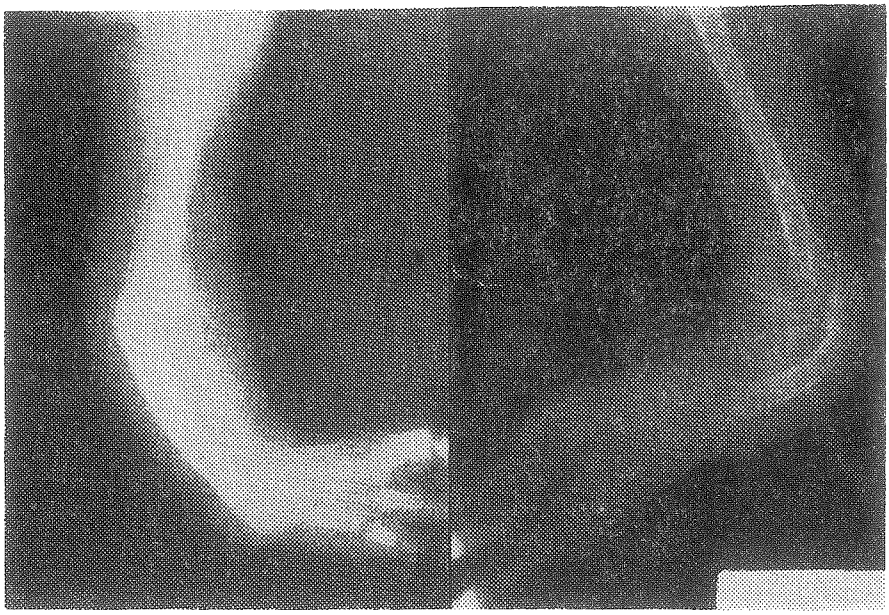

Figure 1
Roentgenograms of both upper limbs showing bilateral absence of radii, first metacarps, and thumbs.

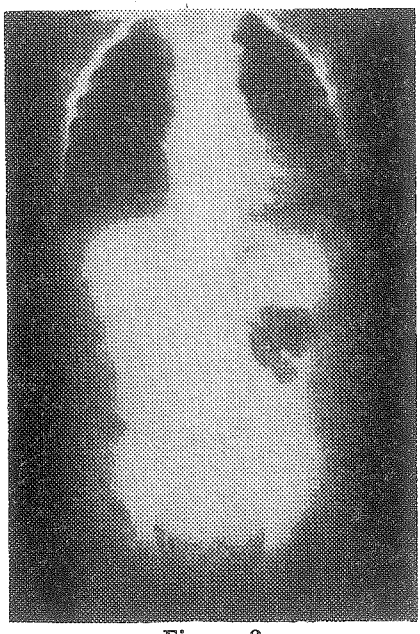

Figure 2
Roentgenogram of the chest and pelvis showing boot-shaped heart and bilateral dislocation of the hips.

The findings of a complete blood count were normal.

The X-ray examination revealed boot-shaped heart, bilateral dislocation of the hips, and bilateral radial paraxial hemimelia (the absence of radii, first metacarps, and thumbs). (Figure 1,2).

Comment

In previously reported cases of congenital absence of the radius; hip dislocation and cyanotic heart disease were not simultaneously associated with this phenomenon, and the present case derives its interest from that circumstance.

Some surgeons advise that this deformity be left untreated in order to allow the child to develop the best function possible.[1] We agree with them with the present knowledge of the case.

For the patient's other problems, surgical therapy will be applied.

REFERENCES

1. Riordan, D. C.: Congenital Absence of the Radius. J. Bone Joint Surg., 37-A: 1129, 1955.
2. Tachdjian, M.O.: Congenital Absence of the Radius. In Pediatric Orthopaedics Philadelphia, W. B. Saunders, 1972, pp. 114.

HACETTEPE BULLETIN OF
MEDICINE/SURGERY
VOLUME 12 / Nos. 3-4 / JULY-OCTOBER 1979

Incidence, Fetal and Maternal Mortality and Current Concepts of Management of Rupture of Gravid Uterus*

Ali Ayhan, M.D. / Hüsnü Kişnişçi, M.D.*****

Introduction

Uterine rupture remains one of the serious life-threatening complications of pregnancy, especially in developing countries, where medical facilities are limited. While physicians may not witness this trouble during residency, they would be prepared to act quickly and effectively, whenever they face such one case. Recent reports suggest that the incidence of uterine rupture may now be increasing in countries where they have insufficient obstetric care.

Despite significant advances in obstetrics, anesthesiology, and clinical pathology, rupture of pregnant uterus continues to threaten both mother and fetus with high morbidity and mortality rates. A greater awareness of this entity aided by prompt diagnosis and definitive treatment is the most crucial factor in reducing such morbidity and mortality.

We have compared our experience with other large studies in the recent literature, especially incidence, fetal and maternal mortality and surgical treatment of ruptured gravid uterus.

* From Department of Obstetrics and Gynecology, Hacettepe University, Ankara Turkey.

** Associate Professor in the same Department.

*** Professor in the same Department.

Materials and Methods

33 Additional patients with uterine rupture were managed at Department of Obstetrics and Gynaecology in Hacettepe Medical School from 1965 to 1980. The data were obtained from patients records.

Results

During this period, there were over 30.021 deliveries at our Department and incidence rupture of 1/909.7 deliveries.

Average age of this series is 27.52 ∓ 0.87 and age distribution is shown in Table I.

TABLE I

UTERINE RUPTURES ACCORDING TO AGE OF MOTHER

Age Group	Scarred Ruptures No.	Unscarred Ruptures No.	Total No.	Total %
20-29	14	8	22	66.7
30+	9	2	11	33.3
Total	23	10	33	100.0

The mean parity is 2.91 ∓ 0.54 and it is given in Table II.

TABLE II

PARITY AT THE TIME OF UTERINE RUPTURE

Parity	Scarred No.	Unscarred No.	Total No.	Total %
0	—	3	3	9.37
1-3	12	6	18	56.25
4+	11	1	12	34.38
Total	23	10	33	100.00

Uterine rupture and its relation to labor is given in Table III.

TABLE III

UTERINE RUPTURE AND ITS RELATION TO LABOR

Uterine rupture type	Labor No.	Unlabor No.	Total No.	Total %
Classical	—	5	5	15.15
Low segment	10	8	18	54.55
Unscarred	9	1	10	30.30
Total	19	14	33	100.00

In our series, the unscarred uterine ruptures accounted for 30.3 % and the others were 69.7 % in Table IV.

TABLE IV

CAUSES OF THE UTERINE RUPTURE

Causes	Number	%
Uterine scar	23	69.69
a- Classical*	5	
b- Low segment	18	
Traumatic and spontaneous	10	30.31
a- Version and Extraction	2	
b- Oxytocin Stimulation	2	
c- Uterine anomalie and Distocia	6	
Total	33	100.00

* The metroplasty has been done in one case.

The mean gestational week is 39.21 ∓ 0.83 in scarred ruptures and is 39.50 ∓ 0.56 in unscarred cases. (t= 0,430 P > 0,05).

The mean weight of babies is 3137,8 ∓ 127.1 grs in scarred ruptures and is 3117,2 ∓ 123.1 in unscarred ruptures. (t= 0.117 P > 0.05)

The fetal mortality rate is 41.17 % and is given in Table V.

TABLE V

FETAL MORTALITY ASSOCIATED WITH UTERINE RUPTURE

Type of rupture	No	Fetal Deaths	Fetal Mortality %
Uterine Scar	23	10	43.47
a- Complete	12		
b- Incomplete	11		
Intact Uterus (traumatic and spontaneous)	10	4	36.36
Total	33*	14	41.71

* One had twin pregnancy.

The fetal mortality rate is 43.47 % in scarred ruptures and is 36.36 % in unscarred rupture cases.

Treatment of uterine rupture in scarred and unscarred cases is shown in Table VI.

The incidence of the ruptures reported has varied widely in the literature Table VII.

TABLE VI

TREATMENT OF UTERINE RUPTURE (ALL TYPES)

Procedure	Type of rupture: Unscarred No.	Scarred: Classical No.	Scarred: Low Segment No.	Total No.	Total %
Repair	6	—	13	19	59.37
Subtotal Hysterectomy	1	3	—	4	12.50
Total Hysterectomy	3	1	5	9	28.13
Total	10	4	18	32*	100.00

* One patient died before surgical procedure.

The maternal mortality rate is 3.03 %.

Discussion

TABLE VII

REPORTED INCIDENCE OF UTERINE RUPTURE IN PREGNANCY

Author [1, 2, 8, 6]	Deliveries	Ruptures	Incidence
Delfs and Eastman	53.574	53	1 : 1010
Voogd et al	17.181	12	1 : 1432
Rendle-Short	15.908	171	1 : 93
Birger-Astedt	407.340	83	1 : 4908
Groen	16.189	144	1 : 112
Schrinsky and Benson	126.770	47	1 : 2695
Kişnişçi and Ayhan	10.714	11	1 : 974

The incidence of our series is 1: 909.7. This incidence is the same as in the literature. Fetal mortality has been reported to be as high as 59 % and maternal mortality rate has ranged from 0 to 17.1 % in the same series Table VIII.

TABLE VIII

FETAL AND MATERNAL MORTALITY IN CASES OF RUPTURED GRAVID UTERUS

Author [2, 3, 4, 8, 6, 7]	Fetal Mortality %	Maternal Mortality %
Margulies and Grapanzano	58.8	7.3
Weingold et all	48.5	17.1
Schrinsky and Benson	36.2	6.4
Yussman and Haynes	14.2	—
Kişnişçi and Ayhan	58.3	—

The fetal mortality rate has been found as 41.71 % Table V. The maternal mortality rate has been observed as 3.03 %.

*Prompt diagnosis, adequate supportive measures such as fluid and blood replacement and rapid laparotomy are essential for reduction in both maternal and fetal mortality once the rupture has occured. It is the most important factor to prevent the uterine rupture.

1) Once a cesarean always a cesarean section

2) The more liberal use of cesarean section

3) After any difficult delivery, uterine exploration is part of the necessary practice of the experienced obstetrician.

The choice of surgical procedure should depend upon the type, extent and location of the rupture as well as the patient's condition and her desire to preserve her child bearing capability 5).

The defect from the rupture was repaired in 59.37 of our patients (Table VI). This procedure is infrequently used by others Table IX.

TABLE IX

SURGICAL MANAGEMENT OF THE RUPTURED GRAVID UTERUS

Authors [2, 3, 8, 7]	TAH %	STAH %	Repaired Defect %
Margulies and Grapanzano	70.7	17.1	7.3
Yussman and Haynes	71.4	—	10.7
Schrinsky and Haynes	29.7	12.7	46.7
Kişnişçi and Ayhan	27.2	45.5	27.3

Repair of the tear must be feasible if the defect is not bilateral or unduly ragged. All patients who undergo suturing must be throughly informed of the site of the defect and possible complications of future pregnancies.

Recurrence rate for rupture varies between 4 and 19 %. Fetal mortality with rupture was 75 % in Sheth's small series when rerupture occurred in the fundus.[5, 6] For subsequent pregnancies, Ritchie has recommended hospitalization at 28 weeks' gestation for patients with a history of previous upper segment rupture and for patients with a previous lower segment rupture, hospitalization at 36 weeks' gestation. Additionally, he recommends elective cesarean section at 36-38 weeks' gestation depending on the previous ruptured site.[4]

* Sudden cessation of labor
Moderate to severe abdominal pain
Regression of the presenting parts
Alterations of uterine contour
Vaginal bleeding and shock

In Summary:

1) In our opinion, all patients with repair of an uterine rupture should have deliveries by cesarean section.

2) Delivery times can now be individualized depending on the lecithin/sphingomylin rations.

3) We would agree with the concept of sterilization at cesarean section for a patient with a history of a previous upper segment rupture.

4) The appearance of a previous lower uterine tear at the time of section helps the surgeon to decide on tubal ligation.

5) Finally we believe that Ritchie's decision is true.

Summary

Thirty-three uterine ruptures of which 23 were scarred are presented.

Its insidence has been found in 1/909.7 deliveries.

The fetal mortality rate was found to be 41.17 % and maternal mortality rate has been seen in 3. 03 %.

REFERENCES

1. Groen, P. B.: Uterine Rupture in Rural Nigeria, Obstet. Gynecol. **44**: 682, 1974.
2. Kişnişçi, H. A. and Ayhan, A.: Gebe uterus püptürleri. Hacettepe Tıp/Cerrahi Bülteni, **5**: 439, 1972.
3. Margulies, D., Grapanzano, T.: Rupture of the Intact Uterus. Obstet. Gyneacol **27**: 863, 1966.
4. Ritchie, H. E.: Pregnancy after rupture of the pregnant uterus, The Journal of Obstetrics and Gyneacology of the British Commonwealth, **78**: 642, 1971.
5. Schrinsky, D. C., Benson, R. C.: Rupture of the pregnant uterus. Obstet, Gyneacol Surv. **33**: 217, 1978.
6. Spaulding, L. B. et al: Current concepts of management of rupture of the gravid uterus. Obstet, Gyneacol. **54**: 437, 1979.
7. Yussman, M. H., Haynes, D. M.: Rupture of the Gravid Uterus, Obstet, Gyneacol **36**: 115, 1970.
8. Weingold, A. B. and Sanford, S. Sherman D. H. et all.: Rupture of the gravid uterus, The Journal of Obstetrics. **125**: 1233, 1966.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 3-4 / July-October 1979 / pp 67 – 71

The Role of Familial Joint Laxity in the Etiology of the Idiopathic Scoliosis

Ümit Akkoyunlu, M.D.*

It is well konwn that the etiology of the idiopathic scoliosis is not definitely clear. There are many, hypothesis on the etiology of the condition. Moreover, genetic factors seems to be in value in the etiology. The high incidence of familial involvement of the joint laxity previously reported in the families showed the idiopathic scoliosis.

There are two main causes of the joint laxity. One of these is physiologic due to hormonal reasons. The other is the familial joint laxity resulting from the defect of connective tissue. The prevalance of familial joint laxity found to be high in the families of idiopathic scoliosis than those of normal population. Harrington and Enneking suggested that the differentiation of the vertebrea is secondary by noting that the primary event could be in the extra-osseous tissues in their histo-pathologic study on cartilage of the patients of the idiopathic scoliosis.

From beginning the points mentioned above the purpose of the study presented here is to show whether familial joint laxity plays role or not in the formation of the idiopathic scoliosis.

Materials and Method

The material consisted of 48 cases of the idiopathic scoliosis and their 226 first degree relatives. The control group is carried out on 443 cases.

All the cases were examined according to Wilkinson and Carter's method.

* Associate Professor in Orthopeadics and Traumatology Division, Hacettepe University, Ankara, Turkey.

Results

8 of 11 male (72.7 %) and 35 of 37 female (94.6 %) cases of the idiopathic scoliosis had the joint laxity (Table I). The frequencies of the joint laxity in the idiopathic scoliosis positive and negative families are shown in Table II and III.

TABLE I

Probands	With FJL	Without FJL
Males (11)	8 (% 72.7)	3 (% 27.3)
Females (37)	35 (% 94.6)	2 (% 5.4)
Total (48)	43 (% 89.6)	5 (% 10.4)

Khi Sq. Exact P: 0.203

TABLE II

THE RATIO OF FJL IDIOPATHIC SCOLIOSIS POSITIVE FAMILIES

Probands	With FJL	Without FJL
Males (32)	21 (% 65.6)	11 (% 34.4)
Females (30)	24 (% 80.0)	6 (% 20.0)
Total (62)	45 (% 72.6)	17 (% 27.4)

Khi Sq.: 0.938 P < 0.05

TABLE III

THE RATIO OF FJL IN IDIOPATHIC SCOLIOSIS NEGATIVE FAMILIES

Probands	With FJL	Without FJL
Males (56)	30 (% 53.6)	26 (% 46.4)
Females (50)	34 (% 68.0)	16 (% 32.0)
Total (106)	64 (% 60.4)	42 (% 39.6)

Khi Sq.: 1.724 P < 0.05

152 cases in the total 216 cases from a group (70.4 %) had the familial joint laxity (Table IV). The ratio in question was 10 % in controls (Table V). The concentration of both characters in the families of idiopathic scoliosis is shown in Table VI.

TABLE IV

THE PREVALANS OF IN IDIOPATHIC SCOLIOSIS FAMILIES POPULATION

Population	With FJL	Without FJL
Males (99)	59 (% 59.7)	40 (% 40.3)
Females (117)	93 (% 79.5)	24 (% 20.5)
Total (216)	152 (% 70.4)	64 (% 29.6)

Khi Sq.: 9.306 0.001 P < 0.005

TABLE V

THE PREVALANS OF FJL IN CONTROL GROUP

Population	With FJL	Without FJL
Males (234)	18 (% 7.7)	216 (% 92.3)
Females (209)	26 (% 12.4)	183 (% 87.6)
Total (443)	44 (% 10.0)	399 (% 90.0)

Khi Sq.: 2.27 P < 0.05

TABLE VI

THE PREVALANS OF BOTH IS AND FJL IN FAMILIES POPULATIONS OF IDIOPATHIC SCOLIOTIC PROBANDS

Groups	Population number	IS	Population Number	FJL
Probands	48	48 (% 100)	48	43 (% 89.6)
Ist. degree relatives	226	18 (% 7.9)	216	152 (% 70.8)
Ist. deg. rel. in IS positive familiese	58	18 (% 31)	62	45 (% 72.6)
Ist. deg. rel. in İS neg. familiese	168	—	106	64 (% 60.4)
Control Group	32 914	68 (% 0.2)	443	44 (% 10)

Discussion

The prevalance of the joint laxity in the families of idiopathic scoliosis is 7 times high than those of controls just as it was shown in Tables IV and V. This finding shows that, the joint laxity is a strikingly important factor in the formation of the idiopathic scoliosis. Besides, the familial joint laxity and the idiopathic scoliosis shows familial concentration just as they were given in Table VI. This finding also supports that both of the characters are hereditary. In addition, there is a strong correlation between them.

The joint laxity markedly transmitted than the idiopathic scoliosis because of the same ratio of the condition is observed in both families of the idiopathic scoliosis positive and negative.

The results presented here shows that the familial joint laxity plays important role in the formation of the idiopathic scoliosis. Indeed, Harrington and Enneking have noted that the primary event in the idiopathic scoliosis should be investigated in the extraosseous tissues.

As a result the study presented here supports the findings of Harrington and Enneking. In other words, the familial joint laxity plays an important role in the formation of the idiopathic scoliosis.

REFERENCES

1. Abalmasova, E., et al.: Familial forms of idiopathic scoliosis, Am. Dig. for Orthop. Lit. 2nd quarter 10, 1971.
2. Akkoyunlu, Ü.,: Genetic investigation in idiopathic Scoliosis-Theses Hacettepe University 1975.
3. Beighton, H. P., Horan, T. F.: Dominant inheritance in familial generalised articulair hypemobility. J. Bone and joint Surg., **52 B**: 145, 1970.
4. Carter, C.: Familial joint laxity and recurrent dislocation of the patella, J. B. and Joint Surg. **40 B**: 94, 1958.
5. Codorniu, A. H. R.: Idiopathic scoliosis of congenital origin, J. Bone and Joint Surg. 51 No. **6**: 1236, 1969.
6. Cowel, H. R., McEwen, G. D.: Familial patterns in idiopathic scoliosis, J. Bone and Joint Surg. 51 A No. **6**: 1236, 1969.
7. Cowel, H. R., et al.: Genetic aspects of idiopathic scoliosis, Clin. Orthop. and Relater Res. No. **86**: 125, 1972.
8. Esteve, R.: Idiopathic scoliosis in identical twins, J. Bone and Joint Surg. **40 B**: 97, 1958.
9. Feber, A.: Skoliose bei eineiign zwillingen, Der. Erbarts **2**: 102, 1935.
10. Faber, A.: Untursuchungen uber die erblichkeit der skoliose, Arch. Orthop. Unfalchir. **36**: 217, 1936.

11. Fisher, R. L., De George, F. V.: Twin study of idiopathic scoliosis, Clin. Orthop. and Related Res. No. 55: 117, 1967.
12. Filho, N. A., Thompson, M.: Genetic studies in idiopathic scoliosis, J. Bone and Joint Surg. 53 A No. 1: 199, 1971.
13. Frances V. De George, Robert Fisher: Idiopathic scoliosis: Genetic and Environmental Aspects, J. Med. Genetics., 4: 251, 1967.
14. Garland, H. G.: Hereditary scoliosis, British Med. J. 1: 328, 1934.
15. Haffner, J.: Eineiig zwillinge, Acta. Rad. 17: 529, 1936.
16. Hull, B. L.: Scoliosis in binovulair twins, J. Bone and Joint Surg. 43 B: 285, 1961.
17. Murdoch, G.: Scoliosis in twins, J. Bone and Joint Surg. 41 B: 736, 1959.
18. Rodney, K. B.: Homocystinuria, J. Bone and Joint Surg. 51 A: 1564, 1969.
19. Scott, T. E., Baily, R. W.: Idiopathic scoliosis in twins, J. Mich. Med. Soc. 62: 283, 1963.
20. Telkmann, B.: Idiopathicshe skoliose bei zwillingen, Z. Orthop. 86: 290, 1955.
21. Veliskakis, K.: Increased generalised Joint laxity in idiopathic scoliosis, J. Bone and joint Surg. 55 A: 435, 1973.
22. Weiser, M.: Spiegelbildichee skoliosen bei zwillingen, Z. Orthop. 76: 264, 1947.
23. Weist, E.: Etude clinique et EMG la musculature paravertebral de deux jumeaux univitelliens dont present une scoliose idiopathique, Chir. Orthop., 40: 138, 1954.
24. Wilkinson, J., Carter, C.: Persistent joint laxity and congenital dislocation of the hip, J. Bone and joint Surg. 46 B: 40, 1964.
25. Wynne-Davis, R.: Familial idiopathic scoliosis, J. Bone and Joint Surg. 50 B: 24, 1968.
26. Wynne-Davis, R., Riseborogh, E. G.: A genetic survey of idiopathic scoliosis, J. Bone and Joint Surg. 55 A: No. 5, 1974.
27. Wynne-Davis, R., james, J. I. P.: Scoliosis in elderly, J. Bone and Joint Surg. 51 A, No. 3: 446, 1969.
28. Wynne-Davis, R.: Family studies and the cause of congenital club foot, J. Bone and Joint Surg. 46 B: 445, 1964.
29. Wynne-Davis, R.: Acetabularie dyplasia and familial Joint laxity, tow etiological factors in CDH. J. Bone and Joint Surg. 52 B: 704, 1970.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 3-4 / July-October 1979 / pp 72 – 78

Current Concepts of Management of Carcinoma in Situ of the Cervix Uteri*

Ali Ayhan, M.D. / Eflatun Gökşin, M.D.*** / Kunter Yüce, M.D.******

Carcinoma in situ is allesion with cells of malignant morphology which have not invaded the underlying stroma although extention into glands is a common feature.[5] Patients with carcinoma in situ should have an almost 100 % 5-year survival. For only this reason, malignant changes in the epithelium of the cervix should be uncovered prior to invasion.

The wider use of cytologic screening and colposcopic examination have been disclosing an increasing number of patients with carcinoma in situ and a resultant improvement in the 5-year survival rates for carcinoma of the cervix.

It is advisable to report the results of large series of such cases to ascertain the best method of treating patient with carcinoma in situ.

Prompt initiation of therapy requires early diagnosis, the choice of treatment should depend upon the severity of the disease.[1]

It is the purpose of this paper to describe the diagnostic procedure, the type of treatment used and the findings on follow-up of our patients.

Material and Methods

The series comprises 26 cases of carcinoma in situ of cervix registered between 1964 and 1980, at the Department of Obstetrics and Gynecology of Hacettepe Medical School.

* From Department of Obstetrics and Gynaecology, Hacettepe University Ankara, Turkey.
** Associate Professor in the same Department.
*** Professor in the same Department.
**** Specialist Assistant in the same Department.

The cases were investigated according to the demographic peculiarities, the method of therapy and follow-up later.

Results

26 cases were diagnosed as carcinoma in situ of cervix at the Department of Obstetrics and Gynecology of Hacettepe Medical School between 1964 and 1980. These cases were 0.0112 % of all patients examined and were 13 % of 195 invasive forms of cervical carcinoma in the same period.

The mean age of these series was 41.42-1.36 and age distribution was shown in Table I.

TABLE I
AGE DISTRIBUTION OF PATIENTS

Age Groups	Number of Patients	%
34 and below	4	15.38
35-44	13	50.00
45 and above	9	34.62
Total	26	100.00

All patients were married and became pregnant once or more. Their gestational numbers were given in Table II.

TABLE II
GESTATIONAL NUMBERS OF PATIENTS

Number of Gestation	Number of Patients	%
4 and below	6	23.07
5 and above	20	76.93
Total	26	100.00

There is a relation between cervical carcinoma and the first coital age. The Turkish population is a traditionalist community, so the marital age may reflect the first sexual intercourse. The distribution of the marital age is seen in Table III.

Vaginal discharge in 32 %, menometrorrhagia in 20 % and contact bleeding in 7.7 % of all cases were found. (But there are no specific symptoms and signs in carcinoma in situ of the cervix. For this reason, diagnosis depends upon the cytologic screening, colposcopic examination and biopsy). The results of cytologic screening were shown in Table IV.

TABLE III

DISTRIBUTION OF MARITAL AGE

Marital age	Number of Patients	%
19 and below	15	57.69
20-24	8	30.76
25 and above	3	11.55
Total	26	100.00

TABLE IV

RESULTS OF CYTOLOGIC SCREENING

Cytologic Results	No of Patients	%
Negative (Class I-II)	2	7.70
Suspected (Class III)	5	19.23
Positive (Class IV-V)	19	73.07
Total	26	100.00

The biopsy of the cervix has been applied in all cases and their specimens were examined by Pathology Department. The results of these demonstrated carcinoma in situ. Cone biopsy was usually recommended for the investigation of cases with positive or suspicious cytology.

TABLE V

SURGICAL PROCEDURE USED

Surgical Procedures	Number of Patients	%
Radical Conisation	6	23.09
Simple Hysterectomy	9	34.61
Modified Radical Hysterectomy	11	42.30
Total	26	100.00

Conisation had been previously done in 16 out of the 19 cases treated by hysterectomy. The study of the uterus removed showed that 5 of 16 cases (31.2 %) had remaining foci of carcinoma in situ after the conisation.

Invasive carcinoma has been detected in 2 patients (7.7 %) during the follow-up. (Follow-up durations: All cases one year, 68 % of all cases three years 52 % of all cases five years and 32 % of all cases six or more years.) Invasion in one case was found on the vaginal cuff and the other in the cervix. These two patients have undergone the suitable treatment and they are in remission.

Discussion

The cervical carcinoma would occur in 2 % out of all women between 20 and 50 years old.[25, 28] The incidence of carcinoma in situ of the cervix varies from 0.03 % to 34.4 %. in the literature.[12, 15, 19, 22, 24, 28] This is 0.0112 % in our clinic.

The over all ratio carcinoma in situ to invasive form is between 1/0.1 and 1/9.1 in the studies,[2, 5, 18, 19, 27] This ratio has been found as 1/7.5 in our cases. Both incidence and ratio does not differ from other clinics.

The age distribution of the carcinoma in situ in the literature ranges from 31.8 to 42.6 years old.[10, 11, 12, 18, 25, 28] The youngest patient was 18 years old and the oldest one was 84 years old in these studies.[9, 10] The majority of our cases were between 35 and 44 years old. The same results has been found in Kolstad's series.[18]

The first sexual intercourse and related pregnancy is an important etiologic factor in cervical carcinoma. 94-95.1 % of all cases with carcinoma in situ in the large series were married and became pregnant once or more.[11, 29] Our patients were married and had pregnancy once or more (Table II and III).

As known, carcinoma in situ has no specific symptoms and signs. Some authors reported that more than one third of the patients had no symptoms.[3, 10, 18, 25, 29] There were no specific symptoms in our cases, but some of our patients had unrelated symptoms. Therefore, to make a diagnosis depended upon cytologic screening, colposcopic examination biopsy.[6, 13, 14, 18, 20, 30] Cytologic screening (from posterior fornix and cervix) was the most important diagnostic procedure in our study. False negative smear has been found in 7.7 % of all cases. This condition varies from 1.4 and 31.9 % in the literature.[3, 12, 15, 18, 21, 28]

The methods of treatment in carcinoma in situ used are as follows:

a) Radical conisation
b) Simple hysterectomy with 2 cm. vaginal cuff excision
c) Modified radical hysterectomy
d) Cryosurgery, cryocautery, electrocautery, electrodiathermy and CO_2 lasser.
e) Radiation (Radium and external X-ray)

The indications for last methods (such as cryosurgery) have been limited in the treatment of cervical in situ carcinoma because, the healing rate after cryosurgery varies from only 81 to 97 %.[9]

Radical conisation is an available treatment but there is no quarantee of cure. Recurrent rate ranges from 2.7 to 33.3 % in the literature after conisation.[1, 8, 11, 14, 16, 18, 24, 29] This event has been found as 7.7 % in our study.

The hysterectomy specimens following conisation reveal residual carcinoma in 12-60 % of cases in the large series. Residual disease was found in the cervix in 31.2 % of hysterectomy specimens after conisation in the presented study. Conisation as the treatment of carcinoma in situ is inadequate in many patients. For this reason, all cases treated by radical conisation must be followed up with regular cytology.

Carcinoma in situ spreads to the upper vagina in 3,4, 21 % of cases.[5, 11, 14, 29] Therefore, simple hysterectomy is also inadequate in the treatment of cervical carcinoma in situ. The vaginal extension, in one case has been found two years later after simple hysterectomy in our series.

Lymph node involvement has not been found in any patients in the literature.[2, 3, 11, 18, 29]

The best method of treatment is modified radical hysterectomy and extirpation of 1/3 upper vaginal cuff. The recurrence rate after MRH is 30 times less than after conisation and 3 times less than after simple hysterectomy.[11, 29] Lymph node dissection is unnecessary.

Summary

26 cases with carcinoma in situ were investigated. The incidence has been found as 0.0112 %. The mean age of these series was 41.42-1.36. There were no specific symptoms to make a diagnosis. Cytologic screening has been found as the most important diagnostic procedure. In 7.7 % of all cases false negative smear was seen. Residual tumor was seen in 31.2 of hysterectomy specimens after the conisations. Recurrence rate was 7.7 %. There was no mortality and important morbidity.

REFERENCES

1. Adelman H. C., Hadju S. I.: Role of conisation in the treatment of cervical carcinoma in situ. Am. J. Obstet Gynecol **98**: 173, 1967.
2. Beecham C. T., Beiler D. D.: Treatment of cervical carcinoma. Am. J. Obstet Gynecol **124**: 281, 1976.
3. Benson R. C. (Ed.): Current Obstetrics and Gynecologic treatment and diagnosis. 1 st ed. Canada, Lange, 1976, pp. 181.
4. Bertini B., Hornstein M.: The epidemiology of trichomoniasis and the role of this infection in the development of carcinoma of the cervix Acta Cytol (Baltimore) **14**: 325, 1970.

5. Boyes D. A., Worth A. J., Fidler H. K.: The results of treatment of 4389 cases preclinical cervical squamous carcinoma. J. Obstet. Gynecol. Br. Commonw. **77**: 769, 1970.

6. Burghardt E.: Premalignant conditions of the cervix. Clinics in Obstet Gynecol **3(2)**: 257, 1976.

7. Danforth D. N. (Ed.): Obstetrics and Gynecology 3 rd ed., Maryland, Harper and Row, 1977, pp. 910.

8. Davis R. M., Cooks J. K. and Kirk R. F.: Cervical conisation: an experience with 400 patients. Obstet Gynecol **40**: 23, 1972.

9. Einerth Y.: Cryosurgical treatment of dysplasia and carcinoma in situ of the cervix uteri. Acta Obstet Gynecol Scand **57**: 361, 1978.

10. Gusberg S. B., Marshall D.: Intraepithelial carcinoma of the cervix: A clinical reappraisal. Obstet Gynecol **19**: 713, 1962.

11. Hall S. E., Boyce J. G., Nelson J. N.: Carcinoma in situ of the cervix uteri Obstet Gynecol **34**: 221, 1969.

12. Hertig A. T., Young B. A.: A debate. What is cancer in situ of the cervix. Is it the preinvasive form of the carcinoma? Am. J. Obstet Gynecol **64**: 807, 1952.

13. Jaeger J.: Dysplazi ve karsinoma in situ'un teşhis ve tedavisi. Ankara Jinekoloji Derneği Semineri Bayrak Matb., 1969, Ankara.

14. Jordan J. A.: The diagnosis and management of premalignant conditions of the cervix. Clinics in Obstet Gynecol **3(2)**: 295, 1976.

15. Kaminetzky H. A., Swerdolow M.: Carcinoma in situ: Factors in its diagnosis. Obstet Gynecol **19**: 721, 1962.

16. Kaufman R. H.: Frozen section evaluation of the cervical conisation specimen. Clinical Obstet Gynecol **10(4)**: 838, 1967.

17. Kolinski J.: Correlation between trichomoniasis and cervix neoplasms in women. Gynecol Pol. **46**: 181, 1975, (Eng. Abst.).

18. Kolstad P.: Carcinoma of the cervix Am J Obstet Gynecol **96**: 1098, 1966.

19. Laskey P. W., Meigs J. W., Flannery J. T.: Uterine cervical carcinoma in Connecticut 1935-1973. Evidence for two classes of invasive disease. J Natl Cancer Inst **57**: 1037, 1976.

20. Mestwerdt: Serviks karsinomunun en erken devrelerinin kolposkopik, sitolojik ve histolojik teşhise göre tedavi imkanları Ankara Jinekoloji Derneği Sem. Bayrak Matbaası, 1969, Ankara.

21. Van Naegell J. R., Parker J. C., Hicks L. P., Conrad R.: Diagnostic and therapeutic efficacy of cervical conisation. Am J Obs. Gyn. **124**: 134, 1976.

22. Population Reports. Supp to series A Number 4, May 1977, USA.

23. Terrill B. S., Kantor H. I.: A correlated study of carcinoma in situ. South M. J. **57**: 883, 1964.

24. Topek N. H.: Surgical treatment of carcinoma in situ of the cervix. Clinical Obstet Gynecol **10**: 853, 1967.

25. Twombly G. H.: Tumors of the female generative tract. Cancer, diagnosis and treatment. (Ed. Field J. B.) 1 st ed. USA, Little Brown, 1959, pp. 355.

26. Vaclavinokva V., Hedman A. K., Nasiell K.: Follow-up studies in dysplasia and cancer in situ of the cervix uteri. Acta Obstet Gynecol Scand **57**: 69, 1978.

27. Wall J. A., Collins V. P., et al.: Carcinoma of the cervix. Am. J. Obstet Gynecol 96: 57, 1966.

28. Wall J. A., Kaufman R. H.: Carcinoma in situ of the cervix. South M. J. 57: 876, 1964.

29. Way S., Hennigan M., Wright V. C.: Some experiences with pre-invasive and micro invasive carcinoma of the cervix. J. Obstet Gynecol Brit. Cwlth 75: 593, 1968

30. Wespi H. J.: Karsinomatöz olmayan ve olan değişikliklerde kolposkopinin rolü. Ankara Jinekoloji Dernegi Sem. Bayrak Matbaası, 1969. Ankara.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 3-4 / July-October 1979 / pp 79 – 92

Free Serum Proline and Hydroxyproline Determination by ^{14}C Tracing in Acute Viral Hepatitis and its Clinical Significance*

Fikri Canoruç, M.D.**

Introduction

Collagen constitutes 25-33 % of the total body proteins. It is a protein found abundantly in human and animal tissues.[1-3] In contrast to his abundancy, data about its properties and metabolism has been obtained in the last 25-30 years.

After having been discovered that some fractions are syntetized and catabolized rapidly in Collagen, studies on Collagen were intensified and the majority of these studies were carried out on hydroxyproline that is considered as index of collagen metabolism.[4-8]

Studies, investigating the values free proline and hydroxproline in liver diseases and comparing them with the findings in normal are not numerous in literature.

The values of proline and hydroxyproline had been calculated directly until M. Rojkindh and E. Gonzales.[9] Introduced modern and sensitive methods by which the specific radioactivities of the values proline and hydroxyyroline marked by ^{14}C in collagens and non collagens are measured.

---

* These studies were performed in the Biochemistry Research Laboratory of Radiobiology Department of The Medical School of Hacettepe University Ankara Turkey.

** Anadolu University, School of Medicine, Division of Gastroenterology, Eskişehir, Turkey.

It is after this discovery mentioned above that, the values of proline and hydroxyproline could be calculated and their clinical significance be discussed.[9, 10] in some of the liver diseases.

Thinking that the determination of the values of serum free proline and hydroxyproline in acute viral hepatitis which is very important in the etiopathogenesis of postnecrotic cirrhosis and chronic hepatitis could be significant and helpful,

Our aims with regards to the points mentioned above are:

1. To determine the values of free serum proline and hydroxyproline during the icterus and posticterus stages in acute viral hepatitis using ^{14}C tracing method and compare them with those determined in the group.

2. To follow up the values of proline and hydroxproline in acute viral hepatitis in various stages and determine its clinical significance.

Material and Method

This study was performed on the control and patient groups indicated below. In all groups cases without any chronic and systemic disorders such as chiefly clinic and laboratory findings (biochemical, hematologic, radiologic) in addition to bone, kidneys, endocrine and collagen tissue disorders were subjected to our study.

I. Control Cases: Proline and hydroxyproline determinations were based on a total of 28 cases of the staff and practician doctors from the medical school of the University of Hacettepe, without any organic disorders and especially without icterus in their past history.

7 of the cases were women, 21 men with an average age 28 varying between 21 and 49. These cases subjected as control groups had no clinical and laboratory findings related with liver disease.

All liver function tests were normal.

II. The Cases With Acute Viral Hepatitis: 1975, 45 patients from the infectious clinics of Ankara Numune Hospital and Eskişehir Air Force Hospital were studied 14 of the patients were women, 31 were men. Their average age was 28 ranging between 16 and 55.

The descriptions were based on the historical review, physical examination and liver function tests.

The values of free serum proline hydroxyproline and simultaneous transaminase (SGOT, SGPT) in this group.

I. 45. patients in their jaundice term

II. 3-4 weeks after the first serum (45 patients)

III. The values of serum aminoacid (proline, hydroxyproline) were calculated during the period of 4-3 months subsequent to jaundice term of the patients (7 patients)

III. Cases With Chronic Hepatitis: There were 6 patients in this group with a histopathologic diagnosis of aggressive hepatitis. One of the patients who had aggressive hepatitis was a woman, the other five were men. The average age 35, ranging between 28-37.

IV. Cases With Cirrhosis: In 1975, the levels of serum free aminoasit (proline, hydroxyproline) of 15 patients with postnecrotic and 5 with alcoholic cirrhosis, in the internal clinic of Hacettepe University were measured. 5 of the cases of postnecrotic cirrhosis were women, one man. Their average age was 46, between 35-53.

Six of the case with postnecrotic cirrhosis and two with alcoholic cirrhosis had tissue diagnosis. In the other cases the diagnosis based on the case history, physical examinations, liver function tests, hematological, radiological, isotop studies and endoscopic findings.

The case histories of the cases with alcoholic cirrhosis had alcohol being used for 15 years; These with postnecrotic cirrhosis had jaundice history. In these cases, biopsy couldn't be carried out due to various contrindications (prothrombin time period, pancytopenia. jaundice).

V. The Group Who Had Infectious Hepatitis: The values of serum aminoacid (proline, hydroxyproline) of 10 doctors, with the diagnosis of definite acute viral hepatitis 3 years ago, treated in Gastroenterology department of Medical School of Hacettepe University, with no complaints now, were calculated. Two of these were women and eight men. Their average ago was 29 varying, between 25-34. The liver function tests were normal. (SGOT, SGPT, biluribin, alkalen phosphatase).

In all group, liver function tests and free serum proline and hydroxyproline values of the patients measured simultaneously.

The method by Rojkind and Gonzales[9, 10] was used to determine serum free aminoacids (proline, hydroxyproline). Studies were performed in the Biochemistry Research Laboratory of radiobiology department of the Medical School of Hacettepe University.

The Way Traced For The Test: Approximately 10 ml blood from every case contrifuged for 5 minutes with a frequency of 1500 and serum preserved in deep-freeze till the test time.

Serums Dissolved before the test. Tests were done duplicated.

The process carried on as follows:

Oxidation With Chloramine-T

2 ml of dissolved serum were put in pyrex tubes (18x200 mm). 0.1 mol DL. hydroxyproline-^{14}C were added to the serums. This was put into the serum through an automatic pipette of 0.1 ml. or Eppendorf pipette. Later on an amount of 1 ml chloramin-T solution, prepared recently, were added to the serums and preserved for 20 minutes at normal room temperature. The reaction ceased after adding of 5 ml of 2 M Sodium thiosulfate. The solution, stirred in vertex, was restirred after the addition of 1 ml I M sodium hydroxide. Later on the solution was saturated with 2.0 gm NaCl and Stirred in vortex with 6 ml toluene for about 30 seconds. Thus the examination of the oxidation products of proline is maintained.

To obtain two equal separations, samples were put into counting bottles, containing 2 ml toluene layer and 5 ml 3x-scintilation liquid, after being centrifuged for 5 minutes at a frequency of 2000. 1 ml of the remaining toluen was put in thermoduric test tubes through pipette for a ninhidrin analyse, the rest of the solution being extracted twice with 6 ml toluen, the oxidation products of proline were (discarded). Subsequently to convert the oxidation products of hydroxyproline into pyrrole, test tubes sealed with glass marbles inserted into over boiling water bath. Thirty minutes later tubes were cooled at normal room temparature. 2 ml of Pyrrole, obtained from hydroxyproline was reextracted with 6 ml toluene, was put into counting bottles. 1 ml of it into test tubes for the calorimetric calculation.

Calorimetric Calculation of Proline: The test tube containing 1.0 ml toluen phase was added 2 ml H_2O, 3 ml glacial acetic acid (CH_3COOH) and 3 ml ninhydrin solution and inserted into overboiling water sealed with glass balls 60 minutes later test tubes cooled under mad water and 4 ml toluene was added, after being stirred.

Some time was given to have a separation between organic and inorganic phases. When an complete seperation occured the color of the toluene phase was 515 nm.

For the colorimetric determination of hydroxyproline 1 ml of toluene layer containing pyrole was added Ehrlich reactive and then stirred in vortex mixer. Keeping it for 30 minutes at normal room tomperature for the color formation, 560 nm was read.

The radioactive calculation of the samples was carried on with Inc. Illinois, U.S.A. liquid Scintillation Spectrometer model 3380 Packard Instrument Company.

The colorimetric determination of the samples was carried on with Coleman Instrument A Division of the Perkin Elmer Corporation Illinois, U.S.A. Model 6/20.

The student's "t" test was used for the importance of the difference between averages in statistical calculations.[11]

Results (Table I, Figure 1)

I. The values of serum free proline and hydroxyproline obtained in control cases.

a. The average value of serum proline was 0.045 ± 0.0054 (± SE) μ mol/ml

b. The average value of serum hydroxyproline was 0.0054 0.0005 μ mol/ml

II. Patients with acute viral hepatitis

A. In the serums of jaundice period:

a. The average free proline valve was 0.134 ± 0.004 μ mol/ml and the difference due to the control group was statistically significant ($P < 0.001$).

b. The average value of serum free hydroxyproline was obtained as 0.014 ± 0.0006 μ mol/ml. The difference due to the control group was statistically very significant ($P < 0.001$).

B. In the serums of convalescence period, 3-4 *weeks subsequent to the jaundice period*

a. The average serum free proline value was 0.113 ± 0.004 μ mol/ml. and the difference from the control group was considerably important ($P < 0.001$).
The difference had statistical importance in comparing with the values of jaundice period ($P < 0.01$).

b. The average value of serum hydroxyproline was 0.012 ± 0.0005 μ mol/ml. The difference regarding with the control group had statistical significance ($P < 0.001$). On being compared with the values of jaundice period the difference seemed statistically important ($P < 0.01$).

TABLE I

COMPARISON OF ALL GROUPS ACCORDING TO IMPORTANCE

Groups		Number of cases	Proline: Average (±S, E) (μ mol/ml.)	Proline: Significance	Hydroxyproline: Average (±S, E) (μ mol/ml.)	Hydroxyproline: Significance
Controls		28	0.045 ± 0.005	—	0.054 ± 0.0005	—
Viral Hepatitis	I	45	0.134 ± 0.004	P < 0.001*	0.014 ± 0.0006	P < 0.001*
	II	45	0.113 ± 0.004	P < 0.001* P < 0.01**	0.012 ± 0.0005	P < 0.001* P < 0.01**
	III	7	0.069 ± 0.01	P < 0.001** P > 0. 05*	0.065 ± 0.0006	P > 0.05* P < 0.01**
Positive History of Viral Hepatitis		10	0.045 ± 0.005	P > 0.05*	0.0049± 0.0002	P > 0.05*
Chronic Hepatitis		6	0.152 ± 0.021	P < 0.001*	0.014 ± 0.002	P < 0.001*
Postnecrotic Cirrhosis		15	0.051 ± 0.005	P > 0.05*	0.0049± 0.0006	P > 0.05*
Alcoholic Cirrhosis		5	0.180 ± 0.02	P < 0.001*	0.017 ± 0.002	P < 0.001*

* Significance against to control group
** Significance against to first stage of viral hepatitis

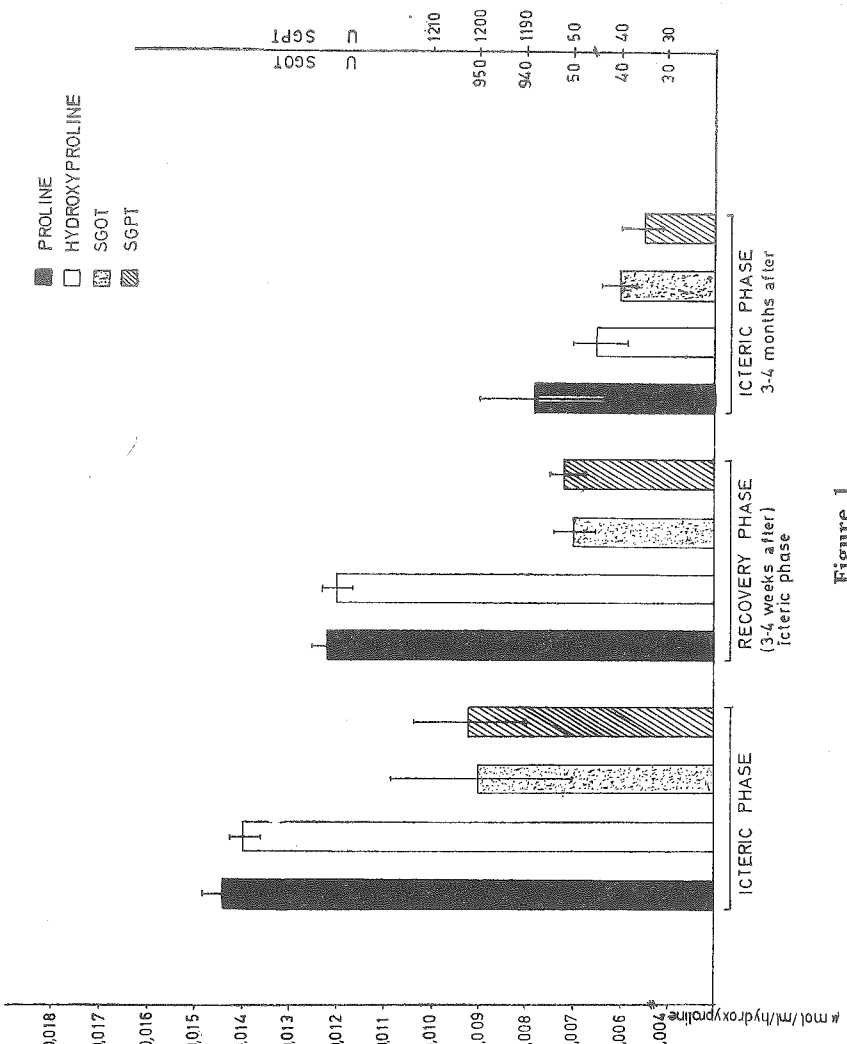

Figure 1
The relationship of proline, hydroxyproline and serum transaminase value during acute viral hepatitis.

C. In the seven cases treated for longer periods:

a. The average value of proline was 0.069 ± 0.01 μ mol/ml. The difference regarding with the control group was not statistically significant ($P > 0.05$).

b. The average value of hydroxyproline was 0.0065 ± 0.0006 (± SE) μ mol/ml. Compared with the control cases the difference was not significant statistically ($P > 0.05$).

III. Cases with Cronic Hepatitis

a. The average value of serum free proline was 0.152 ± 0.021 μ mol/ml. and the difference regarding with the control group was very significant statistically ($P < 0.001$).

b. The average value of serum free hydroxyproline was 0.014 ± 0.002 μ mol/ml. and the difference regarding with the control cases was quite significant ($P < 0.001$).

IV. Cases with Cirrhosis

A. Cases of Postnecrotic Cirrhosis:

a. The average value of serum free proline was 0.051 ± 0.005 μ mol/ml. The difference relating with the control group was not significant statistically ($P > 0.05$).

b. The average value of serum free hydroxyproline was 0.0049 ± 0.0006 μ mol/ml. The difference relating with the control cases was insignificant statistically ($P > 0.05$).

B. Cases of Alcoholic Cirrhosis:

a. The average value of serum free proline was 0.180 ± 0.02 μ mol/ml. The diffrence relating with the control group was very significant statistically ($P < 0.001$).

b. The average value of serum hydroxyproline was 0.017 ± 0.002 μ mol/ml. The difference hydroxyproline in the control group was very significant ($P < 0.001$).

V. Cases associated with previous jaundice (1-3 years).

a. The average value of serum proline was 0.045 ± 0.005 μ mol/ml. The difference relating with the control group was not statistically significant ($P > 0.05$).

b. The average value of serum hydroxyproline was 0.0049 ∓ 0.0002 μ mol/ml. The difference between control cases was statistically insignificant ($P > 0.05$).

Liver function tests on the patients with acute viral hepatitis:

A proper correlation and parallelism observed between the values of serum proline and hydroxyproline and transaminases (Figure 1).

a. Both the levels of proline and hydroxyproline and the liver function tests were high during the jaundice period of acute viral hepatitis.

b. During the period of consalescence a considerable decrease was observed both in the levels of proline and hydroxyproline and SGOT, SGPT and bilurubin compared with the levels of jaundice period.

Discussion

In our research a very close correlation was found between the values of serum free hydroxyproline obtained in the control group and the ones in[10] control groups obtained in a research carried out by a method explained in literature. While the average value of hydroxyproline in our research was 0.0054 ± 0.0005 ($\pm$ SE) μ mol/ml. it was 0.0067 ± 0.006 μ mol/ml. in the research mentioned. As seen, there is a close relation between the values. There was not such a correlation between the average proline values. While in our research the average proline values were 0.045 ± 0.005 μ mol/ml., in the above mentioned research[10] the values were 0.158 ± 0.005 μ mol/ml. Since there is no other research using the sensitive ^{14}C tracing method in both researches. It is difficult to explain the difference in proline values. And it was decided that research carried out with this sensitive and new method will enlighten the case.

In our research, the values of serum proline and hydroxyproline during the jaundice period of acute viral hepatitis showed a considerable increase compared to the values in the control group (Table I, Figure 1)

During the convalescense the values of serum free proline and hydroxyproline were also higher than the control group ($P < 0.001$).

However, a considerable decrease was found compared to the values of the jaundice period.

In the cases followed up longer periods ($P > 0.05$), it was observed that the values of serum free proline and hydroxyproline were reduced as low as the control level only 3-4 months after the jaundice period.

These findings proved that the collagen metabolism varied in acute viral hepatitis.

It was thought that the resultant findings of our research and the acute liver cell corruption would be a dominant cause for the increasing values of serum proline and hydroxyproline indicating the alteration in collagen metabolism in acute viral hepatitis.

1. In acute viral hepatitis the values of serum proline and hydroxyproline showed a parellelism with the clinical recovery state of the disease liver funciton tests and especially with transaminases (SGOT, SGPT). The values of proline and hydroxyproline were observed to fall down gradually to the normal values together with the clinical recovery and normalizing of transaminase values (Figure 1).

2. In acute viral hepatitis the values of serum proline and hydroxyproline showed a considerable increase in accordance with the activity of disease while they were normal in our 15 cases of postnecrotic cirrhosis group ($P > 0.05$).

With The Findings found in Our Research:

a. Since the levels of amino acid in serum increase due to liver damages.[12]

b. And the values of serum proline and hydroxyproline increased in a considerable amount in acute alcoholic hepatitis as in acute viral hepatitis causing acute liver cell damages.[10]

We ended up with the conclusion that the cause that increases the values of serum proline and hydroxyproline ought to be the acute liver cell damages.

Besides, liver cell damage in an important factor to increase the values of serum proline and hydroxyproline in cases of acute alcoholic hepatitis, in which alcohol seems to be a dominant factor.

Alcohol actually accelerates the collagen metabolism by increasing the activity of protocollagen proline hydroxylase enzyme.[13, 14]

With the recent researches, some factors were discovered to accelerate the collagen metabolism like alcohol in acute liver cell damages.[15] It was reported that three factors that stimulate the collagen from the damaged liver cell were released. The first two of these factors were found to stimulate protocollagen prelin oxidizing enzyme (factor 1-2) and also. Collagen biosynthesis by affecting fibroblasts directly.[15]

In another research, it was shown that, in acute viral hepatitis, the activity of protocollagen proline oxidase enzym might increase.[16]

With the date mentioned above, in acute viral hepatitis, these factors presumably accelerate the collagen metabolism by being secreted from the damaged liver cell. At the end, soluble collagen which is the main source of hydroxyproline, is formed. Thus the increase in the soluable collagen causes the increase of hydroxyproline in serum.[7, 8, 17]

In our cases the amounts of proline and hydroxyproline in the liver tissue could not be calculated. In our cases of postnecrotic cirrhosis, however, the serum values were normal ($P > 0.05$). With our literature knowledge, it was discovered that, in cases of cirrhosis, the values of serum amino acids were normal while collagen increased in liver.[18, 19, 20] This and the previous date made us think that the liver cell damages, rather than the amount of collagen, take an important part in increasing the values of serum proline and hydroxyproline. As a result of the higher dose of release of the previously mentioned collagen-stimulating factors in acute liver damages such as in acute viral hepatitis with regards to post necrotic cirrhosis, the difference between the two disease has arised as far as serum proline and hydroxyproline values are concerned.

In the previous chapter, with the date we have, it was found out that the values of serum free proline and hydroxyproline, compared with normal values, has increased a considerable amount in acute viral hepatitis. A difference was observed between the serum values due to the activation of the disease. Besides this, it was believed that the variation in these values would reflect the variations occurred in liver functions and collagen metabolism.

In our research besides the results obtained there is another finding that must be taken into consideration. This is the very high values of serum proline and hydroxyproline in chronic aggressive hepatitis caused by acute viral hepatitis.[21, 26] ($P < 0.001$).

In our research there is no other patient group examined regarding the period from the diagnosis of acute viral hepatitis to the development of chronic agressive hepatitis. However, when the findings obtained in our studies are taken into consideration, the calculation of the values of serum proline and hydroxyproline in acute viral hepatitis and pursuing these values at regular intervals, would be a significant test, in the prognosis of the disease, especially in indicating a tendency to the chronic aggressive hepatitis. That is:

1. The values of proline and hydroxyproline, reaching the highest level during the jaundice term of acute viral hepatitis, decrease as recovery progresses and become normal during the third and fourth months.

2. The values of proline and hydroxyproline in the presently healthy group, and in the one who had jaundice previously are normal.

3. Because of the considerable increase of serum proline and hydroxyproline in chronic aggressive hepatitis, the measuring of the values of serum proline and hydroxyproline, in acute viral hepatitis, at regular intervals, may lead us to be informed of the upcoming chronic aggressive hepatitis. The increasing of the values of serum proline and hydroxyproline is likely to happen because of the continuation of the previously mentioned collagen stimulating factors[15] And this may be worthy in showing the collagen synthesis activity which is an indication of the continuing liver cell damage. Finally, summerizing all what we have ended up with:

The measuring of the values of serum proline and hydroxyproline in acute viral hepatitis and following them up at regular intervals could be a test or an index indicating the prognosis of the disease especially the cause of chronic aggressive hepatitis.

Summary

^{14}C Tracing which is a new and sensitive method, was utilized for determination of free serum proline and hydroxyproline levels in patients:

I. During icterus in 45 patients with acut viral hepatitis.
II. 3-4 weeks after icterus (post icteric period).
III. Serums taken (3-4 months after) in 7 cases of Jaundice that could be traced for long time and 28 normal controls.

And the values of serum free proline, hydroxyproline were determined in six chronic agressive hepatitis, 15 postnecrotic 5 alcoholic cirrhosis and 10 cases that had jaundice (1-3 years ago). Results were as follows:

1. Serum free proline and hydroxyproline levels were found to be significantly elevated in patients with acute viral hepatitis, during icteric and post icteric recovery period (3-4 week after icterus).

2. Serum proline and hydroxyproline levels were significantly lower in patients during post icteric recovery period, than those with icterus.

3. In patients whom we were able to follow-up for 3 to 4 months, levels were found to be within normal limits, and the difference from normal controls were not significant.

4. Normal levels were found in patients with a past history (1-3 years) of jaundice ($P > 0.05$).

5. In chronic agressive group, very high levels of proline and hydroxyproline were found.

A proper correlation and parallelism observed between the values of serum proline, hydroxyproline and transaminases (SGOT, SGPT) and bilurubin.

It was concluded that proline and hydroxyproline levels are reflection of the degree of paranchymal tissue damage of the liver and is parallel to other liver function tests.

Periodic determination of serum proline and hydroxyproline levels were found to be valuable both in showing the prognosis of acute viral hepatitis, and the shift of this disease into chronic aggressive hepatitis.

REFERENCES

1. Traub, W, Piez, K. A.: The chemistry and structure of collagen. Adv. Protein chem **25**: 243, 1971.
2. White, A., Handler, P., Smith E. L.: Connective Tissue in Principles of Biochemistry. 4rd edition Kogakusha Company Ltd., Tokyo 1968, p. 871.
3. Haschmeyer, R. H., Haschmyeer A. E. V.: Fibrous Proteins in Proteins. A guide to study by physical and chemical methods. A Wiley-interseience publication John Wiley and sons. New York. 1973, p. 410.
4. Woesner, J. F. Jr.: The determination of hydroxyproline in tissue and protein samples containing small proportions of this aminoacid. Arch Biochem. Biophys **93**: 440, 1961.
5. Grant, M. E., Prockop D. J.: The biosynthosis of collagen. New England J. Med. **286**: 194, 242, 1971.
6. Bornstein, P.: Disorders of connective tissues. In Duncan's Diseases of Metabolism Genaus and Metabolism Edited by P. K. Bondy 6th ed. W. B. Saunders Company Philadelphia, 1969, p. 654.
7. Sjoerdsma, A., Udenfriend, S., Kesier, H., et al.: Hydroxyproline and collagen metabolism. Clinical implications. Combined clinical staff conferance at the National Institues of Health. Ann. Intern. Med. **63**: 672, 1965.
8. Kivirikha, K. I.: Urinery excretion of hydroxyproline in health and disease. Int. Rev. Connect Tissue. Res. **5**: 93, 1970.
9. Rojkind, M., Gonzalez, E.: An improved method for determining specific radioactivities of of proline ^{14}C and hydroxyproline- ^{14}C in collagen an in noncollagenous proteins. Anal biochem **57**: 1, 1974.
10. Mata, J. M., Kershenobich, D., Villareal, M. D. et al.: Serum free proline and free hydroxyproline in patients with chronic liver disease. Gastroenterology **68**: 1265, 1975.
11. Snedecor, G. W.: Statistical Methods five edition. Ames, Iowa State University Press, 1959, p. 239.
12. Iber, F. L., Rosen, H., Levenson, S. M., et al.: The plasma aminoacids in patients with liver faillure. J. Lab. Clin. Med. **50**: 417, 1957.

13. Walker, F., Shand, J.: Influence of alcohol on collagen synthesis in vitro. Launt 29: 233, 1972.

14. Feinman, L., Lieber, C.S.: Stimulation of hepatic collagen formation by ethanol consumption. Am. Soc. Clin. Invest. p. 28 a, 1971 (Abstract).

15. McGee, J. O. D., Patrick, R. S.: Stimulation of the collagen biosynthetic Pathway by factors isolated from experimental ly-injured liver. Nature New Biology 243: 121, 1973.

16. Stein, H. D., Keiser, H. R., S Joerdsma, A.: Proline-hydroxyproline activity in human blood. Lancet 1: 106, 1970.

17. Prockop, D. J., Kivirikko, K. I.: Relationship of hydroxyproline excretion in urine to collagen metabolism. Annals of Internal Medicine 66: 1243, 1967.

18. Huberman, A., Recio, A., Rojkind, M.: Collagen biosynthesis in normal and collagen biosynthesis in normal and cirrhotic art liver slices. Proc. Soc. Exp. Biol. Med. 131: 200, 1969.

19. Rojkind, M.. Diaz de Leon, L.: Collagen biosynthesis in cirrhotic rat Liver slices Biochim Biophys Acta. 217: 512, 1970.

20. Kershenobich, D., Fierro, F. J., Rojkind, M.: The relationship between the free pool of proline and collagen content in human liver cir.hosis. J. Clin Invest 94: 2246, 1970.

21. Sherlock, S.: Posthopatitis cirrhosis. Lancet 1(6509): 817, 1948.

22. Schaeter, J. W., Schiff, L., Gall, E. A., et al.: Progeession of acute hepatitis to postnecrhosis cirrhosis. Amer. J. Med. 42: 348, 1967.

23. Boyer, J. L., Klafskin, G.: Patera of necrosis in acute viral hepatitis. New. England J. Med. 283: 1063, 1967.

24. Klatsik, G.: Subacut hepatitis necrosis due to anicteric infections with the hepatitis virus. Amer. J. Med. 25: 333, 1958.

25. Redekar, A. G.: Chronic hepatitis. The medical clinics of North America 59: 863, 1975.

26. Sherlock, S.: Virus Hepatitis in Diseas of the Liver and Biliary System. 4rd edition. Revriod third printing. Blackwell scientific Publ. Oxford and Edinburgh, Great Britain 1971, p. 317.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 3-4 / July-October 1979 / pp 93 – 95

The Incidence of Idiopathic Scoliosis in Turkey

(A Study of 45 000 Minifilms of the Chest Made during A Survey for Tuberculosis)

Ümit Akkoyunlu, M.D.*

Between 1965-1969 45,000 chest roentgenograms were taken in the state of Ankara during a survey for pulmonary Tbc. by a research center of the ministry of health and social welfare in Turkey. All of these roentgenograms were taken by mobile X-Ray units on 40-50 exposure roles of 70 millimeter X-Ray films. The author examined 45,000 minifilms, in 68 of which he found evidence of idiopathic scoliosis (24 male and 44 female).

In recording the amount of curvature Cobb technique was used to measure the degrees, No curvature in the angle was less than 10 degrees was recorded.

The author recorded that only 32,914 of 45,000 minifilms were properly determined because that many errors may have resulted from basing a study of soft tissue.

In these minifilms (32,914), the cervical and dorso-lomber vertebraes were examined in wich the curvatures due to congenital anomalies and infection of the bone were excluded.

The incidence of the idiopathic scoliosis found to be 2 per 1 000 or 0.2 %. In the analysis of the data according to sex it was found that 44 of the subject were females and 24 were males.

Discussion

In the review of the literature, the author found little information on scoliosis statistics in English and American literature and no information on idiopathic scoliosis statistics given in Turkey. In 1947, Dewar of

* Associate Professor in Orthopaedics and Traumatology Divison, Hacettepe University, Ankara, Turkey.

Toronto reviewed 20,000 minifilms made during a survey for tuberculosis in the Province of Ontario. His study was never completed because as he states, "It was not dependable" He found an incidence of scoliosis of approximately 4 percent., an incidence which he felt was too much high and which is slightly more than double the incidence found in the other series.

In some textbooks there are a few references on scoliosis statistics. In 1929, Jones and Lovett state, "Figures with regard to the frequency with which scoliosis is found in the population as a whole are lacking, except for some figures brought forward by Shanz." In 1910, Shanz stated that of 189,000 army recruits examined in Germany over a five-year period, 7.2 per 1000, or less than 1 percent, were disqualified for spinal curves of all kinds. Selective service figures show that of 2,096,551 registrants examined in the United states from July 1,1950 through December 31,1951 2.4 per thousand were disqualified for curvatures of the spine. This is one third of the German rate, a variation which is undoubtedly due to the difference between the disqualifying factors for military service in the United States today and those for service in the old German army. In 1955 Shands and Eisberg stated that the rate of scoliosis (all types) per 1 000 was nineteen, but only five per 1000 had the moderate and severe curves which might be considered disqualifying for military service. This rate represents the mean figure between the German and Selective Service rates.

Whitman states that Drachmann found an incidence of 1.3 percent among over 28.000 school children in Denmark. Whitman further states that of 3,252 persons with scoliosis 21.5 percent were males and 78.5 percent were females.

Mc Murray, writing on scoliosis says that: In young children the deformity is found to occur in the same proportions in male and female, but later, when adolesence is reached, there is a persistent increase in the percentage of females affected as compared with males, to such an extent that in children requiring treatment the proportions are five females to one male. Our average proportion of 1.8 females to one male, and Shands three females to one male is definitely lower than the proportion of McMurray's.

In Shands and Eisberg survey they stated that, the etiology of the 230 curvatures that appeared in the first 15,000 minifilms examined were as follows: approximately two thirds, the postural of functional type; one fourth, idiopathic; and remaining 10 percent, almost equally divided among congenital, paralytic and post-thoracoplasty.

General population incidence idiopathic was studied by Wynne-Davies in Edinburg, Scotland. Her survey included children from two weeks to 18 years of age. The results are shown in table I the total incidence of late onset of scoliosis was 1.8 percent. Our results as is seen in Table I is approximately the same results found by Wynne-Davies.

TABLE I

INCIDENCE OF IDIOPATHIC SCOLIOSIS

Number of Population	Population Incidence		
	Male	Female	Total
32,914	0.2	1.3	1.8
7,894	0.3	3.9	1.8

It is believed that the population of Ankara is a mixture of Urban and rural, and this population is a typical of the population found along most of Turkey. Therefore, these statistics present as accurate a picture as can be obtained of the incidence of idiopathic scoliosis in any general population of Turkey.

REFERENCES

1. Drachmann: Cited in Whitman.[7]
2. Jones, Robert, and Lovett, R. W.: Orthopaedic Surgery. Ed. 2, London, Oxford University press, 1929, p. 718.
3. McMurray, T. P.: A Practice of Orthopaedic Surgery, Baltimore William Wood and Co., 1937, p. 215.
4. Schanz, A.: The Journal of Bone and Joint Surgery, Vol. 37 A, No. 6 1955.
5. Steindler, A.: Diseas and Deformities of the spine and Thorax St. Louis, C. V. Mosby Co., 1929, p. 168.
6. Tachdian, M.: Pediatric Orthopedacs. W. B. Saunders Co. London 1972.
7. Whitman, Royal: A Treatise on Orthopaedic Surgery. Ed. 6, Philadelphia, Lea and Febiger. 1919, p. 145.
8. Wynne-Davies: J. Bone and Joint Surgery, **50-B**: 24, 1968.

Hacettepe Bulletin of Medicine/Surgery
Volume 12 / Numbers 3-4 / July-October 1979 / pp 96 – 102

The Problem of Urethral Catheterization After Vaginal Hysterectomy Plus Kelly-Kennedy Plication*

Ali Ayhan, M.D. / Kunter Yüce, M.D.*** / Mithat Erdoğan, M.D.******

Introduction

Pelvic relaxation refers to diminished anatomic support of the uterus, bladder, urethra, vagina, rectum and perineum. This condition includes descensus uteri (I°, II° and III°), urethro-cysto-recto and enterocelle. There is little doubt that the chief etiologic factor is genetic and that the most important precipitating factor is child-bearing, including the pressure of the fetus during the last half of pregnancy, as well as, the trauma of labor and delivery. The most important symptom of the pelvic relaxation specially in conjuction with urethro and cystocelle is real stress incontinence. The treatment of this condition is surgical. There are many vaginal and abdominal techniques in this surgical procedure. But some of them are not used because they have some infavourable sequelae and poor long-term results. Recently, we have observed the increasing use of the vaginal hysterectomy and anterior-posterior repair Kelly-Kennedy plication as the major method of surgical treatment of symptomatic pelvic relaxation. In this surgical techniques, the most important problem is the duration of urethral catheterization. Because if the catheter is left in place for a long time the possibility of infection increases, and if it is removed early the spontaneous urine may be late

* From Department of Obstetrics and Gynaecology, Hacettepe University, Ankara, Turkey.

** Associate Professor in the same Department.

*** Specialist Assitant in the same Department.

**** Professor in the same Department.

from various factors (edema, echymosis in proximal urethra and vesical neck), in addition the residue urine volume may increased.

In order to determine the appropriate duration of urethral catheterization, its infection possibility and residue urine volume, this study was planned and performed.

Materials and Methods

This study was performed at the Department of Obstetrics and Gynecology of Hacettepe Medical School, from 1975-1980. Seventy-one cases underwent vaginal hysterectomy and anterior-posterior repair plus Kelly-Kennedy plication were studied. Five of them have underwent Manchester-Fothergille operation with Kelly-Kennedy plication. The data were obtained prospectively.

Their ages ranged from 25 to 72 years. The mean age was 47.6 years. The mean parity was found as 4.9 and theirs varied from 2 to 14.

All of them had pelvic relaxation and real stress incontinence. The real stress incontinence was demonstrated by Marshall-De Bruin or Bonney tests.[2, 10, 12]

After the cleaning of vulva with dilue benzalchonium chloride, the urine for culture was taken by mid-stream technique from all patients 24 hours before the operation.

All procedures were performed by the same persons and in the same operation room. The techniques and instruments used were the same.

At the end of surgical procedure, a sterile disposable indwelling Foley catheter was inserted. After that the catheter was connected by means of sterile rubber tubing to a clean open drainage collection bottle placed on the floor under the patient's bed. Neither antibiotics nor sulphonamide were used in any of the cases during this study.

All cases were divided in two groups:

Group one: 39 patients were in the first group. Their catheters were left in for 24 hours and removed directly. The urine for culture was taken at this moment.

Group two: 32 patients were in the second group. Their catheters were left in for 48 hours and removed after the exercise of bladder (after 48 hours the exercise of bladder was started and continued for 24 hours, by catheter was clamped for two hours and then opened for 15 minutes

during 16 hours. After that it was drained for 8 hours and removed). The urine for culture was taken in the same manner.

After removing of the catheters, spontaneous mixtion was waited, both spontaneous and residue urine measured, if residue urine was more than 100 cc., the catheter was reinserted.

Results

Only 4 cases (5.6 %) have preoperatively a positive urine culture. There was a significant difference between preoperative and postoperative urine cultures in both the first and the second groups. (Table I and II).

TABLE I

THE RESULTS OF PRE AND POSTOPERATİVE URINE CULTURES IN THE FIRST GROUP

		Postoperative cultures		
	Results	(+)	(−)	Total
Preoperative	(+)	1	2	3 (7.6 %)
Cultures	(−)	11	25	36
	Total	12 (30.7 %)	27	39
		X^2: 6.23	P < 0.05	

TABLE II

THE RESULTS OF PRE AND POSTOPERATIVE URINE CULTURES IN THE SECOND GROUP

		Postoperative cultures		
	Results	(+)	(−)	Total
Preoperative	(+)	1	—	1 (3.1 %)
Cultures	(−)	20	11	31
	Total	21 (65.6 %)	11	32
		X^2: 20	P < 0.05	

These results have suggested that urethral catheterization provokes urinary infection. A positive correlation was found between the duration of catheterization and infection (Table III).

TABLE III

DURATION OF CATHETERIZATION AND INFECTION

Duration of catheterization	Postoperative cultures (+) No	%	(−) No	%	Total No	%
24 hours	12	36.4	27	71.1	39	54.9
72 hours	21	63.6	11	28.9	32	45.1
Total	33	100.0	38	100.0	71	100.0

X^2: 8.54 $P < 0.05$

The mean residue urine volume was 52.5 ± 48.5 in the first group and 41.2 ∓ 33.2 cc. in the second group. The statistical difference couldn't be found between the first and the second group (Table IV).

TABLE IV

THE MEAN RESIDUE URINE VOLUMES

Volume	1st group	2nd group
mean	52.53 cc	41.25 cc
S.D	48.56 cc	33.26 cc
	t: 1.15	P > 0.05

All pathogens grown in the preoperative cultures were E. Coli. Pathogens grown in the postoperative cultures were shown in Table V.

TABLE V

PATHOGENS IN THE POSTOPERATIVE CULTURES

Pathogens	1st group No	%	2nd group No	%	Total No	%
E. Coli	8	66.7	16	76.2	24	72.7
Staph. coag. (+)	2	16.7	1	4.8	3	3.1
Enterococ. and aero.	2	16.6	—	—	2	6.1
Pseudomo. and pyoce.	—	—	4	19.0	4	12.1
Total	12	100.0	21	100.0	33	100.0

There was no statistical correlation among the age, parity and infection (Table VI and VII).

TABLE VI

CORRELATION OF AGE AND INFECTION

Age groups	Postoperative cultures (+) No	%	(−) No	%
40 and less	7	21.2	13	34.2
41 and more	26	78.8	25	65.8
Total	33	100.0	38	100.0

X^2: 1.474 P > 0.05

TABLE VII

CORRELATION OF PARITY AND INFECTION

Number of delivery	Postoperative cultures (+) No	%	(−) No	%
4 and less	18	54.5	21	55.3
5 and more	15	45.5	17	44.7
Total	33	100.0	38	100.0

X^2: 0.0036 P > 0.05

Discussion

The treatment of pelvic relaxation in connection with real stress incontinence is surgical. Although a lot of operative tecniques have been described, we prefer vaginal hysterectomy plus Kelly-Kennedy plication in these cases as authors.[2, 4, 6, 7, 10, 11, 12] In these procedures, duration of the catheter inserted in the bladder is the most important problem about infection and atony of the bladder. There is the same problem in the other clinics. For this reason, we will hope this study to be useful to many surgeons.

In this study, the incidence of bacteriuria was found 5.6 % prior to the catheterization. This figure varies from 10 % to 21 % in the literature.[8, 9, 12] There is no direct correlation between these results and the duration of bladder catheterization.

Although several workers contend that indwelling Foley catheterization with or without systemic chemoprophylaxis is a relatively innocuous procedure when sterile technique is observed, increasing number of

investigators have reported high rates of infection of the urinary tract after indwelling catheterization in medical, surgical, urologic, gynecologic and other patients. Infection mostly produces only local inflammation with no serious consequences, and eventually, if all goes well, it dies out some months after the patient has left hospital. Occasionally it may lead to serious and sometimes fatal results by way of secondary hemorrhagia, pyelonephritis and septicemia.[5, 8, 9] Bacteria points out 40-98 % following pelvic operations and the use of the indwelling catheter postoperatively.[1, 3, 6, 8, 9, 12] The results of this study showed that urinary infection chance increases with duration of the catheterization (Table II and VI).

In the present study, the most important pathogen was E. Coli (72.7 %). This figure is in accordance with literature.[1, 3, 5, 8, 9]

The operative trauma from plication of pubovesicocervical fascia causes edema of the urethral tube, especially at the urethrovesical junçtion, thus contributing to obsruction for spontaneous voiding to occur. It is necessary to a wait the return of parasympathetic function. For that, an indwelling Foley catheter is necessary for a time. Some surgeons point out this time 3-5 days.[12] But this study showed that, a time like that was unnecessary. There was no difference about urinary retention volume between 24 hours or more catheterization (Table IV).

As a result, a long term urethral catheterization is not necessary after the vaginal hysterectomy and anterior-posteriorrepair plus Kelly-Kennedy plication. 24 hours is sufficient for prevention of atony of bladder. In addition, extension of duration of catheterization increases the infection rate.

Summary

A study, in order to determine the most appropriate duration of urethral catheterization, after the vaginal hysterectomy and anterior posterior repair plus Kelly-Kennedy plication was presented. The incidence of bacteriuria prior to catheterization was 5.6 %. This raised to 30.7 % in the patients who have catheterized for 24 hours, and to 65.6 % for 72 hours. Most important pathogens was found as E. Coli. There was no difference about residue urine volume between 24 hours or more catheterization. Unnecessity of extension of the duration of catheterization and danger about infection were stressed.

REFERENCES

1. Akalın E.: Thesis for specialty in internal medicine. Hacettepe Medical Faculty. Department of Internal Medicine. Ankara. 1975.

2. Baden W. F., Walker T. A.: Physical diagnosis in the evaluation of vaginal relaxation. Clinical Obstet-Gynecol. **15**: 1055, 1972.
3. Cücük M.: Factors acting upon bacteriuria after the transurethral instrumentation. Thesis for specialty in urology. Hacettepe Medical Faculty Department of Urology. Ankara. 1980.
4. Kelly H. A.: Incontinence of urine in women. Urol. Cutan Rev. **17**: 291, 1913.
5. Last P. M., Harbison P. A.: Bacteriuria after urological instrumentation Lancet **1**: 74, 1966.
6. Lee R. A.: The vaginal approach to anterior vaginal relaxation. Clinical Obstet Gynecol. **15**: 1099, 1972.
7. Lee R. A.: Recurrent stress incontinence of urine: Preoperative assessment and surgical management. Clinical Obstet. Gynecol. **19**: 661, 1976.
8. Martin C. M., Bookrajian E. N.: Bacteriuria prevention after indwelling urinary catheterization. Arch. Int. Med. **110**: 703, 1962.
9. Muller A., Gillespie W. A.: Postoperative infection in urology. Lancet **2**: 608, 1958.
10. Novak E. R., Jones G. S., Jones H. W.: Relaxation, incontinence, fistula and malposition. Novak's Textbook of Gynecology. 9th edition. Baltimore U.S.A. The Williams Wilkins Com., 1975, pp. 292.
11. Pratt S. H.: Common complications of vaginal hysterectomy thoughts regarding their prevention and management. Clinical Obstet. Gynecol. **19**: 645, 1976.
12. Te Linde R. W., Mattinly R. F.: Urethrocelle, cystocelle and stress incontinence of urine. Operative Gynoecology 4th ed., U.S.A., Lippincott Comp. 1970, pp. 534.