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MITOCHONDRIAL ALTERATIONS OF THE RENAL PROXIMAL TUBULE EPITHELIUM IN RATS EXPOSED TO SMOKE*

Cengiz M. Güven • Belgin Can** • Serdar Yardımcı*** Hakkı Taştan**** • Yüksel Saran****

SUMMARY

The ultrastructural changes in the mitochondria of renal proximal tubule (RPT) epithelium produced by smoke were investigated in this study. Modified Walton Smoking Machine was used to generate smoke and the tissues taken from the control and smoke exposured rats were prepared for examining by transmission electron microscope. Most of the smoke exposured rats exhibited fine structural changes in mitochondria of renal proximal tubul (RPT) cells such as variations in size, form and disposition of internal membranes. Mitochondrial changes were thought to be a part of a pathological process, the presence of these changes might be interpreted as a sign of mitochondrial malfunction and degeneration.

Key words: *Smoke, Kidney, Mitochondrion, Ultrastructure.*

Several studies of the renal ultrastructural changes produced by some environmental factors and smoke in experimental animals were elucidated (1,2,3). About 3 million people die each year of smoking in developed countries, half of them before the age of 70 (4). Carcinogenic effects of tobacco led the International Agency for Research On Cancer to conclude that the cigarette smoking of cigarettes was an important cause of cancers of the lung, larynx, liver, oro- and hypopharynx, oesophagus, bladder, renal pelvis and pancreas (1,5,6,7).While the influence of smoke on the other cells has been actively investigated, the effects on renal cell mitochondria received little attention. In the present study the effects of smoke agents on the mitochondria of rat RPT cells were investigated.

MATERIALS AND METHODS

Modified Walton smoking machine described by Kendrich was used to generate smoke (8). Twelve mature male rats (age of 4 months, weight of 250-300g) were kept for 2 hours per day in this machine for a period of 60 days. Six control rats (same age and weight as experimental group) were also placed in this machine for the same period of time but they were only exposed to room air. The animals were sacrificed by ether anesthesia; then dissected and the kidneys were removed and fixed in 2 % glutaraldehyde in a phosphate buffer, pH 7.2, for 24 hours and postfixed in 1 % osmium tetroxide. The material was then dehydrated in graded alcohols, embedded in Araldite CY 212, sectioned with a LKB Ultratome III, stained with uranyl-acetate and lead citrate and examined with a Jeol 100 electron microscope.

RESULTS

The size, shape and internal structure of mitochondria of RPT cells in control group were similar to normal mitochondria that have been described previously (9-10). Most of the smoke exposed rats exhibited fine structural alterations in the mitochondria of RPT cells. They displayed variations in size and form and disposition of internal membranes. The unusual mitoc-

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hondria were abundant in most of the cells. The majority of mitochondria were ovoid and ellipsoid in shape (Fig. 1-5). A few mitochondria exhibited small herniations (Fig. 1-5). Variations in the density of matrix were obvious; in some of the mitochondria the matrix had a uniformly diminished opacity whereas in others it was more dense than normal (Fig. 1-5). Occasionally some of the mitochondria with dense matrix assumed a flattened shape and frequently aggregated. In some of these flattened mitochondria; the membranes of cristae were difficult to resolve (Fig. 1). Certain cells possessed mitochondrial aggregates in the cytoplasm (Fig. 2,3). In some of the mitochondria with normal appearing matrix there were finely particulate material and dense intramitochondrial granules (Fig. 2,3). Some mitochondria showed disorganized cristae and dilated intracristal spaces that contained an ill defined material. Rupture and lysis of mitochondrial membranes and cristae were frequently seen (Fig. 2-5). These degenerated mitochondrial matrixes were electron lucent in appearance (Fig. 2-3).

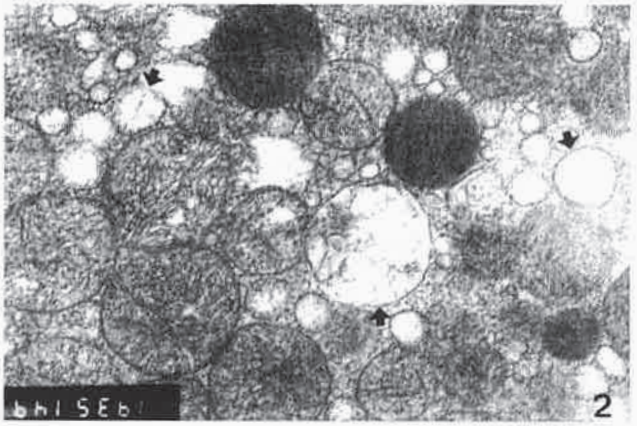

Fig. 2. RPT epithelium in rats exposed to smoke. Mitochondria are mostly accumulated in the cytoplasm. Several degenerated mitochondria with vacuolization and disposition of internal membrane (arrows) X 19000.

DISCUSSION

In the present study, the ultrastructural changes in the mitochondria of RPT epithelium produced by smoke have been investigated. We observed that most of the smoke exposed rats exhibited fine structural changes in mitochondria of RPT cells such as variations in size, form and disposition of internal membranes.

It was suggested that, population attributable risk percentages due to smoking was 90% for lung, 53% for bladder, 54% for oesophagus, 35% for stomach and 33% for pancreas (7). Long term cigarette smoking was a predictor for renal cell carcinoma risk in individuals (11). Up to date the effects of smoke on renal cell mitochondria received little attention.

In general, the number of mitochondria and the complexity of their internal structure vary with the energy requirements for specific functions carried out by the cell. In cells that are relatively inactive, the mi-

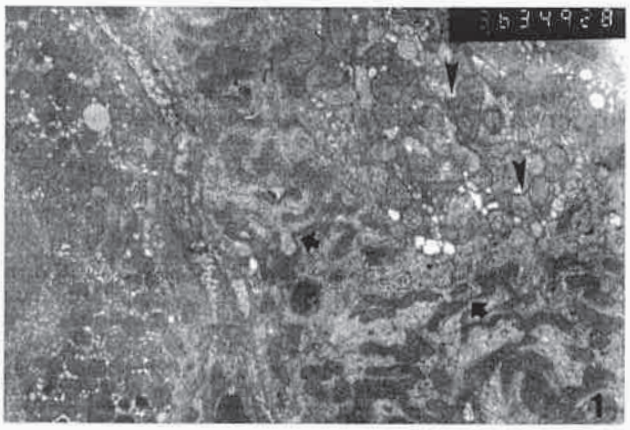

Fig. 1. RPT epithelium in rats exposed to smoke. Mitochondria show variations in size, form and density of matrix. Mitochondrial herniation (arrow head) and flattened mitochondria are seen (arrows) X 3600.

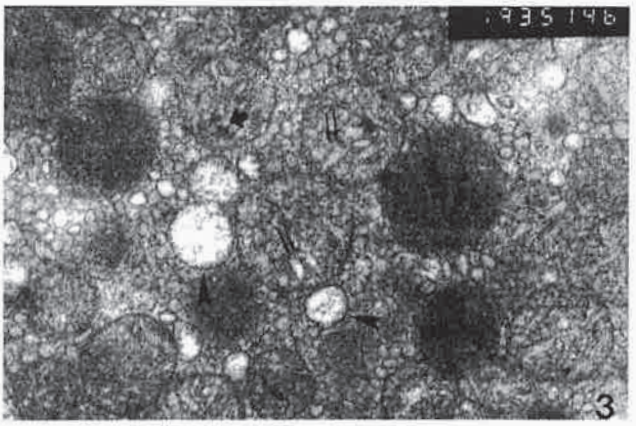

Fig. 3. RPT epithelium in rats exposed to smoke. Degenerated mitochondria are electron lucent in appearance (arrow head). Mitochondrial matrix contain particulate material and dense intramitochondrial granules (arrow). Dilated intra cristal spaces are noticeable (double arrows) X 19000.

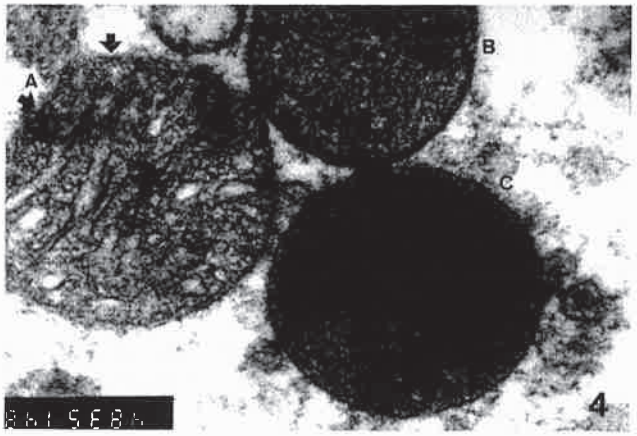

Fig. 4. RPT epithelium in rats exposed to smoke. A: Mitochondrion with fine granulated matrix and dilated intracristal spaces (star) are shown. At arrow - lysis of mitochondrial membrane. B and C illustrate two mitochondria showing advanced degeneration of the structure of the cristae and matrix: B: Only a few cristae can be seen, C: The cristae were disappeared X 48000.

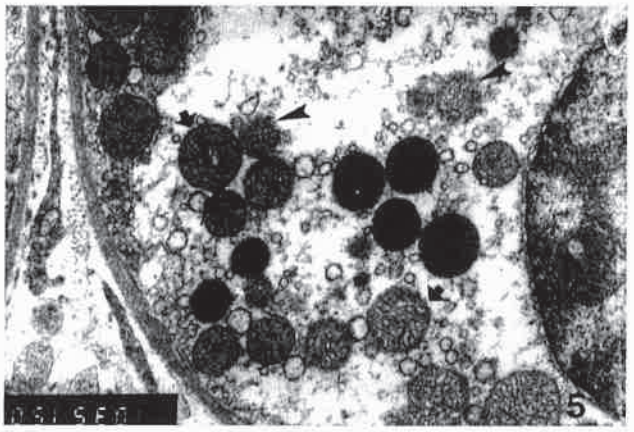

Fig. 5. RPT epithelium in rats exposed to smoke. Mitochondria showing disorganized cristae (arrows), herniation (double arrow) and lysis of mitochondrial membrane (arrowhead) are seen X10000.

tochondria tend to be few in number and their internal structure tends to be simple. On the other hand, cells engaged in active transport, in the synthesis of fat from carbonhydrate, or in the conversion of chemical energy to mechanical work usually have large numbers of mitochondria that contain a profuse number of cristae (12). In other words when a cell line becomes specialized in the course of its ontogenic evolution, its mitochondria also specialize, gradually change their functions(13).The small dense granules in the mitochondrial matrix vary greatly in number. In some cells they appear to be entirely lacking, while in others, they are abundant. Within the same cell type the number may change in different physiological conditions. They are said to be especially prominent in tissues transporting large amounts of ions or water (12). It was thought that large numbers of mitochondria indicated oncocytic differentiation and the large granules of unknown composition occurred in renal cell carcinomas (14). Mitochondrial alterations have frequently been observed in several conditions and in experimentally induced kidney lesions (2,15,16). Mitochondrial enlargement in the proximal tubules with cellular hypertrophy was observed as an initial morphologic change in albuminuria. These mitochondrial abnormalities disturbed adenosine triphosphate (ATP) metabolism in proximal tubules, reducing active transport and causing urinary excretion of low-molecular-weight protein (16). It was seen likely that such changes represented an unspesific reaction to cell injury, probably as a consequence of interference with some basic enzymatic activity.

Thus, all these mitochondrial changes produced by smoke were thought to be a part of pathological process. The previous findings and discussions also explained that the mitochondrial changes represented an unspesific reaction to injury. Consequently, the presence of these changes in the mitochondria of RPT epithelium, might be interpreted as a sign of mitochodrial malfunction and degeneration caused by smoke.

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INTRACELLULAR ANTIOXIDANT DEFENCE IN LUNG CANCER

Sema Yavuzer* • Hakan Fıçıcılar* • Murat Akal • Şinasi Yavuzer****

SUMMARY

Free oxygen radicals play a significant role in pathogenesis of lung cancer. At the systemic and cellular levels, oxygen radicals can cause tissue injury, DNA damage and mutational changes. On the other hand, tissue injuries and cell damage caused by oxygen species can be due to deficient removal of free radicals. So, the antioxidant defence has critical importance in prevention of carcinogenesis. In the present study the antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were evaluated in 21 patients with lung cancer before surgery or radio-chemotherapy and in 12 healthy volunteers.

SOD activity was found significantly lower in the cancer group than that of the control group ($p<0.05$). CAT and GPx enzyme activities were not significantly different in respect to the controls. The results of the present study indicate that the first stage antioxidant defence is defective in lung cancer patients. Supportive antioxidant therapy can increase efficacy of surgical treatment and radio-chemotherapies.

Key words: *Superoxide dismutase, catalase, glutathione peroxidase, lung cancer.*

It is generally accepted that free oxygen radicals may play a significant role in pathogenesis of lung cancer (LC). Also, it has been shown that inhibitors and scavengers of oxygen free radicals can prevent neoplastic transformation (1). Internal and external environments contain tremendous amount of oxidants such as molecular oxygen, superoxide anion, singlet oxygen, hydrogen peroxide, ozone and the oxides of nitrogen, sulphur and carbon. At the systemic and cellular levels, oxygen radicals are believed to involve in tissue injury, in DNA damage and mutational changes.

Lung cells, especially in smokers, are exposed high levels of oxidants. Cigarette smoke contains many oxidants and free radicals, and also it can cause increased generation of reactive oxygen species (ROS) due to activation of phagocytes. So, cellular antioxidant defence has critical importance in prevention of carcinogenesis in the lung. There are few reports about the erythrocyte antioxidant enzyme activities in lung cancer, and also their results are inconsistent. Some studies presented significantly lower SOD and GPx activities in red cells and/or plasma of lung cancer patients (2,3,4). Thus some authors have shown no changes in these red cell antioxidant enzyme activities in lung cancer patients (5,6).

The present study designed to investigate erythrocyte antioxidant enzyme activities in the patients with lung cancer.

MATERIALS AND METHODS

The investigation was carried out in twenty-one male patients (age range were between 39 to 75) hospitalised in the department of Thoracic Surgery. Mean age of the patients with LC was 56.7. An age-matched group of twelve normal healthy subjects volunteered as the control group. All of the patients with LC and the control group members smoked 20-40 cigarettes per day for 10-20 years. In the postoperative period, it was shown that the histological type of LC is squamo-

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us cell carcinoma in all patients. Erythrocyte superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were determined in the heparinized venous blood samples obtained from the subjects. The blood samples of the patients were taken in preoperative period. After separating the plasma from the blood samples, erythrocytes were washed twicely with saline and were hemolised by diluting 1:1.5 (v/v) in distilled water. Then ethanol-chloroform extracts were prepared, and the enzyme activities were spectrophotometrically determined. (7,8,9). The chemicals (EDTA, NaCN, NBT, riboflavin) were obtained from Sigma Chemical Co., and Hitachi Model 100-20 spectrophotometer was used for enzyme assays. For statistical analysis Mann-Whitney U test was used.

RESULTS

The erythrocyte SOD, CAT, GPx activities (mean ± Sd) were determined in the control and patient groups as 2859.88 ± 216.28 U/grHb, 216.48 ± 11.98 k/grHb, 3.27±0.43 IU/grHb and 2279.88 ± 146.62 U/grHb, 195.33 ± 18.72 k/grHb, 3.17±0.35 IU/grHb respectively. In the lung cancer group, the SOD activity was significantly lower than that of the control group ($p<0.05$). CAT and GPx activities were also lower in the patients with LC in respect to the controls but the differences were not statistically significant (Figure 1).

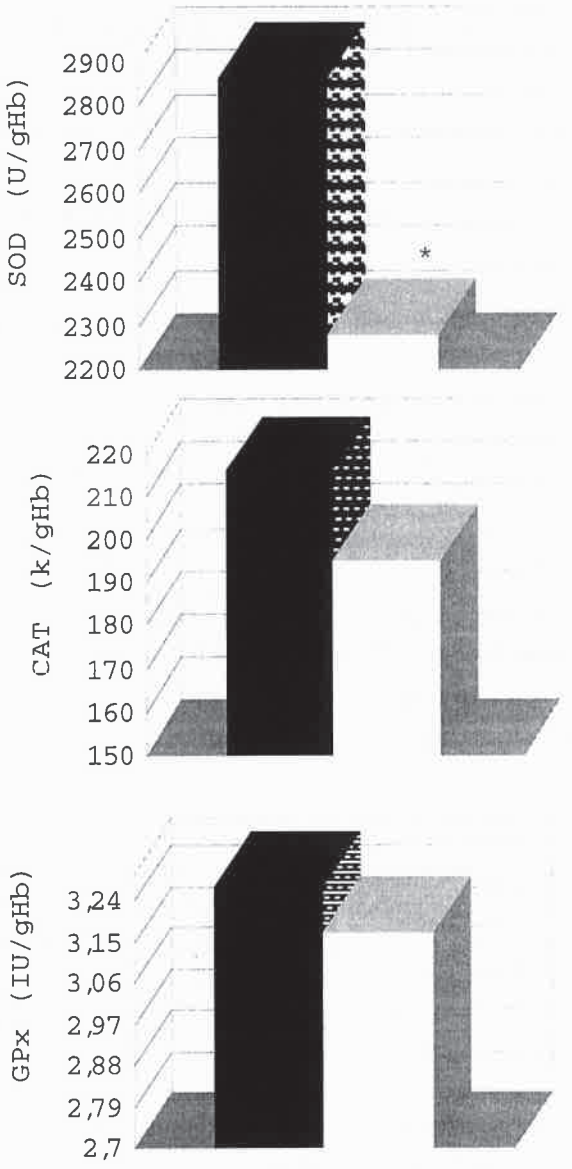

Fig. 1: Erythrocyte SOD, CAT and GPx enzyme activities in the control and the lung cancer groups (*$p< 0.05$).

DISCUSSION

Reactive oxygen species in general and superoxide radical anion ($O_2^{-\cdot}$) in particular, have been incriminated as the possible cause for cancer (10). ROS can promote a series of chemical reactions, such as membrane lipid peroxidation, disrupting sulphydryl bonds in proteins and enzymes, degradation of DNA molecules, formation of hydroxylated DNA bases or DNA strand breaks and so on (11,12). So, cellular antioxidant defence has critical importance in prevention of carcinogenesis.

The body has many overlapping antioxidant defence mechanisms to protect against ROS. These mechanisms include mineral dependent enzymes and small molecules that act as scavengers of ROS. Mineral dependent enzymes are SOD, CAT and GPx. One of such mineral dependent enzymes is Zn-Cu SOD, the copper-dependent $O_2^{-\cdot}$ scavenger. GPx and CAT are selenium-dependent and iron-dependent enzymes respectively (13,14).

In our study erythrocyte SOD activity was found significantly lower in the lung cancer group. CAT and GPx activities were also lower in the patients in respect to the controls but the difference were not statistically significant.

In the literature, some studies presented significantly lower SOD and GPx activities in red blood cells

or plasma of the patients with lung cancer (2,3,4). Thus some authors have shown no changes in these red cell antioxidant enzyme activities in lung cancer patients (5,6).

Very recently (1998) Martin-Mateo et al reported significantly decreased erythrocyte SOD activity in lung cancer patients with respect to healthy individuals and lesser enzymatic activity in advanced stages (1). They suggested that the lesser SOD activity is related with more aggressive tumors. SOD is key enzyme protecting aerobic organisms against oxidative damage. It is widely distributed in all body organs and cells, and acts as a catalyst in dismutation of superoxide radicals (15,16). For example, toxicity and carcinogenity of nickel are well established. Nickel strongly inhibits SOD activity and there is a high incidence of lung cancer in nickel workers (17). Furthermore, it has been shown that hydralazine produces radicals (18) and dramatically enhances free radical activity in the body (19) and induces lung tumour in mice (20). Drozdz et al also found that SOD activity and copper concentration were diminished during lung tumorigenesis by hydralazine in mice (21).

The oxidative damage or modification of DNA can play important role in the induction and/or progression of carcinogenesis. Jaruga et al reported that higher levels of DNA lesion were observed in lung cancerous tissue than in cancer-free surrounding tissues and antioxidant enzyme levels were lower in cancerous tissue. They suggest that the results indicate an association between decreased activity of antioxidant enzymes and increased levels of DNA lesions (22).

In fact, our study shows a significant decrease of erythrocyte SOD activity in the patients with lung cancer in respect to healthy individuals. It is suggested that when the activity of this protective enzyme decreases, that is what we found, the accumulation of superoxide radicals occurred. The free oxygen radicals may induce cytogenic changes, perhaps in related in lung carcinogenesis. SOD together with GPx and CAT constitute the primary antioxidant defence interfering the initiation and propagation of ROS related damage. SOD catalyses dismutation of superoxide radicals ions to hydrogen peroxide (H_2O_2). Both CAT and GPx appear to play important role in regulating the intracellular H_2O_2 concentration. H_2O_2 is detoxified by GPx and CAT. However several studies indicated that GPx system may play a greater role than CAT in protecting target cells from extracellular H_2O_2 (13).

In our study, CAT and GPx activities of red blood cells were lower in lung cancer patients compared with the control group. But the differences between the patient and control groups were not statistically significant. These results are compatible with some literature data. Tho and Candlish were found no significant change in SOD and GPx activity in lung cancer (6). But, Zachara et al (1997) reported that in blood components of lung cancer patients selenium concentration and GPx activity are significantly lower compared with healthy subjects (3). In addition Casado et al presented that CAT activity of blood was lower in the patients with cancer compared with that in the normal population (23).

GPx and CAT must be considered to be complementary to SOD in that they decompose the product of the latter's activity, hydrogen peroxide, which is potentially less destructive than the superoxide radical but it is intolerable in terms of optimal cell function (6). However, when there is a decrease in SOD activity as demonstrated in our study, lesser GPx and CAT activities would presumably be needed to dispose of the H_2O_2 generated by the activity of SOD.

On the other hand, in the present study all members of the lung cancer group are heavy smokers. It is accepted that cigarette smoke is a complete carcinogen (24), it can act both as an initiator and as a promoter. Incidence of lung cancer was strongly dependent on smoking status, showing a dose-response relationship. Exsmokers have a 3 fold greater risk of the disease and smokers of more than 15 cigarettes a day have a 25 fold greater risk in comparison with subjects who had never smoked (25).

Cigarette smoke contains many oxidants and ROS. In addition, smoking causes an increase in oxidative metabolism of phagocytes accompanied by increased generation of ROS, such as superoxide radicals, hydrogen peroxide and hydroxyl radicals. The gas phase of cigarette smoke contains small oxygen and carbon-centred radicals. The principal radical in the tar phase of smoke, a quinon/hydroquinon complex, is capable of reducing molecular oxygen to superoxide radicals (26,27). Thus SOD has a crucial importance in detoxification of superoxide radicals.

As smokers are being subjected to oxidative stress (a consequence of the free oxygen radical generation increase and/or the organism's antioxidants defense decrease) resulting from oxidant and free radicals present in smoke, as well as ROS generated by increased and activated phagocytes, their antioxidant status is likely to be adversely affected (27).

As a conclusion, in the presented study, activity of the first stage antioxidant enzyme, SOD of the patients with lung cancer was found significantly lower than that of the controls. The detoxification of free radical superoxide anion is not sufficient in these patients who are also heavy smokers. Our result suggests that the oxidant-antioxidant balance is altered in the blood of the patients with lung cancer, and supportive antioxidant therapy can increase efficacy of surgical treatment and radio-chemotherapies.

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INCREASED SERUM ARGINASE ACTIVITY IN DEPRESSED PATIENTS

Serenay Elgün* • Hakan Kumbasar**

SUMMARY

In this study, we aimed to determine serum arginase activities in depressed patients (n=30) and matched healthy control subjects (n=30) in order to make a contribution to the understanding of disease mechanism regarding arginine-nitric oxide pathway. Arginase activity was measured spectrophotometrically and depressed patients were found to have significantly higher serum arginase activity compared to controls (p<0.0005).

Results suggest that enhanced activity of arginase in the patient group may lead to a decrease in NO synthesis which may in turn affect certain neuroendocrine mechanisms contributing to the symptomatology of depression.

Key words: *Arginase, depression, serum.*

Arginase (EC 3.5.3.1) is an key enzyme of urea cycle catalyzing the hydrolysis of L-arginine to urea and ornithine in liver however extrahepatic functions of arginase are still unclear [1]. In humans it exists abundantly in liver but is also expressed in other organs, for example; an inducible arginase is found in brain which is an important part of the arginine regulatory system affecting nitric oxide synthase (NOS) activity [2].

L-arginine, substrate of arginase, is also a substrate for nitric oxide synthase, which produces nitric oxide (NO). It is known that NO is generated by various cells including endothelial, smooth muscle cells and neurons [3] and has potent biological activities as a vasoactive, neurotransmitter [3], cytotoxic and platelet regulatory agent [4].

Its neurotransmitter function has been investigated in many aspects and NO has also been recently established as a new important regulator of neuroendocrine function although its exact physiological role stays unclear. The presence of NOS has been demonstrated in several areas of brain particularly the hypothalamic paraventricular nucleus where it is co-localized with the hypophysiotrophic corticotropin-releasing hormone (CRH) [5, 6]. The physiological significance of this co-localization may probably be that NO modulates the release of CRH from hypothalamus as shown in recent studies. It was reported that this modulatory effect of NO appeared to be an inhibitory one [5, 7, 8].

The role of NO has been implicated in various neuropathological conditions including depression [7]. On the other hand excessive CRH secretion from activated paraventricular neurons and increased concentrations in cerebrospinal fluid in patients with depression have also been detected [9, 10, 11]. In addition, in a recent study, hypothalamic paraventricular NOS expression was found to be reduced in depressive patients perhaps leading to the overexpression of CRH [7]. However, the presence of NOS in neurons whether reduced or not, does not give an idea about its biochemical activity in producing NO. Thus, we think that the disease process has to be looked over within this regard.

Arginase is an important part of the arginine-regulating system affecting NO generation, however neither arginase activity nor the relation of this enzyme

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with NO metabolism has been studied in depressed patients so far. In this regard, we decided to investigate the arginine-NO pathway by means of circulating arginase activities in depressed patients assuming that a significant disturbance in enzyme systems of brain might be reflected in blood.

MATERIALS AND METHODS

Fasting blood samples were collected from 30 drug free depressive outpatients (12 males, 18 females) aged between 20-49 years (mean ± SD= 31.6 ± 7.2 years for males, and 34.8 ± 8.1 years for females) who referred to the Department of Psychiatry, University of Ankara, Faculty of Medicine. Depressive patients were diagnosed according to DSM IV criteria.

Healthy control subjects of the same number (15 males, 15 females) aging between 20-47 years (mean ± SD= 32.7 ± 7.1 years for males, and 31.3 ± 7.7 years for females) were excluded for past, present and family history of psychiatric disorders. They were free of any medication for at least 1 month prior to blood sampling. None had ever been taking psychotropic drugs.

All patients and controls were medically healthy. They had normal physical examinations and routine clinical laboratory analyses. They all agreed to participate in this study and gave us informed consent.

Sera were separated from fasting blood samples and stored at -20°C until arginase activity was measured spectrophotometrically according to Chinard's method [12]. The enzyme activity was determined by measuring the amount of ornithine produced from the hydrolysis of arginine by arginase and were expressed as IU/L . The within run and between run precisions of the assay were 3.4% and 4.2%, respectively. Data were analyzed using Student's t-test.

Table 1. The mean ± SD serum arginase activities (IU/L) of the healthy controls and depressed patients and comparison of means by Student's t-test.

	Arginase (IU/L)
Control group n=30	0.30 ± 0.11
Patient group n=30	0.48 ± 0.16
	p<0.0005

RESULTS

As seen from the Table 1, depressive patients were found to have significantly higher serum arginase activities compared to the controls.

DISCUSSION

NO which is thought to play a role in central neurotransmission [3, 5, 6] is, now, being referred to in various neuropathological conditions but there are not so many reports. On the other hand, the overexpression of CRH in depressive patients and its possible impact on symptomatology of depression have been implicated in a number of studies [9, 10, 11]. The most prominent role attributed to NO in brain is its potential inhibitory effect on the release of CRH in hypothalamus [5, 7, 8], while in a recent study it was stated that the total amount of NOS-immunoreactive paraventricular neurons was smaller in depressed patients than in normal cases and this reduction might be related to the supposed regulatory function of NO in the release of CRH which had been shown to be overexpressed in depression [7]. However, as was mentioned above, we do not know about the biochemical activities of NOS and arginase or the presence/amount of the products participating in arginine-NO pathway.

As known, arginase and NOS metabolism uses arginine as a common substrate. Arginase being an arginine depleting enzyme, may inhibit NO generation [2, 13, 14, 15] and we know that NO may play a role in depression [7], but the problem of whether synthesis is impaired or metabolism of NO is augmented has to be clarified. In a recent study, it was suggested that some endogenous substances present in schizophrenic plasma inhibit NO synthesis [16]. But it is not clear which mechanism is involved in depression yet.

Considering these limited data, we thought that it would be reasonable to measure arginase activity in depressed patients. For this aim, we used sera of patients thinking that an imbalance in enzyme systems in brain might be mirrored in blood. It would have been better if we could use cerebrospinal fluid or brain tissue for this aim but it would be neither ethical nor possible. However, experimental animal studies can be made in this regard.

Since both of the groups were medically healthy, it seems possible that the increased avtivity of arginase in depressed patients may be of importance as to possible relation of arginase and NO in depression. The relationship of serum arginase to brain arginase acti-

vity is unclear yet. However, this increase in arginase in serum which may be the reflection of induced arginase activity in brain, possibly indicate that arginine is depleted, leading to a decrease in NO production. The decrease in NO synthesis may in turn cause an impairment of its inhibitory control on the release of CRH in brain. This possible overexpression of CRH in depressed patients will not only stimulate hypothalamo-pituitary-adrenal axis but also be the cause of at least part of some symptoms of depression [11]. If this is the case, then arginase inhibitors might be beneficial in the control of depressive illness.

Analyses should also be performed before and after antidepressant treatment to see the effects of therapy on enzyme activities.

Thus, the subject needs further investigations in larger series all the way, including animal experiments and clinical inhibition trials etc., since it seems likely that the arginine-NO pathway including arginase and NOS, is involved in depression.

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THE EFFECTS OF L-CARNITINE ON LEFT VENTRICULAR FUNCTION AND ERYTROCYTE SUPER OXIDE DISMUTASE ACTIVITY IN PATIENTS WITH ISCHEMIC CARDIOMYOPATHY

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SUMMARY

We studied the effects of L-carnitine on left ventricular systolic function and the erythrocyte superoxide dismutase activity in 51 patients with ischemic cardiomyopathy. They all previously were under the treatment of angiotensin converting enzyme inhibitor, digitalis and diuretics. Patients were randomized into two groups. In group I (n=31) 2 g/day L-Carnitine were added to therapy and L-Carnitine were not given to other 20 of the patients (Group II). In group I (mean age 64.3±7.8 years), 27 of the patients were men, and 4 were women. In group II (mean age 66,2±8,7 years), 17 of the patients were men, and 3 were women. Twenty age matched healthy subjects (mean age: 60.1±5.3 years) constituted the control group. In each group left ventricular ejection fraction by echocardiography, red cell superoxide dismutase activity by specthrophotometric method were measured initially and after a month of randomization. Compared with normal healthy subjects (n=20), patients (n=51) had significantly higher red cell SOD activity (5633±1225 U/g-Hb vs. 3202±373, p<0.001). At the end of one month of L-carnitine therapy, red cell SOD activity showed a more increase in group I (5918±1448 U/g-Hb to 7218±1917, p<0,05). In group II red cell SOD activity showed no significant change after a month of randomization (5190±545 U/g-Hb to 5234±487, p=0.256). One month after randomization there was a significant increase in ejection fraction in both group I and II (37,8% to 42,3, p<0,001 in group I) (41,5% to 43,8, p<0,001 in group II). We conclude that, as a sign of increased free radical production superoxide dismutase activity were furthermore increased in patients with L-Carnitine treatment. L-carnitine treatment in combination with other traditional pharmacological therapy might not have an additive effect for the improvement of left ventricular function in ischemic cardiomyopathy.

Key words: *Ischemic cardiomyopathy; L-Carnitine; erytrocyte superoxide dismutase*

Carnitine is a naturally occurring compound in the body, and is synthesised chiefly by the liver. Tissues with active fatty acid metabolism such as skeletal muscle and the heart, contains the highest levels of carnitine but are incapable of synthesising it [1,2]. Fatty acid metabolism constitutes one of the main sources of energy and, more specifically, is the preferential substrate for cardiac oxidative metabolism. Carnitine is an essential factor in transporting the long-chain fatty acids (acyl CoA) from the cytoplasm into the mitochondria where the beta-oxidation takes place [1-3].

A reduced O_2 supply, which occurs in the course of hypoxia or ischaemia, causes the slowing down of intramitochondrial oxidative processes and thus of beta-oxidation, leading to the accumulation of the intermediate products of oxidative metabolism, particularly acyl CoA [3,4]. Accumulated acyl CoA is capable of inhibiting adenine nucleotide translocase, the enzyme responsible for transporting ATP from the mitochondria, where it is produced, to the cytoplasm, where it is utilised for muscle contraction. [3-5]. In ischaemic conditions, then, there is an overall slowing down in the mitochondrial energy production mechanism [4-6]

L-carnitine, an essential intermediate compound in the physiological transport of long-chain fatty acids

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across the mitochondrial membrane has been found to be below normal levels in the ischemic myocardium. Experimental and clinical studies have shown that in the ischaemic, infarcted or failing myocardium, carnitine depletion occurs rapidly [4,7-9]. The exogenous administration of L-carnitine restores normal levels of intramyocardial carnitine and has been shown to be capable of improving the mitochondrial function of ischaemic cells [7]. It reduces the ischaemia induced increase in long-chain fatty acid concentration and thus lessens its deleterious functional effects [10-12]. The mechanism by which carnitine exerts its action in ischaemic metabolism lies in its ability to react with the acyl CoA which accumulates as a result of the slowing down of beta-oxidation, and, in turn, is capable of carrying acyl groups out of the anoxic cell [4,13]. The anti-ischaemic and antianginal effects of L-carnitine have been shown by the improvement in myocardial metabolism observed during atrial pacing in patients with coronary artery disease and in exercise tolerance in patients with chronic stable angina [14].

Orlando at al. have shown that with oral L-carnitine treatment in patients with chronic cardiac ischaemia there have been an improvement in symptoms, functional NYHA class and in the left ventricle shortening fraction [9].

On the basis of these clinical trials we decided to study the effects of oral L-carnitine administration in patients with ischaemic cardiomyopathy.

METHODS

Patient population: Fifty-one patients with the diagnosis of ischaemic cardiomyopathy were studied. Patients having one or more than one of the diseases such as chronic inflammatory disease, symptomatic peripheral vascular disease, diabetes mellitus, infection, respiratory disease or malignancy were excluded.

Fifty-one patients with the diagnosis of ICMP were included in this study. They all previously were under the treatment of angiotensin converting enzyme inhibitor, digitalis and diuretics. Patients were randomized into two groups. In group I (n=31) 2 g/day L-Carnitine were added to therapy and L-Carnitine were not given to other 20 of the patients (Group II). In group I, 27 of the patients were men, and 4 were women. Nineteen of them had previous history of anterior wall myocardial infarction, ten had previous history of inferior wall myocardial infarction and other rest 2 had a history of anterior and inferior wall myocardial infarction. The mean age of the patients was 64.3±7.8 years. In group II, 17 of the patients were men, and 3 were women. Seven of them had previous history of anterior wall myocardial infarction, 8 had previous history of inferior wall myocardial infarction and other rest 5 had a history of anterior and inferior wall myocardial infarction. The mean age of the patients was 66,2±8,7 years (Table 1). Twenty age matched healthy subjects (mean age: 60.1 ±5.3 years) constituted the control group. In the study group, 43 of the patients had had coronary angiography previously in which 14 of them also had percutaneous transluminal coronary angioplasty and 8 of them had coronary artery bypass surgery.

Echocardiography: All patients underwent a complete physical examination with measurement of blood pressure and heart rate, standard 12-lead ECG at rest and two dimensional M-mode standard echocardiography. In both group of patients ejection fraction of left ventricle was measured by echocardiography initially and also after a month of randomization.

Red cell superoxide dismutase activity: The blood samples of both the controls and the patients were drawn in the morning always at the same time. The blood samples of totally 5 ml from fasting subjects were taken with polyethylene disposable syringes and put into demineralised centrifuge tube, which contained heparin. Plasma and red cell of each sample were separated by centrifugation. The blood samples for Cu-Zn-SOD determination was studied immediately. The red cell SOD activity was determined according to Winterbourn's method by spectrophotometer [15].

Statistical analysis: Student's two-tailed t-test for paired samples was used to compare the differences in left ventricular ejection fraction and red cell SOD activity before and after a month of randomization. Red

Table 1. Basal clinical data in patients

	Group I, *n*=31	Group II, *n*=20	p
Mean age, year	64.3±7.8	66,2±8,7	NS
NYHA class	3.21±0.76	3.32±0.87	NS
Ejection fraction, %	37,8	41.5	NS
SOD, U/g-Hb	5918±1448	5190±545	NS

NYHA: New York Heart Association.
SOD: Superoxide Dismutase Activity

cell SOD activity was compared between patients and normal healthy subjects by unpaired Student's t-test. The data were expressed as mean±SD. A p value <0.05 was considered significant.

RESULTS

There were no side effects observed in patients during one month of follow-up. At the end of 30 days of randomization, the left ventricle ejection fraction showed a significant improvement in L-Carnitine group (37,8% to 42,3, p<0,001). The ejection fraction remained unvaried in one patient. In two-third of the patients the improvement in EF was over 10% and two of them showed a 30% increase. There was no decrease in EF in any patients. In group II the improvement in EF was also significant (41,5% to 43,8, p<0,001) (Table 2).

Compared with normal healthy subjects (n=20), patients (n=51) had significantly higher red cell SOD activity (5633±1225 U/g-Hb vs. 3202±373, p<0.001). At the end of one month of L-carnitine therapy, red cell SOD activity showed a significant increase in group I (5918±1448 U/g-Hb to 7218±1917, p<0,05). In group II red cell SOD activity showed no significant change after a month of randomization (5190±545 U/g-Hb to 5234±487, p=0.256) (Table 2).

DISCUSSION

Except the carnitine deficiency syndromes, carnitine's use in heart diseases remains equivocal. [16-18]. The rationale for the use of L-carnitine in patients with ischaemic heart disease initially originated from the finding that myocardial carnitine concentrations were lower in these patients [7,8]. Depressed myocardial uptake of 123-β-methyl iodophenyl pentadecanoic acid, which may reflect depressed fatty acid catabolism in viable myocardium, is frequently observed in patients with ischaemic heart disease. Watanabe at al have shown that, three months of oral L-carnitine treatment have increased the depressed 123-β-methyl iodophenyl pentadecanoic acid uptake and have improved myocardial ischemia [19]. In the study of Fujiwara at al. it was shown that intravenously administered L-carnitine stimulated cardiac metabolism and increased coronary blood flow during exercise in patients with ischaemic heart disease [20]. Beyond this, there is increasing evidence suggesting a beneficial effect for carnitine therapy in a number of cardiovascular disorders including angina pectoris, acute ischaemia, congestive heart failure, and also hyperlipidemia [2,4,7,9,14,18,20-23].

Table 2. Clinical data 1 month after randomization

	Basal	1 month after	p
Group I, n=31			
EF %	37,8	42.3	<0.001
SOD U/g-Hb	5918±1448	7218±1917	<0.05
Group II, n=20			
EF %	41.5	43.8	<0.001
SOD U/g-Hb	5190±545	5234±487	0.256

EF: Ejection Fraction
SOD: Superoxide Dismutase Activity

It was shown that there is a role of oxygen free radicals in the pathogenesis of postischemic myocardial dysfunction (myocardial "stunning", reperfusion injury) after acute myocardial infarction. [24-26]. Free radicals could also be implicated in the development and progression of chronic myocardial dysfunction. Underlying coronary artery disease may predispose to stunning. Repeated episodes of stunning may lead to permanent myocardial dysfunction [27,28]. Additional factors could also favour free radical generation in congestive heart failure. Pressure or volume overloads may lead the protracted cycles of generalised myocardial ischaemia and reperfusion and thus the generation of oxygen free radicals [29,30]. Adrenergic activity is increased in congestive heart failure. Catecholamines may augment free radical generation by increasing mitochondrial respiration and undergoing autooxidation [31,32]. Free radicals cause impairment of myocyte metabolism and contraction and also cause endothelial dysfunction and induce arrhytmias [33-35]. Both lipid peroxidation and thiol group oxidation can cause these derangement as a pathological process. In the study of Diaz-Velez at al. plasma malondialdehyde levels, a marker of lipid peroxidation, were found abnormally elevated in patients with chronic heart failure and were strongly associated with the chronicity of the heart failure state [36].

Several lines of evidence show that administration of SOD, free radical scavenger, attenuates the free radicals induced myocardial injury [37,38].It was shown that Propionyl-L-Carnitine suppresses formation of hydroxyl radicals in an ischaemia reperfusion

model, and attenuates peroxidative injury [39].The free radical scavenging properties of Propionyl-L-Carnitine haven't been seen with L-Carnitine [40,41]. In our study, echocardiographic improvement in patients under the treatment of L-carnitine in addition to conventional therapy is no more different from patients under conventional treatment. In our study, compared with normal healthy subjects, in patients with ischaemic cardiomyopathy, the red cell SOD activity was higher ($p<0.001$), and this might be attributable to enhanced oxygen free radical generation in failing heart. One month after L-carnitine therapy, we observed that there was a significant increase in red cell SOD activity. This increase in red cell SOD activity after L-carnitine treatment may be attributable to enhanced β-oxidation of fatty acids, and so on increased free radical generation. This may suggests a negative effect of L-carnitine in the energy metabolism of the failing heart. Otherwise, the increase in SOD activity with L-carnitine treatment seems to be the result of increase in free radical generation more which already has been enhanced in failing heart.

Angiotensin converting enzyme inhibitors have been shown to cause a reduction in oxygen free radical production [42]. In group II, patients were under treatment of ACE inhibitor, diuretics and digitalis and the red cell SOD activity showed no significant change after a month of randomization (5190±545 U/g-Hb to 5234±487, $p=0.256$).

A more widely recognised salutary action of L-carnitine is its ability to reduce the accumulation of long-chain acyl CoA in the ischaemic mitochondrial matrix [8,10-12,43]. This accumulation is thought to be partially responsible for the loss of myocardial contractility. Additionally, L-carnitine has been reported to improve pyruvate metabolism, to reduce lactate production and acidosis [8,12]. Despite some ambiguity regarding the mechanism, oral L-carnitine has been shown to improve global left ventricle performance in patients with ischaemic cardiomyopathy [4,21,44,45]. The available short-term controlled studies in patients with stable coronary artery disease indicate that L-carnitine improves exercise tolerance and increases the ischaemic threshold. Cherchi at al have shown that L-carnitine administered to patients with stable angina pectoris has increased the exercise test tolerance on the cycle ergometer and has reduced S-T segment depression at maximum load [46]. In the study of Taillard at al, in patients with dilated cardiomyopathy dramatic improvement of the cardiac function was assessed by radionuclide methods, during oral L-carnitine therapy [45]. A recent multicenter investigation in patients with anterior myocardial infarctions (CEDIM trial) has shown attenuation in left ventricular enlargement with L-carnitine therapy [7].

In the study of Ghidini at al elderly subjects suffering from heart failure, secondary to ischaemic and/or hypertensive heart disease were given oral L- carnitine for 45 days in addition to traditional therapy. A distinct improvement was observed in patients with respect of their functional status [23].

It is concluded that, exogenous administration of L-carnitine can restore adequate intramyocardial carnitine levels but has no effect on improvement of left ventricular global performance in patients with ischemic cardiomyopathy. This may suggests that L-carnitine is not a useful therapeutic agent in combination with traditional pharmacological therapy for the treatment of patients with heart failure and ischaemic heart disease.

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PSYCHIATRIC DISORDERS IN LEPROSY PATIENTS IN TURKEY

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SUMMARY

The aim of this study was to compare leprosy patients (n=66) with healthy controls (n=19) for the occurrence of psychiatric co-morbidities. Comparisons were done with Zung depression, Speilberger anxiety and Symptom check list 90R self-rating scales. The leprosy group, in comparison with the healthy controls, had had significantly higher mean scores in depression and anxiety scales; Moreover, global assessment of psychopathology revealed higher levels of psychiatric symptoms with Symptom check list 90R in leprosy group. Our findings suggested that leprosy patients should be included into the risk groups for psychiatric problems and further studies concerning about psychiatric issues are indicated in order to to increase our understanding.

Key words: *Anxiety, Depression, Leprosy, Psychiatric Disorders*

Hansen's disease or leprosy is a major worldwide public health problem that has profound psychological effects on it's victims (1). Surveys from different cultures reveal that knowledge of leprosy is limited and that much stigma still exists in ordinary people (2, 3, 4). In Ethiopia attitudes toward leprosy were compared with attitudes to epilepsy. Most of the responders neither were willing to employ or work with a person having the disease nor would allow their children to associate with a playmate suffering from leprosy (2). In addition to the prejudisms and misconceptions that threatens leprosy patients emotional well-being, generally after the emergence of physical deformities, they usually encounter with further decline in their societal status and even may lose their job (4,5).

The stigma around leprosy doesn't appear to be confined to the ordinary people; studies investigating leprosy patients attitude toward leprosy also reveals some stigmatization and misconceptions about Hansen's disease (6,7,8). In one of these studies, few patients applied the bacterial theory as cause of their disease but traditional beliefs and religious ideas found to play an important role in their understanding of Hansens's disease (7). In another study from Pakistan, more than half of the noncompliant patients, reaches up to 30 % of all diagnosed patients, denied having the disease (8).

Threats to the integrity of self and body-image generally induce adjustment responses. The diagnosis of "leprosy" is definitely a major threat that provokes adjustment responses not only because of the stigma and misconceptions around it but also because of the need for the reevaluation of oneselves future expectancies, social roles, relationships. Adjustment process may result with either adaptive or maladaptive responses. Adaptive responses restores the indivievals psychological equilibrium; however, in case of maladaptive responses, a broad spectrum of psychological difficulties ranging from minor interpersonal conflicts to major psychiatric disorders can be seen (9). Denial (8), discriminating themselves from others (7), depressive and paranoid personality traits (10), personality structure disposed to neuroticism (11) are among relatively minor responses that are reported.

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To date, depression and anxiety has been the most frequently searched psychiatric diagnosis and all studies revealed high incidence of depression and anxiety in leprosy patients (12,13,14,15,16). In a study (16), 100 leprosy patients assessed for psychiatric comorbidity and 76 % received the diagnosis of either neurotic depression (55 %) or anxiety neurosis (21 %). Of 24 patients assessed for the suicidality 14 revealed suicidal ideation, additional 2 reported previous attempts (17). Leprosy patients and patients on renal dialysis were found to be similar to but significantly different from the healthy controls in terms of anxiety and psychological stress levels (13). In a well designed study, 50 % of 56 recently diagnosed leprosy out-patients, 37% of 19 controls with another stigmatized dermatological condition (vitiligo), but only 8% of 12 controls with a comparable non-stigmatized condition (tinea versicolor) met DSM-III-R criteria for depressive, anxiety or somatoform disorder (12). Among leprosy patients, single, unemployed, socio-economically backward and patients with physical deformities (16) and with shorter duration of illness (17) were found to be more vulnerable to psychiatric problems.

In this preliminary study, the investigators aimed at screening the leprosy patients for psychiatric problems with self-rating scales for to clarify areas of psychopathology that needs further evaluation. To cover various aspects of psychopathology, self-rating scales for global assessment of psychopathology, depression and anxiety were used.

MATERIAL AND METHOD

This study was done in the Leprosy education and Research Center of the Faculty of Medicine, University of Ankara, located in the city of Ankara and Elazığ Leprosy Hospital, located in the city of Elazığ, both in Turkey. Sixty-six inpatient leprosy patients agreed to fill out self rating scales and to participate the study. Control group consisted of 19 healthy volunteers without any known psychiatric or medical illnesses. There were no statistically significant differences in age, sex distribution between control and leprosy group.

Zung Depression Scale (18) and Speilberger State-Trait Anxiety Scales were used to identify cases with depression and anxiety, respectively. Zung Depression scale had been translated and adapted into Turkish and distributed to the psychiatry clinics of Turkey by CIBA Company. Speilberger State-Trait Anxiety Scales had also been translated into Turkish and reliability-validity studies had been completed (19). These self rating scales provide cut-off-points about the level of depression and anxiety and scores above cut-off points usually well correlated with relevant clinical diagnosis.

Symptom Check List 90R (SCL-90R) is another self rating scale used in the study. This scale covers many areas of psychopathology and provides us an index for global assessment of psychopathology. Validity and reliability studies of Turkish version of SCL-90R was completed (20).

The SPSS PC+ program was used for statistical analysis. Two tailed Student T-test was used for comparisons of the means of control and leprosy patients group on Zung, Speilberger and SCL-90R scales. Statistical analysis of the influence of sociodemographic characteristics on the rating scales were done with Chi-square or ANOVA statistics.

RESULTS

Sociodemographic characteristics of patients were presented in Table 1.

In comparison to control group, leprosy patients received significantly ($p<0.0001$) higher scores on Zung Depression Scale (Table 2).

Of all patients, only 20 found to be free of depression; However, the remainder, a total of 46 patients, got scores included in either mild, moderate or severe levels of depression (Table 3).

Speilberger state and trait anxiety scale scores were generally in normal ranges for both Control and Leprosy groups. In patients group, 2 and 7 patients received higher than normal scores for state and trait anxiety, respectively. However, comparison of means of two groups revealed significantly higher levels of state and trait anxiety levels in leprosy group (Table 4).

Variables listed on Table 1; marital status (single or married), occupation, living with significant others or alone, being on or off antileprosy medication, the type of leprosy, having a deformity and if any, its severity, were not found to be factors effecting either the level of depression or anxiety. On the other hand, being female has clear-cut negative effects on both depression and anxiety scales ($p<0.01$).

Table 1. Socio-demographic Characteristics*

Sex	Age	Diagnosed at the age of
Male: 52 (%78.8) Female: 14 (%21.1)	Mean:53.53 sd: 10.33 Range: 3-75	Mean:18.51 sd: 9.05 Range: 5-39
Duration	**Occupation**	**Living with**
Mean:34.54 sd: 11.64 Range: 3-52	Yes: 18 (%29) No: 45 (%71)	Significant others: 18 (%29) Alone: 44 (%71)
Marital Status	**Type of Leprosy**	
Married: 26 (%41) Single: 38 (%59)	Tuberculoid, Borderline tuberculoid: 12 (%20) Borderline lepromatous, Lepromatous: 47 (%80)	
Antileprosy medication	**Leprosy in relatives**	
On: 28 (%44) Off: 35 (%56)	Present:: 23 (% 36.5) Not present: 40 (% 63.5)	
Deformity **None: 15 (%24), Mild-Moderate: 10 (%16), Severe: 38 (%61)		

* In some socio-demographic characteristics the number of subjects evaluated were less than 66.

** None: No deformity, Mild-Moderate: Cases with between no and severe deformity, Severe: Facial paralysis, Clover nose, hand & foot deformities, amputations

Both depression and anxiety scales can provide insight to some aspects of psychopathology. Symptom Check List-90R (SCL-90R), on the other hand, provides a global view for the assessment of psychopathology in broader perspective. SCL-90R scale results of patients and controls are presented in Table 5. As expected, highest level of significance reached at depression subscale; Interpersonal sensitivity, phobic anxiety and general index of psychopathology (GSI) were other subscales significantly differing from Control group.

CONCLUSION

To our knowledge, only limited number of studies concerning about the psychiatric problems of leprosy patients exist in the literature. Available studies are from different cultures with various ethnic, religious and racial backgrounds. This variability limits our ability to reach definite conclusions.

Increased level of depression seems to be a common finding in many studies as well as in this study. We may conclude that, based on the consistency of the same finding in various settings, leprosy patients are vulnerable to depression in any given culture. Both Zung Depression Scale, 69.7 % of all patients showed depression at the clinical level, and SCL-90R depression subscale revealed statistically significant increase in depression levels in leprosy group. Methodological differences among studies make it impossible to compare our results to reach further understanding of the characteristics of the depressive patients; we have used a self rating scale with cut-off points for both the existence of depression and the level of severity of depression, DSM-III-R diagnostic

Table 2. Comparisons of depression scores.

	Zung Depression Scale				
	N	Mean	sd	t value	p
Leprosy Group	66	54.03	8.643	4.19	0.0001
Control Group	19	44.78	8.410		

Table 3. The level of depression

Level of depression (Zung Dep. Scores)	N	%
<50 = none	20	30.3
50 - 59 = mild	27	40.9
60 - 69 = moderate	16	24.2
>70 = severe	3	4.5

Table 4. Comparisons of trait and state anxiety scores

Speilberger Trait Anxiety Scale

	N	Mean	sd	t value	p
Leprosy Group	66	46.50	7.106	3.24	0.003
Control Group	19	404.78	6.680		

Speilberger State Anxiety Scale

	N	Mean	sd	t value	p
Leprosy Group	66	47.12	8.962	2.36	0.026
Control Group	19	40.21	11.807		

criteria used in the other (12) and unspecified criteria in another (16). Comparisons with other chronic diseases provide further support to increased frequency of depression in leprosy patient and indicated similarities between leprosy and other long-lasting diseases (8,13,14). Although being single, unemployed, the presence of physical deformities (16) and shorter duration of illness (17) were found to be related to depression, our analysis didn't support these relationships. In addition, no statistically significant difference was found between depression and age, type of leprosy, the presence of leprosy in relatives, being on or off medication. However, females were more likely to be depressed in our study. Another aspect of depression, increased suicidality was also reported (17) but not assessed in this study.

Both mean State and Trait anxiety scores were significantly higher in leprosy group, making the diagnosis of anxiety disorder another important aspect of psychopathology. These results are comparable with other studies on Leprosy or many other long-lasting illnesses (9,12, 13,14). None of the sociodemographic features listed in Table 1 but female sex found to be related to the anxiety scores. Similar to that seen in depression, female patients found to be significantly more anxious than males. However, when cut-off points taken into consideration, in contrast to that seen in depression scales, only 2 and 7 patients received higher than normal scores for state and trait anxiety, respectively; these results indicated that most patients, although more anxious than controls, didn't have anxiety levels at pathological levels.

Significant difference between control and Leprosy groups found on General symptom index (GSI) on SCL-90R; GSI provides information about the global severity of psychopathology deriven from the answers given to 90 questions covering 9 different aspects of psychopathology. Higher GSI scores seen in leprosy patients indicates that psychological difficulties are not confined to depression and anxiety but the existence of more generalized pathology of mental health is likely. Similarly, Kumar (14) also suggested the possibility of psychiatric diagnosis other than depression in leprosy. Among subscales of SCL-90R, interpersonal sensitivity and phobic-anxiety were other aspects of psychopathology requiring special interest. Increased

Table 5. Comparisons of SCL-90R scores for the global assessment of psychopathology

Symptom Check List-90R Scores

	Leprosy Group		Control Group			
	mean	sd.	mean	sd.	t value	p
Somatization	1.12	0.53	0.83	0.64	1.80	0.08
Obs-Comp	1.26	0.56	1.16	0.47	0.78	0.43
Interpersonal	1.47	0.69	1.00	0.56	3.09	0.004
Depression	2.45	0.62	1.99	0.52	3.25	0.003
Anxiety	0.91	0.54	0.81	0.64	0.61	0.54
Hostility	0.82	0.70	0.78	0.48	0.24	0.81
Phobic-anx	0.87	0.57	0.56	0.38	2.73	0.009
Paranoid	1.03	0.65	1.04	0.56	0.07	0.94
Psychosis	0.98	0.57	0.78	0.49	1.50	0.14
GSI	2.93	0.45	2.69	0.43	2.20	0.03

interpersonal sensitivity and phobic-anxiety may be related to the findings reported in other studies such as increased isolation (7), depressive and paranoid personality traits (10), increased neuroticism (11).

In conclusion, we may speculate that leprosy patients could be included in the risk groups for psychiatric problems. The methodological limitations of this preliminary study makes it impossible to discuss the features making any leprosy patient predisposed to any special psychiatric diagnosis. On the other hand, not only the results of this study but also our clinical experiences with Leprosy patients supports the view about the need for new and detailed studies on psychiatric problems of these patients. The fact that psychiatric symptoms and syndromes can cause breakdown of the capacity to use the resources for treatment that are already limited, makes this issue even more important.

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CEREBROVASCULAR ACCIDENTS AND THE ROLE OF FACTOR V MUTATION IN CHILDREN

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SUMMARY

Cerebrovascular accidents constitute a far smaller proportion of the neurologic diseases of childhood than of adulthood. We studied protein C, protein S, antithrombin III, resistance to activated protein C and Factor V Leiden in patients with cerebrovascular accident of unknown etiology.

Over a 5-year period 13 children aged 13 months to 12 years trated for ischemic stroke were enrolled in the study. The diagnosis was based on clinical neurologic deficits, computed tomography scan, magnetic resonance imaging and angiography in some cases.

Moyamoya disease was determined in one patient and protein C level was low in three patients and all the patients with low protein C level were male. Protein C resistance was found in one patient and his factor V 1691 G→A was negative. Two patients were heterozygous for Factor V 1691 G→A.

Key words: *Childhood stroke, Factor V Leiden*

Cerebrovascular accidents (CVA) constitute a far smaller proportion of the neurologic diseases of childhood than adult. The incidence is 2.52 per 100.000 population. Occlusive vascular diseases, collagen disorders, congenital heart diseases, fibromuscular dysplasia, mitochondrial diseases are mainly the causes of CVA (1-6).

Several hereditary conditions share a predisposition to vascular thrombosis, the result either from increased deposition or decreased dissolution of fibrin. These conditions include several forms of antithrombin III deficiency, inherited defects of Protein S, Protein C.

Recently, a new inherited defect, involving the Protein C/Protein S system, has been associated with a poor anticoagulant response of plasma to activated protein C resistance (APCR). In the majority of adult and children with venous thrombosis mutation in Factor V gene has been reported in association with APCR (7-11). The cause of APCR is a point mutation in the Factor V gene, which in turn causes the change in Arg 506 to Gin (12-15). The reported prevelance of this mutation in the Factor V gene varies in different populations (7,10). It seems that the defect itself is sufficient to cause thromboembolism (8).

Because of the infrequent occurrence of APCR in general population it is necessary that some patients with CVA also have this risk factor for thrombosis. In adults the impact of APCR for the thromboembolic events has been well documented (8,10,16).

In this report we evaluated our patients with CVA within the last 4-year period and the role of the defects in the regulation of coagulation was found in some patients.

PATIENTS AND METHODS

A total of 13 patients with CVA were reviewed who had been observed at Ankara University Medical School in the Department of Pediatric Neurology. The

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diagnosis was based on clinical findings, computarized tomography (CT) scan, magnetic resonance imaging (MRI), and angiography in some cases.

Plasma protein C, protein S and antithrombin III were assessed in 10 patients and APCR and FV Leiden in 5 patients. Quantitative analysis of protein C in plasma was studied by clotting assay (Bioclot, TM protein C). Normal ranges in this method are 60%- 140%. The assessment of protein S was the same as protein C. The expected normal range for protein S is 55%-160%. Functional levels of AT-III in plasma was determined by an amidolytic method using a synyhetic chromogenic substrate (chromostrate TM). Normal values of AT-III are expressed as 80%-120%.

DNA was extracted by conventional methods and polymerase chain reaction of exon 10 of Factor V gene was performed according to previously described method using primers 5' TCAGGCAGGAACAACACC 3' and 5' GTTACTTAAGGACAAAATACCTGTAAAGTC3'. Amplification was performed for 35 cycles with annealing temperature of 58C (Ericomp USA). Amplified DNA was digested with Hind III enzyme (Promega, USA) at 37C and subjected to 2% agarose gel electrophoresis (12).

RESULTS

We reviewed 13 patients with CVA; their ages at initial diagnosis were between 13 months-12 years (mean: 6.5 years±2.4 years). Five were female and eight were male. Prominent symptoms were hemiparesis, seizure or combination of those.

Patients' characteristics and neuroradiologic findings were summarized in Table 1. Angiography was performed in 7 patients. Moyamoya was determined in one patient (no:6). Protein C level was normal in 7 patients. Three patients protein C levels were 42.5%, 54.4% and 27.5% rerpectively (no:10,12,13). Protein S was normal in 9 patients, and in one patient (no:11) it was 0%. AT-III was low (65%) in one patient (no:10). APCR and FV Leiden were investigated in five patients. FV gene mutation was found in 2 patients (40%, 2/5) (no.9 and13). APCR was determined in only one patient (no:10).

DISCUSSION

Risk factors and pathogenesis of the CVAs may not be demonstrated in every case in childhood. Moreover cerebrovascular diseases in children unlike that adults is uncommon. When it occurs the consequences are severe. We followed 13 patients with CVA during the 4-year period and could not determine the etiology of CVA in 12 patients.

In adults the impact of APC resistance for thromboembolic events is well documented but in children information about the role of FV 1691G→A in the pathogenesis of thrombosis is scarce. In two published studies APCR was identified as a strong risk factor for thrombosis affecting 46% and 25% respectively (7,8).

In our study we studied FV gene mutation in 5 patients and found heterozygosity in 2 patients (40%). The prevelance of FV gene mutation in Turkey is 9.8% (17). This high incidence in our patients is probably due to the type of thrombosis, that they are all stroke patients. It is shown that in infants and children FV mutation can be a risk factor for arterial thrombo-embolism in contrast to adult patients.

Protein S deficiency is rather rare in childhood stroke.There are only four reports with 5 patients and two of the patients were thought to have acquired protein S deficiency (2-5). In our study population one of the patients had protein S deficiency. Our measurements were made in a stable stage which is at least after two months of the insult. Since we could not determine the protein S levels of the parents of this patient we could not say that this was inherited protein S deficiency.

In 3 (23%) of our patients protein C deficiency was found. In one patient it was associated with APCR but FV 1691G→A mutation could not be detected in this patient. This lack mutation can be due to another mutation yet to be unknown. In the other patient low protein C level was associated with FV 1691G→A mutation.

We conclude that in the pathogenesis of childhood stroke protein C deficiency and APCR resistance play an important role and should be determined in all stroke patients.

Table 1. Patients' Characteristics

No of patient	Sex	Age (y / m)	Neurologic findings	Neuroradiologic findings	Angiography	Proteni C	Protein S	Antithrombin III	APCR	Factor V Leiden
1	M	4 y	Right hemiparesis Seizure	Hypodense area in the left centrum semiovale	-	-	-	-	-	-
2	M	12 y	Left hemiplegia	Intraventricular hemorrhage	-	-	-	-	-	-
3	M	3 y	Left hemiparesis	Ischemic area in right parietal cortex	-	-	-	-	-	-
4	F	10 y	Left hemiparesis Seizure	Hypodense area in throughout right middle cerebral artery and linear hypodense regions	N	N	N	N	-	-
5	F	13 y	Right hemiparesis Seizure	Acute infarct on left cerebral artery level	Obstruction of left middle cerebral artery	N	N	N	-	-
6	F	7 y	Right hemiplegia	Left basal ganglia infarction	Moyamoya	N	N	N	-	-
7	F	11 y	Left hemiparesis	Right temporoparietal infarction	N	N	N	N	-	-
8	M	15 m	Right hemiplegia	Large infarct on left hemisphere	N	N	N	N	-	-
9	F	13 m	Left hemiparesis Seizure	Chronic infarct on right arterial media level	-	N	N	N	N	+/-
10	M	8 y	Left hemiparesis	Right parietal infarct	N	↓ (42.5 %)	N	↓ (65 %)	+	-/-
11	M	18 m	Left hemiparesis	Ischemic area in right cerebral hemisphere	-	N	↓ (0 %)	N	N	-/-
12	M	12 y	Left hemiparesis Seizure	Hemorrhagic infarct in right cerebral hemisphere	N	↓ (54.4 %)	N	N	N	-/-
13	M	10 y	Right hemiparesis	Infarct at left hemisphere	N	↓ (27.5 %)	N	N	N	+/-

M: male, F: female, y: years, m: months, N: normals

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EFFECTS OF THE LOCAL ANESTHETIC AGENTS ON THE BLADDER AND URETHRAL MUSCLES*

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SUMMARY

Objectives: In urology local anesthetics are used for topical anesthesia of urethra and bladder before endoscopic procedures.But there is a few animal study about local effectiveness of these agents on bladder or urethra muscle. In this study we investigated topical effects of local anesthetics.

Methods: We tried to determine effects of prilocaine, lidocaine and bupivacaine on urethral and bladder smooth muscle strips which are derived from rats.

Results: Lidocaine (20mg/ml) and prilocaine(60mg/ml) have significant inhibitory activity at these concentrations ($p<0.05$) but they were found ineffective at low concentrations as local anesthetic agents.Bupivacaine has excitatory effect at low concentrations(1-3mg/ml) versus high concentrations which has inhibitory effect similar to other local anesthetic agents. KCl solution(80 mEq/L) was used for depolarization.

Conclusion: We found that the concentration of local anesthetics on tissue level is important for the effects of anesthetics and at high concentrations, they have inhibitory effects.

Key words: *Bladder,Urethra,detrussor paralysis,local anesthetics*

Local anesthetic agents are generally used for pain relief due to surgical procedures, trauma and chronic pain syndromes by regional (spinal or epidural), topical (skin and mucosa) and intravenous route(1). It is known that these agents blocks cellular Na channels and inhibits neural transmission(2). In clinical doses (1-4mM tissue concentrations) these agents inhibit neural transmission beside enzymatic (phospholipases, protein kinases) and receptor (β adrenergic, cholinergic) activities. Therefore local anesthetics have antihistaminic, antispasmolytic, narcotic and anticholinergic effects(1). Urine passage depends on both renal urine production and spontaneous peristalsis of ureter and bladder muscles. The peristaltic motions depend on the local mediators more than extrarenal mural stimulation(3). Previous studies reported that volatile anesthetic agents can decrease urinary peristalsis due to MAC potens level(4).

Lidocaine, prilocaine and bupivacaine are used commonly in everyday clinical practice for regional anesthesia and in the recent years topical bupivacaine application into the bladder is gaining popularity(5,6). Bupivacaine was found effective in decreasing bladder contractions compared to lidocaine which was found at ineffective.

In this experimental study we aimed detecting tissue level effects of the local anesthetic agents on isolated rat bladder and urethra stripes.

METHODS

Spraque-Dawley male rats weighing between 200-250 gm were used in this study. Rats were sacrificed by cervical dislocation. Bladders and urethra of the rats were resected en-bloc serially then transferred to Petri dish which filled with Krebs solution. Specimens were cleaned away from blood, connective and mucosal tissues. Vertical incisions were performed about 1cm lenght and 0,2 cm width. 2 and 3 stripes were gained from each specimen. Stripes were fixed in the

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* The study was done in GMMA Investigation Department with the permission of Investigation Committee

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Krebs tissue media which one end was stabilized at the base of Petri dish and the other end fixed to the hook of Force 45-196A transducer. Krebs solution was composed of NaCl 120.7, KCl 5.9, CaCl2 1.25, MgCl2 1.2, NaHCO3 15.5, NaH2PO4 1.2, Glucose 11.5 mM/lt. Tissue media containing 10 ml Krebs solution and media aeration was supplied with %95 O2 -%5 CO2. Temperature was kept at 37 °C. Accomodation of the tissue stripe to the environment takes more than one hour. 1 gm tension was applied to the tissue while in accomodation period. Bladder contractions were transmitted to an amplificator and then to the NEC-Sanei polygraph for recording. Contraction responses to KCl ED50 doses were evaluated at the end of the accomodation period. ED50 was 80 mM/L for KCl solution. Six doses of lidocaine, prilocaine and bupivacaine were tested in different concentrations(1,3,10,20,30,60 µg/ml). Tissue stripes were exposed to the anesthetic agents about 1 min. duration. Then amplitudes were evaluated in response to each agent. Tissues were rinsed three times after every attempts in various concentrations. Obtained data were assessed as % values based on KCl ED50 contractions. Statistical analysis was performed by Wilcoxon Signed Rank-Sum Test.

RESULTS

Inhibitory effect was detected in prilocaine group at 20-60µg/ml (Table I).

In bupivacaine group; small doses (1-3µg/ml) have stimulatory effect but high doses (30-60µg/ml) have inhibitory effect ($p<0.05$) (Table II). This effect could probably depend on one of the secondary effects rather than local anesthetic effect.

In lidocaine group, an inhibitory effect was detected after 2 µg/ml dose ($p<0.05$) (Table III).

DISCUSSION

In this study we investigated the direct motor effects of the various local anesthetic agents on bladder and urethral contractions.This is a well recognised effect of local anesthetic agents.t Previous studies have examined the effect of local anesthetics on the detrussor instability and bladder capacity.But they have given conflicting results on the influence of local anesthetics on detrussor contractility. They tend to suggest that sensory blockage is the more likely mechanism (7,8,9,10) However,the permeability of the bladder mucosa is well -known and our study shows us one could make detrusor paralysis only by using local anesthetics permeating into the muscle layer through the mucosa at high intravesical doses, but at lower se-

Table I: Prilocain Group

DOSE	0.5 µg/ml	1µg/ml	3µg/ml	10µg/ml	30µg/ml	60µg/ml
RESPONCE	–	–	–	–	↓	↓
SIGNIFICANCE	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

Table II : Bupivacain Group

DOSE	0.25µg/ml	0.50µg/ml	1.5mg/ml	5mg/ml	15mg/ml	30m/ml
RESPONCE	↓	↑	↑	–	↓	↓
SIGNIFICANCE	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.05$

Table III: Lidocain Group

DOSE	0.2µg/ml	0.6µg/ml	2µg/ml	6µg/ml	20µg/ml	60µg/ml
RESPONCE	-	-	↓	↓	↓	↓
SIGNIFICANCE	$p > 0.05$	$p > 0.05$	$p< 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

rum concentrations. The mechanism is the direct effects of anesthetics on detrussor muscle.They can inhibit smooth muscle contractility.So, together with electromotile drug administration methods, local anesthetics could be used for detrussor instability, hyperreflex bladder, increasing bladder capacity and intravesical operations(11,12).We do not investigate the exact doses for these kinds of applications.But if you increase the mucosal permeability by any kind of method, you can obtain total detrussor paralysis.This is clinically more important conclusion of the study,especially outpatient procedures than which sensorial fibres affected from local anesthetic agents.

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THE INCIDENCE OF POSTOPERATIVE DEEP VEIN THROMBOSIS FOLLOWING ABDOMINAL SURGERY

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SUMMARY

We investigated the incidence of postoperative deep venous thrombosis (DVT) in 200 patients who underwent an elective abdominal surgery. DVT was diagnosed in 27 of 200 patients. Of 27 patients with DVT, 17 did not have any clinical findings and diagnosis was made on the basis of color doppler USG findings. Ten of 27 patients had clinical findings of DVT and diagnosis was confirmed with doppler USG. The overall incidence of DVT was 13.5% and probability of correct diagnosis of DVT based on clinical findings alone was less than 40%, suggesting that clinical examination is notoriously unreliable in establishing the diagnosis of DVT and is clearly inadequate for making a more subtle determination of extent of disease. In this study, duration of operation (>45 minutes), hospital stay (>5 days) and age of the patients were found to be important risk factors for postoperative DVT. Color doppler USG is a highly sensitive and specific non-invasive technique for diagnosis and follow-up of patients with DVT.

Key words: *Deep vein thrombosis, abdominal surgery, color doppler USG*

The diagnosis and management of venous thrombosis and pulmonary embolism may challenge the skills of the most experienced clinicians, in both ambulatory practice and inpatient service. Thromboembolic disease is common, may present either insidiously or abruptly, and is associated with considerable acute and long term morbidity and mortality (1). There is no clear-cut best approach to either diagnosis or treatment. Therefore, objective diagnostic methods are frequently applied for diagnosis of deep venous thrombosis (DVT). Color doppler ultrasonography (USG) is increasingly used for diagnosis of DVT with a sensitivity rate of up to 90% -95% (2).

DVT following abdominal surgery is not uncommon, and sometimes may lead to fatal complications. The predisposing factors and exact incidence of postoperative DVT is not clearly known. Autopsy studies showed that cause of death in 10% of cases was pulmonary emboli (PE), and in 83% of these PE was associated with DVT and 24% of these patients had surgery in a week prior to death.[3] Unfortunately, prior to death, DVT has been determined in only 19% of these patients with PE (3). Sometimes chronic venous insufficiency may be a morbid result of DVT despite intensive treatment. It has been reported that postphlebitic syndome may develop in 50%-60% of patients with proximal vein thrombosis, 5 to 7 years after the event (4).

In literature, patients who underwent surgical operations have been stratified as low, moderate, and high risk patients as follows (5):

Low - risk patients

1. Operation time < 30 min.
2. Age < 40 and operation time > 30 min.
3. No additional risk factors.

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Moderate - risk patients

1. Age > 40
2. Major general, gynecological, or urological surgery.
3. Younger patients with additional risk factors.

High - risk patients

1. Major trauma
2. Surgery of hip, knee, or lower limb
3. Operation for pelvic or abdominal surgery for malignancy.
4. Those undergoing major surgery with a history of previous venous thromboembolism or thrombophilia.

Based on the type of the surgical intervention, the incidence of DVT may change. It has been reported that the incidence of DVT following abdominal surgery was 15%-35% as compared to 40-55% following hip surgery (6). Postoperative DVT is a common problem and may cause fatal complications, but early diagnosis and treatment may significantly decrease the incidence of these complications. Recent studies showed that perioperative prophylaxis of thromboembolism using heparin or pneumatic compressions in patients undergoing general surgical interventions decreased the incidence of postoperative DVT (7)

As far as our knowledge, there is yet no study indicating the actual incidence of postoperative DVT in general surgery patients in Turkey. In this study, we attempted to evaluate risk factors and the incidence of postoperative DVT using physical examination findings and color doppler USG in 200 patients undergoing abdominal surgery.

MATERIALS and METHODS

We determined the incidence of postoperative DVT in 200 patients who underwent abdominal surgery with intratracheal anesthesia. In this study, all patients were more than 35 years old. Of 200 patients, 117 were female and 83 were male.

Common risk factors, which were evaluated in this study, for postoperative DVT are given in Table 1.No mechanic or pharmacologic DVT prophylaxis were performed in order to establish the actual incidence of postoperative DVT in general surgery patients in Turkey. Superficial and deep vein systems of lower extremities were evaluated with color doppler USG the day before and 7 days after the surgery. Color doppler USG evaluation included the presence of reverse flow patterns at several levels of deep and superficial veins

Table 1. Common Risk Factors for DVT and Distribution of These in Patients with and without DVT

RISK FACTORS	PATIENTS with DVT		PATIENTS without DVT	
	number	%	number	%
Age>60	15	56	44	25
Presence of malignancy	9	33	57	33
Diabetes mellitus	6	22	31	18
Obesity	10	37	32	18
History of previous DVT	3	11	4	2
History of previous PE	0	0	0	0
Congestive heart failure	8	29	12	7
COPD	4	15	14	8
Pregnancy (3rd trimester)	0	0	0	0
Number of births (>3)	15*	80*	39*	23*
Oral contraceptives	3	11	9	5
Presence of varicose veins	7	26	26	15
Chronic venous insufficiency	2	7	11	6
Fungal infection of lower extremities	2	7	9	5
Duration of surgery > 40 min.	19	70	80	46
Duration of hospital stay >5 days	23	85	107	62
Smoking	8	29	63	36
NSAIDs Intake	4	15	30	17

Abbreviations DVT : Deep vein thrombosis, PE: Pulmonary emboli,
COPD : Chronic obstructive Pulmonary Disease
* : Calculated for female patients

and at saphenofemoral and saphenopopliteal junctions. Presence of thrombi, superficial venous dilation and evidence of excessive collateral venous circulation were also evaluated.

Patients with vascular disease, thrombocytopenia (< 100 x 10^9/L), essential thrombocytosis (> 500 x 10^9/L) or myeloproliferative syndromes, and receiving anticoagulant treatment were not included in this study. Patients who underwent an orthopedic, gynecologic or neurosurgical operation in last 6 months were excluded from this study. Patients were ambulated as early as possible following surgery.

RESULTS

Twenty seven of 200 (13.5%) patients without DVT in preoperative evaluation received a diagnosis of DVT during the postoperative evaluation on day 7. Of these 27 patients, 19 were women and 8 were male. Seventeen of 27(63%) did not have any sign or physical finding of DVT except a positive color doppler USG study. The color doppler ultrasonographic views of a normal lower extremity and femoral thrombi are shown in figures 1,2, and 3 respectively. Ten of 27(37%) had clinical findings of DVT and this was confirmed with color doppler USG. We determined that physical examination has 37% sensitivity as compared to color doppler USG in diagnosis of DVT. The location of thrombus formation on the basis of color doppler USG evaluation in 27 patients are shown in Table 2. Of 27 patients, 13 had isolated distal vein(calf) thrombosis, 7 had isolated proximal (thigh) vein thrombosis, and 7 had distal and proximal thrombosis. Risk factors in 27 patients with DVT are given in Table 1. Fifteen of 27 patients(55.5%) with DVT were more than 60 years old. Nineteen of 15 women(80%) with DVT had history of 3 births. Duration of surgery was more than 45 minutes in 19 of 27 patients(70%) with DVT. Duration of hospital stay was more than 5 days in 23 of 27 patients(85%).

DISCUSSION

It is widely accepted that contrast venogram is a gold standard for diagnosis of DVT. Because of invasiveness of contrast venogram and side effects of contrast agents non-invasive techniques are increasingly being preferred.[1] Non-invasive techniques such as I^{125} fibrinogen uptake test, plethysmography and color doppler USG are increasingly used in clinical settings (1,8). Furthermore, high sensitivity and specificity with MR scanning in the diagnosis of DVT has already been reported (9) Color doppler USG, as a diagnostic tool in DVT is a non-invasive, cheap, practical, and easily accepted method by patients. We have to emphasize that the success of color doppler USG is highly operator dependent and diagnostic sensitivity, in expert hands, in the diagnosis of proximal vein thrombosis of lower extremity is 95% (2,8). Several studies, using compressibility as a diagnostic criteria and contrast venography as a referance method, reported a 98-100% specificity rates with color doppler USG in the diagnosis of DVT in patients with a clinical suspicion of thrombosis (2,6,8)

The non-invasive tests, except radioactively labeled fibrinogen uptake scanning (rarely useful clinically) are insensitive (less than 50%) to isolated calf DVT (10,11). A negative non-invasive evaluation presents the clinician with two options. The patient can undergo contrast venography, which remains the definitive test for DVT, or can be followed with serial non-invasive tests performed every 1 to 3 days until proximal extention has been ruled out (usually 7 to 14 days) (11). Patients at low risk can be followed non-invasively. High-risk patients with a presentation strongly suggesting DVT or risk factors for associated morbi-

Table 2. Location of Thrombosis with Color Doppler USG

LOCATION of THROMBOSIS	NUMBER of PATIENTS	CLINICAL FINDINGS	
		Positive	Negative
Isolated distal (*calf*) vein thrombosis	13	2	11
Isolated proximal (*thigh*) vein thrombosis	7	4	3
Distal and proximal (*calf and thigh*) vein thrombosis	7	4	3
TOTALS	27	10	17

Table 3. Distribution of Patients According to The Types of Operations and Time of Surgery

OPERATIONS	Mean Operation Time (minute)	Patients with DVT		Patients without DVT	
		Number	%	Number	%
Cholecystectomy	45	7	26	44	25
Other biliary procedures	60	2	7	15	9
Gastric resections	110	3	11	19	11
Vagotomy + Drainage	90	0	0	12	7
Colonic resections	90	6	22	25	14
Hepatic operations	140	4	15	16	9
Pancreatic operations	130	1	4	4	2
Surrenalectomy	120	0	0	3	2
Splenectomy	75	0	0	6	3
Ventral hernia repairs	60	2	7	14	8
Others	50	2	7	15	9

dity should be considered for venography. It may be appropriate to administer anticoagulant therapy to such patients until venography can be done. There is a considerable date base to support the use of serial non-invasing testing while withholding anticoagulation in managing low-risk patients with suspected calf-only DVT. Withholding therapy in the presence of a documented distal DVT is less clearly and acceptable option, however. Patients with significant local symptoms, a high probability for complications of thromboembolism, and relatively low risk for complications of anticoagulation should certainly be treated (7,11).

Several authors reported that the incidence of postoperative DVT in patients more than 40 years-old following an abdominal surgery was 14-35%, where as this incidence was up to 60% in patients more than 60 years-old (12). In the current study, 15 of 27 patients (55.5%) with DVT were more than 60 years-old. In 70% of patients with DVT, duration of surgery was more than 45 minutes. In 85% of patients with DVT, duration of hospital stay was more than 5 days. Seventynine percent of female patients with DVT had a history of more than 3 births. These data suggest that increased age, duration of surgery, hospital stay and number of births for female patients are significant risk factors leading to DVT.

This study also showed that the incidence of DVT in 200 patients entering to this study was 13.5% and the probability of correct diagnosis on the basis of clinical findings alone is less than 40%. This suggest that clinical examination is notoriously unreliable in establishing the diagnosis of DVT and is clearly inadequate for making a more subtle determination of extent of disease.

In summary, color doppler USG is a non-invasive, cheap, repeatable, and easily appliable diagnostic tool for surgical patients. In high risk patients, the predictive value for a positive test with color doppler USG is greater than 95% for diagnosis of thrombosis. Age, duration of surgery and hospital stay, and number of births are important risk factors leading to DVT. Clinical examination alone is notoriously unreliable in establishing the diagnosis of DVT.

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AN ALTERNATIVE METHOD TO PROSTHETIC SPHINCTER IN THE TREATMENT OF INCONTINENCE; GRACILIS URETHRAL MYOPLASTY- A REVIEW ARTICLE

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SUMMARY

Using the neurovascular intact gracilis muscle as an urethral wrap instead of prosthetic urethral sphincter is a novel surgical method in the treatment of severe incontinence. The functional urethral closure of gracilis urethral myoplasty (GUM) provides dryness, permits intermittent self catheterization if necessary, and avoids the risk of erosion associated with the artificial sphincter. Electrical stimulation (ES) of the transposed muscle using intramuscular electrodes and a subcutaneously placed pulse generator can alter the molecular physiology of the gracilis muscle from predominantly fast-twitch to fatigue resistant slow twitch fibers that is necessary for long-term sphincter function. This procedure has been done in several centers. We are going to introduce this surgical method with its clinical results.

Key words: *Urinary incontinence, graciloplasty, electrical stimulation*

Gracilis muscle was first used in the treatment of incontinence 71 years ago [1]. Since gracilis muscle is prominently composed of fast twitch fibers that are strong but easily fatigued, the patient can only keep up their continence during contractions. Recently, neurostimulation has been used to reinforce skeletal muscle function. Fast twitch fibers are converted to fatigue resistant,slow twitch fibers with constant low current electrical stimulation. This method was described in the use of latissimus dorsi muscle for cardiomyoplasty [2]. Gracilis muscle myoplasty was first applied to patients who had faecal incontinence to create a new sphincter mechanism. This technique utilized a nuerovascularly intact gracilis muscle as a circumferential perianal wrap. Constant low current stimulation provided through intramuscular electrodes connected to a pulse generator was used to permit the gracilis muscle to act as a sphincter[3]. Janknegt and associates developed this method for the treatment of urinary incontinence[4].

OPERATIVE TECHNIQUE AND MUSCLE TRAINING

The patient is placed in the dorsal lithotomy position. Two medial incisions are made parallel to long axis of the gracilis muscle. The muscle is mobilized and tendinous portion is incised immediately cranial to the knee joint. Great care is taken to avoid manipulation or damage to gracilis muscle's neurovascular pedicle located along the proximal medial one third of the muscle belly. Intraoperative electrical stimulation during dissection confirms the location of neurovascular pedicle, and assures the viability of the muscle.

The gracilis muscle is tunneled subcutaneously without tension to the perineum. A perineal inverted "U" incision exposes the periurethral region allowing mobilization of bulbous urethra and encircling of muscle. The gracilis is threaded around the urethra a 360 degree wrap closely approximating the gracilis muscle and urethra. The bulbous urethral wrap is stabilized by delivering the distal portion of the gracilis

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muscle through a tunnel created in the proximal portion of the muscle belly adjacent to the urethra (Similar to the locking mechanism of artificial urethral sphincter). (Fig. 1) If the bladder neck is selected to wrap the gracilis muscle, pfannenstiel incision is necessary to prepare a space around the bladder neck. The distal edge of the gracilis muscle is then sutured to the contralateral pubic bone with non absorbable sutures.

Two intramuscular electrodes are sutured into the muscle belly near the nerve insertion site. Two electrodes are secured when maximal contraction is observed during intraoperative testing. Insulation sheaths are secured and electrodes are tunneled to the lower abdomen where they are connected to a subcutaneously positioned pulse stimulator (Itrel III™, Medtronic, Minneapolis, MN). (Fig. 2) This procedure can be done at the time of the operation or 6-8 weeks later.

After implantation, a stimulation program is initiated that will transform the gracilis fibers from easily fatigued, type II fast-twitch fibers to type I fatigue resistant, slow-twitch fibers. Intermittent stimulation is started with a cycle of 0.125 seconds "on" and 2 seconds "off" with a burst frequency of 25 Hz. The voltage is adjusted at the level of contraction perception (1-4 volts). The stimulation cycle is increased every two weeks with an external programmer. By 8-12 weeks the cycle is 100% "on" with frequency being decreased to 15Hz.

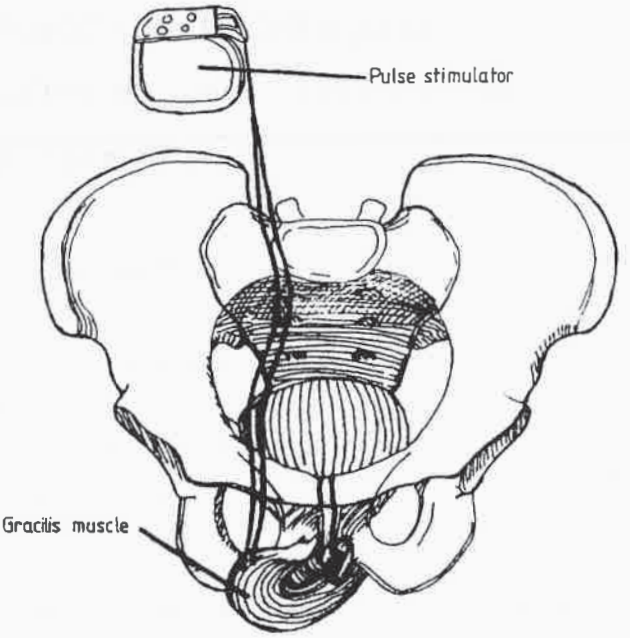

Fig. 2. The pulse generator is placed subcutaneously in the lower abdomen and connected to two intramuscular electrodes.

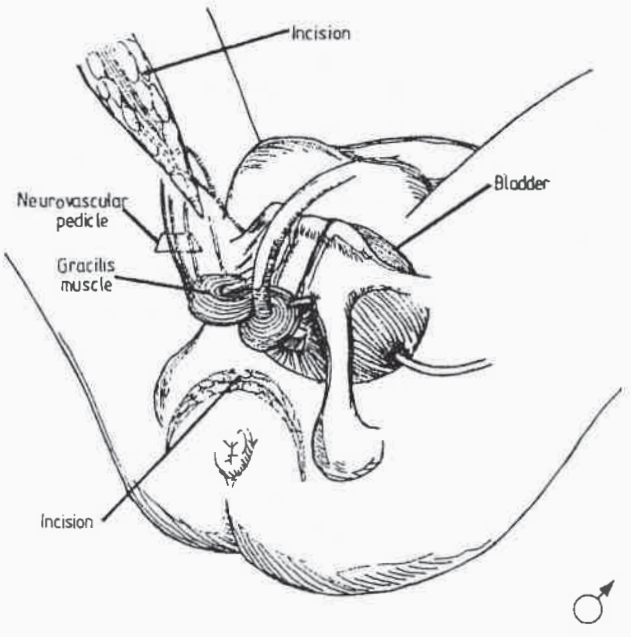

Fig. 1. Illustrations of gracilis muscle wrap around the bulbous urethra with the tendinous insertion of the gracilis muscle is sutured to contralateral pubic bone.

The dynamic urethral myoplasty is set to "on" stimulation setting except during micturition. The pulse generator is turned "off" by the patient with a programmer for micturition.

DISCUSSION

Gracilis urethral myoplasty has been studied in several animal experiments to establish its feasibility.

GUM with or without ES were compared in a rat model[5]. GUM with electrical stimulation achieved significantly greater leak point pressure and leak point volume than control and unstimulated gracilis myoplasty. Split sling graciloplasty, was compared with conventional graciloplasty in a rabbit model[6].. This technique was described by Rosen et al.[7]. Although achieved pressures are higher with the split sling graciloplasty the difference was not statistically significant when compared to the conventional graciloplasty.

Results of gracilis wrap placement around the bladder neck and bulbous urethra with or without ES were compared in the billy goat model[8]. Stimulated graciloplasty around the bulbous urethra was found superior to the bladder neck graciloplasty.

Four clinical studies exist in the literature on gracilis urethroplasty applied with or without electrical stimulation in the treatment of severe incontinence. The first study belongs to Janknegt and associates in 1992 [9]. After this preliminary report Williams et al. performed electrically stimulated gracilis urethral myoplasty in 4 patients. They wrapped the gracilis muscle around the bulbous urethra and implanted the electrodes and stimulator at the same time. Although 3 of the four patients gained continence postoperatively, dense urethral strictures occurred in all patients within four months. Corrective surgery for urethral strictures was necessary but only one patient was able to keep up continence after surgery. Two patients needed permanent suprapubic catheters and one needed urinary diversion. The authors correlated frequent occurrence of urethral stricture with urethral ischemia because of the location of the wrapping[10].

In the Maastrich study, Janknegt et al. performed the gracilis urethral myoplasty in 7 patients [4]. They wrapped the gracilis muscle around the bladder neck. While this method showed complete success in three patients and partial success in 1 patient, three patients did not show any improvement postoperatively.

In addition to seven patients, two boys with a spina bifidas resulting in total urinary as well as faecal incontinence were treated [11]. One gracilis muscle was wrapped around the bladder neck and the other around the anal canal. Six weeks later, electrodes and pulse generators were implanted. One patient was continent for faeces and urine after the operation. He was planned for intermittent self catheterization for urinary retention. The second patient did not use diapers any more but he still had several wet episodes a week. He remained continent for faeces if he applied a regime of defecating twice a day.

Chancellor and associates performed GUM in neurologically impaired 5 incontinent patients[12]. All patients had spinal injuries. Surgery was successful in four patients at mean 16 months follow-up. Three of four patients managed with intermittent catheterization and one managed by ileocystostomy. Electrical stimulation was added to the treatment in a second operation in one patient whose mild incontinence disappeared completely. Another patient needed collagen injection after GUM and he became completely dry. Collagen injection into the gracilis wrap is technically easy and can further improve continence.

Chancellor and associates also used the same technique for the treatment of postprostatectomic incontinence in three patients[13]. All three patients had radical retropubic prostatectomies combined with external beam radiotherapy for prostate cancer. Two patients were dry after the dynamic GUM. The third patient who did not have ES reported only 20% incontinence. All patients were able to urinate without needing self catheterization.

Gracilis muscle is ideally suited for urethral myoplasty because of its location along the superficial median aspect at the thigh and high insertion level of the neurovascular bundle. The gracilis muscle normally helps to rotate the thigh inward. Removing the gracilis from the leg does not cause any mobility problems. No lower extermity deficits were found in the studies in which gracilis muscle was used to create anal sphincter.

The advantage of the dynamic urethral myoplasty is the usage of well vascularized autologous tissue which may reduce the risk of infection, urethral erosion and mechanical failure associated with artificial sphincters.

Location of gracilis wrap still remains controversial. While the placement of bulbous urethral wrap is easier than bladder neck, such an approach may increase the incidence of urethral stricture development postoperatively [14]. We prefer the bulbous urethral wrap which is easier than bladder neck urethral myoplasty.

As a result, dynamic urethral myoplasty is an exciting and promising treatment for severe stress incontinence for both genders. It may even be feasible for patients who are not candidates for the artificial urinary sphincter or had failed sphincter operations.

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A CASE OF GIANT PROSTATIC HYPERPLASIA SUCCESFULLY MANAGED WITH COMBINED SUPRAPUBIC AND RETROPUBIC PROSTATECTOMY TECHNIQUE

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SUMMARY

Giant prostatic hyperplasia is a very rare pathologic entity with only 11 reported cases of prostate glands exceeding 500 g. We reported a case of a giant prostatic hyperplasia treated by combined suprapubic and retropubic techniques with no associated complications and low morbidity.

Key words: *Giant prostatic hyperplasia, combined suprapubic and retropubic techniques, morbidity.*

Benign prostatic hyperplasia (BPH), the most common benign tumor in men, is responsible for urinary symptoms in the majority of men older than 50 years of age and results in the need for a prostatectomy in 20 to 30 percent of men who live to age 80 years (1). However, prostate sizes greater than 100 g are uncommon occurring in only 4 percent of men over 70 years of age (2). We reported here the uncomplicated removal of a 542-g prostatic adenoma by combined suprapubic and retropubic techniques.

CASE REPORT

A sixty seven-years-old man admitted to our clinic with acute urinary retention. On questioning, he had long-standing symptoms of bladder outlet obstruction, and he was catheterizing himself intermittently. On physical examination, he had a palpable bladder with the sign of suprapubic tenderness. Digital rectal examination revealed grossly enlarged benign prostate Bladder catheterization returned 850 ml of urine. Ultrasonography showed a thickened bladder wall with bilateral normal kidneys and a very large prostate. His creatinine level was 1 mg/dl and this was believed to be due to intermittent catheterization. IVP had showed bilateral normal kidneys with bilateral moderate dilatation of lower ureters and bilateral fishhook sign. Cystography showed a large prostatic protrusion into the bladder and an irregular bladder vall. IPSS was 33.

The patient was surgically treated by combined suprapubic and retropubic prostatectomy with blood loss estimated at 400 ml and no transfussions necessary. A large adenoma was enucleated entirely in one piece (Fig. 1). When evaluated on the third month postoperatively the patient was voiding satisfactorily to completion and was continent. Uroflowmetry revealed a peak folw rate of 28 ml/s and IPSS was 1.

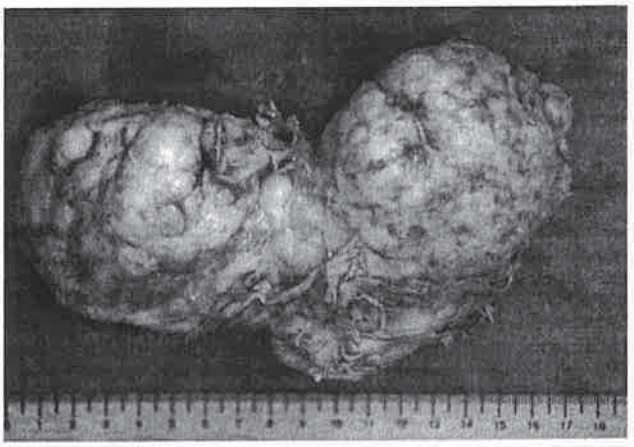

Fig. 1. Prostatic adenoma, gross weight 542 g.

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Pathologic examination of the specimen was notable for a gross weight of 542 g and measured as 17.3 cm, 10.1 cm and 9.6 cm in three dimensions. Histologic examination revealed a predominance of glandular hyperplasia with numerous dilated glands and smaller amounts of stromal hyperplasia. Focal areas of chronic inflammation and infarction with two small focuses of PIN II were seen. In addition to these findings, small amounts of prostatic calculus were seen in the lumen of the dilated glands.

CONCLUSION

The natural history of the disease predicts that larger specimens will occur with advancing age (2). However, giant prostatic hyperplasia (GPH) is an extremely rare pathologic entity throughout the world with only 11 reported cases of prostate glands exceeding 500-g (2). Our patient was the youngest reported case with GPH.

While Kawamura et al. accepted the term giant-prostate for glands exceeding 200-g (3), we accepted it to be over 500 g since Fishman and Merrill reported that such specimens are extremely rare throughout the world (3).

Searching for the type of the open procedure chosen in literature for those GPH, 11 cases found and 8 of them treated by suprapubic, 2 of them retropubic and only 1 of them by combined technique. Suprapubic prostatectomy has been recommended previously for removal of such very large adenomas, with an 820-g specimen being the largest report (4). However, we performed the combined technique and this was thought to allow enucleation of the adenoma entirely in one piece with significantly decreased hemorrhage and morbidity. Direct visualization of the prostatic fossa an the bladder concomitantly serves as and advantage for removing such large adenomas and fully management of unexpected gross hemorrhage or prostatic fossa and bladder injury.

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PULMONARY EDEMA AFTER MULTIPLE VENOUS AIR EMBOLI

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SUMMARY

Acute pulmonary edema following venous air embolism is not very common, but well documented. This complication of venous air emboli is reported to occur rapidly or several hours later, but usually only after multiple episodes. We report an 18 year - old male patient who developed postoperative pulmonary edema following multiple episodes of venous air emboli while in the sitting position for correction of Arnold-Chiari malformation.

Key words: *Pulmonary edema, sitting position, venous air embolism.*

Neurosurgical procedures in the sitting position is a setting in which venous air embolism (VAE) has been frequently described (1-4) and is reported to be between 21% and 40% (5). This high incidence has led some surgeons to advocate the use of prone position. The sitting position is, however, still preferred in many units since its advantages include better access to the patient and his airway, reduced hemorrhage and improved surgical exposure.

Site of air entry in VAE is found in only 35% of cases and incidence of severe morbidity or mortality is about 1% (6). A rare complication of VAE is pulmonary edema which usually occurs rapidly or several hours later but only after multiple episodes of air trapping (7).

CASE REPORT

A fit 18-year old male patient was admitted to the hospital with a 2-year history of headaches and dizziness. Past medical history was not contributory and laboratory studies, physical examination, chest X-ray (CXR) and ECG were normal. Tomograms showed descent of the cerebellar tonsils to the level of the arch of the atlas and the diagnosis of Arnold-Chiari malformation was made. The patient was classified ASA physical status I, preoperatively.

The patient received 0.5 mg atropin and 75 mg meperidin IM one hour before the operation. Anesthesia was induced with 0.5 mg alfentanil, 500 mg thiopental and tracheal intubation was facilitated with 8 mg vecuronium. Maintenance was achieved with 50% N_2O and 1% isoflurane in O_2. Additional doses of vecuronium were used for muscle relaxation.

Monitoring consisted of ECG, pulse oximeter, direct arterial pressure, end-tidal carbon dioxide ($PETCO_2$) and urinary catheter. Elastic stockings were used to minimize venous pooling. The patient was placed in the conventional supine sitting position. Normal saline was infused to maintain hemodynamic stability. During dissection of the dura $PETCO_2$ decreased from 29 mmHg to 20 mmHg and arterial O_2 saturation (SpO_2) fell from 99% to 77%. No change was observed in blood pressure, heart rate, minute volume or airway pressure. Diagnosis of venous air embolism was made, N_2O was discontinued and the patient was ventilated with 100% O_2. Bilateral neck compression was performed and possible site of air entry was packed with sponges. End-tidal carbon dioxide and SpO_2 gradually returned to normal in fifteen minutes. In the

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following 2 hours, two more similar episodes occurred, showing the same changes in $PETCO_2$ and SpO_2. Hypotension or arrythmias did not occur. At the end of the operation $PETCO_2$ was 27 mmHg and SpO_2 was 97%. The patient was awake and fully cooperative after extubation. However in the following half hour the patient showed respiratory distress; on auscultation of lungs, rhonchi and inspiratory rales were present. Arterial blood gas analysis revealed respiratory acidosis and CXR showed bilateral diffuse alveoler and interstitial infiltrates of both lungs (Fig 1). The patient was intubated again and taken to the intensive care unit. Controlled mechanical ventilation with positive end expiratory pressure (PEEP) was initiated. A thermodilution pulmonary artery catheter was inserted and pulmonary pressures and central venous pressure were found to be normal. Analysis of tracheal aspirates showed high amount of protein. Oxygenation improved progressively and CXR obtained 12 hours later showed clearing of infiltrates. Inspired fraction of oxygen (FiO_2) and PEEP was gradually lowered and the mode was changed to assisted spontaneous ventilation. As the patient could not effectively expectorate secretions which made weaning difficult, assisted spontaneous ventilation was continued for 10 more days until CXR and auscultation of lungs became normal (Fig 2). The patient was discharged on the 14^{th} day of intensive care without any sequelae.

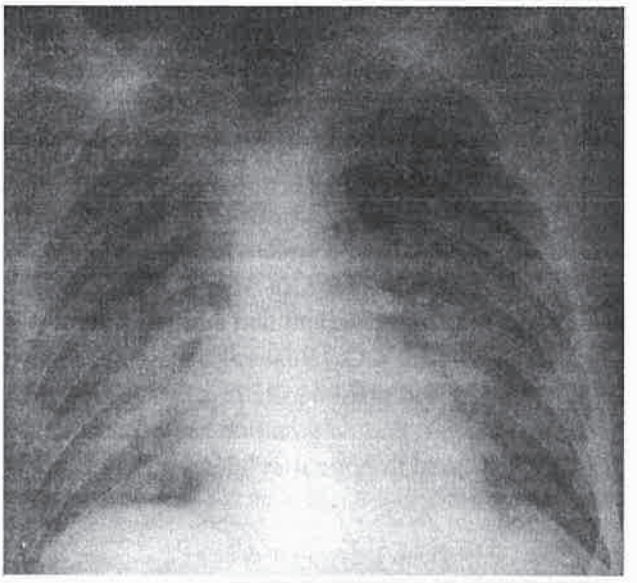

Fig 1. CXR showing bilateral diffuse alveoler and interstitial infiltrates of both lungs.

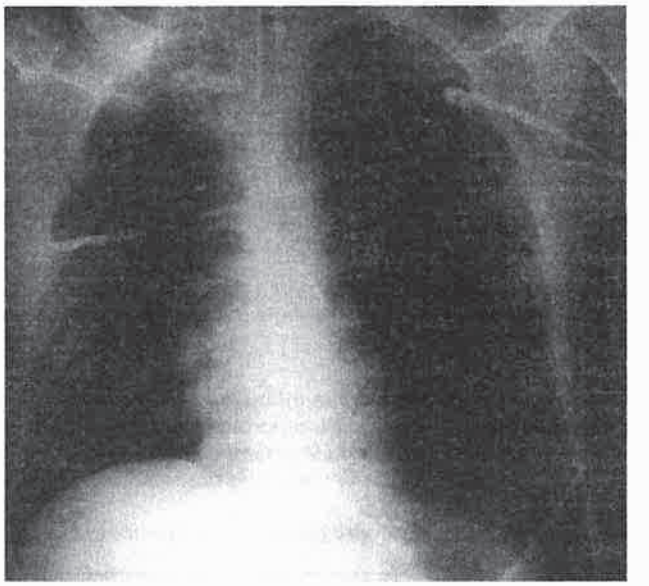

Fig 2. CXR showing clearing of infiltrates.

DISCUSSION

Venous air embolism was diagnosed in this patient after sudden fall of both SpO_2 and $PETCO_2$ during the course of an uneventful anesthetic and operation in the sitting position. A Doppler which is a more sensitive detector for VAE was unavailable unfortunately. Although case reports describe dramatic response to aspiration of air via right atrial catheters immediately upon suspicion of the diagnosis, many studies show that air retrieval is not a major factor in improving hemodynamics (8,9) and it does not justify its replacement if not already in place (10). As we had not placed a central catheter preoperatively we thought that the time required to place one for air retrieval, would be too long to allow intervention to be successful.

The differential diagnosis of the pulmonary changes observed in our patient in the postoperative period included infection, aspiration, neurogenic edema and noncardiogenic edema. Infection was unlikely in view of diffuse involvement and rapid resolution of the pulmonary changes. There was no evidence of aspiration during induction or maintenance of anesthesia. Neurogenic cause was thought unlikely as CT scan of the brain was normal postoperatively. The patient was given 4000 ml of fluid during the operation and as CVP was normal, fluid overload could not have been the cause of pulmonary edema. The patient

was young and healthy with no cardiac problems preoperatively.

Bedford and coworkers (9) evaluated 100 patients, detected 80 episodes of VAE. Of these, 36 demonstrated increased pulmonary artery pressure (PAP) and of whom a minority became hypotensive. All patients with hypotension were found to have high PAP and a correlation was noted between the severity of hypotension and the degree of increase in PAP. In our case, during episodes of VAE, the presence of stable mean arterial pressure may indicate that PAP was normal throughout the operation.

It is known that pulmonary edema is caused by an increase in capillary permeability and /or an increase in hydrostatic pulmonary vascular pressure. Initially pulmonary edema due to VAE was thought to be caused mainly by pulmonary hypertension (11,12). However, recent animal studies (13) favor direct toxicity of air bubbles on the pulmonary endothelial cells due to the release of toxic oxygen metabolites from leucocytes, resulting in increased capillary permeability with normal pulmonary artery occlusion pressures. The increase in capillary permeability causes edema fluid to be rich in protein. In our case the diagnosis of pulmonary edema secondary to VAE was supported by the occurence of multiple episodes of VAE, the development of pulmonary edema a few hours later than the last episode and the detection of high protein content in tracheal aspirates.

We conclude that if VAE occurs perioperatively, the clinician should closely observe the patient for the possible development of pulmonary edema in the postoperative period.

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VARICOCELE TESTICULOPATHY: A NOVEL CAUSE FOR TREATMENT FAILURE IN HYPOGONADOTROPIC HYPOGONADISM

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SUMMARY

37 year-old-male with acquired hypogonadotropic hypogonadism, primary infertility, and decreased libido after 20 months of continued gonadotropin therapy presented with bilateral varicoceles. Bilateral subinguinal varicocelectomy with operative microscope was done. Serum testosterone level, total motile sperm count, 24-hour sperm motility, and libido markedly improved. The couple spontaneously conceived four months post-operatively.

Varicocelectomy improved both spermatogenesis and testosterone synthesis in a man with acquired hypogonadotropic hypogonadism. Varicocele is a reversible cause for gonadotropin treatment failure.

Key words: *Gonadotropin therapy, hypogonadotropic hypogonadism, infertility, surgery varicocele*

Hypogonadotropic hypogonadism (HH) is a rare cause of male infertility. Failure of the pituitary to secrete FSH and LH in these men results in deficient testosterone synthesis and diminished spermatogenesis. Men with HH who have no interest in fertility are treated with testosterone replacement therapy. When parenthood is desired gonadotropin replacement therapy is administered to support both sperm and androgen production. We present a patient with acquired HH who became unresponsive to HCG/HMG therapy due to varicocele testiculopathy. Microsurgical varicocelectomy reversed the end organ failure.

CASE REPORT

A 37 year old man presented with primary infertility and decreased libido. At the age 27, he developed HH following an intracerebral hemorrhage from an arteriovenous malformation. He was maintained on testosterone replacement therapy until age 35 when his regimen was switched to gonadotropins to induce spermatogenesis. High doses of Profasi, 2cc every other day(2000 USP Units/cc, HCG, Serono Laboratories, Randholph MA, 02368) and Pergonal 2 amps every other day(75 IU FSH and 75 IU LH per ampule, Serono Laboratories, Randholph MA, 02368) were required to maintain normal levels of serum testosterone. The patient noted a diminished libido shortly after the hormonal support was changed. In addition to timed intercourse the patient and his wife underwent 8 cycles of IUI including three cycles with super ovulation. One IUI cycle resulted in a chemical pregnancy but embryo loss occured at 8 weeks. After 20 months of gonadotropin treatment the patient presented for evaluation.

On physical exam both testes were small in size and left testis was soft in consistency. A large visible grade III varicocele was noted on the left and a grade II varicocele was palpated in the right spermatic cord. Serum total testosterone level was low 3.9 nmol/L(range 7.0-34.0). Two semen analysis showed low volume ejaculate with sperm densities ranging from 23-48 million/ml.. A sperm migration assay showed poor motility at 24 hours (Table 1)(1).

The patient underwent bilateral subinguinal varicocelectomy with the operative microscope. Two months postoperatively his serum testosterone was

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Table 1. Semen Analyses and Serum Testosterone Levels.

	PREOPERATIVE	POSTOPERATIVE
Ejaculate volume (cc)	0.75	2.0
Sperm count (million/ml)	48	67
Sperm Motility (%)	55	70
Total motile sperm count (x 10^6)	20	94
Sperm Morphology (%)	34	29
Post swim-up sperm count (million/ml)	5	6.5
24-hour motility (%)	10	90
Total Testosterone (nmol/L)	3.9	37.7

elevated 37.7 nmol/L (range 7.0-34.0) despite no change in gonadotropin support. His 3-months post-operative semen analysis significantly improved and excellent 24-hour motility was observed in the sperm migration assay (Table 1). His partner spontaneously conceive 4 months after surgery. An improvement in libido was also noted.

DISCUSSION

Normal serum testosterone levels can be achieved with gonadotropin therapy in men with hypogonadotropic hypogonadism. Successful induction of spermatogenesis occurs in 55%-80% of these men. Pregnancy rates are equally high. Pretreatment testicular volume and previous cryptorchidism can effect the spermatogenic renewal. Prior androgen replacement therapy, should not affect the subsequent testicular response to gonadotropins (2). This is the first report on the effect of varicocele on HH treatment.

Varicocele accounts for the most common reversible etiology of male subfertility. Several large studies have detailed the detrimental effect of varicocele on spermatogenesis. Varicocelectomy improves the sperm count and motility in the majority of men. Repeated studies also have demonstrated decreased serum testosterone levels and altered leydig cell function in men with a clinical varicocele(3). Varicocele repair raises testosterone levels. Grade I varicoceles (palpable only with valsalva) exert a greater reversible effect on leydig cell function, resulting in a larger increase in serum testosterone following repair. The improvement in leydig cell function appears to be independent of improved sperm production(4).

In our patient, in the environment of consistent and uniform exogenous gonadotropin stimulation, the bilateral varicoceles impaired both missions of the testes: testosterone synthesis and spermatogenesis. Although initially normal, serum testosterone levels declined over time, and became manifested by libido loss and low ejaculate volume. Microscopic subinguinal varicocelectomy resumed leydig cell responsiveness within two months. Testosterone levels rebounded to 37.7 nmol/L (range 7.0-34). Enhanced libido and normal ejaculate volume quickly ensued. Clearly the varicocele was responsible for the development of leydig cell inadequacy, and the varicocelectomy for the restoration of function.

The patient also benefited from a significant improvement in his semen parameters and sperm function. Following surgery the total motile sperm count increased almost five-fold (19.8 - 93.8 million). The 24-hour sperm motility in the sperm migration assay improved from 10% to 90%. Most significantly the wife conceived spontaneously four months post-operatively.

The incidence of varicocele is 15% in the male population. The incidence in men with HH unknown. We recommend that all men with HH undergo examination for a varicocele by an andrologist prior to initiating gonadotropin therapy. Varicocelectomy should be considered in patients with an impaired response to treatment.

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HODGKIN'S DISEASE AND AUTOIMMUNE HEMATOLOGIC DISORDERS

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Haluk Gökçora** • Cemil Ekinci***

SUMMARY

Depletion of platelets and red blood cells may occur in advanced Hodgkin's Disease. Immune thrombocytopenia and autoimmune hemolytic anemia are rarely the cause of these complications. Two such cases, one with a fatal outcome are presented in a retrospective analysis of 191 Hodgkin's Disease patients, treated at the University of Ankara, School of Medicine, Department of Pediatric Hematology and Oncology, between 1964 and 1997. The histologic subtypes were mixed cellularity in one patient with immune thrombocytopenia and nodular sclerosis in another with autoimmune hemolytic anemia. Awareness of such disorders and management of predisposing conditions is a bonus to the patient being treated for Hodgkin's Disease.

Key words: *Autoimmune hemolytic anemia, Childhood, Hodgkin's Disease, Immune thrombocytopenia*

Thrombocytopenia and anemia of varying intensity are common in the course of Hodgkin's Disease (HD). Thrombocytopenia may occur in advanced disease, as a result of bone marrow infiltration, a side effect of chemo or radiation therapy, less commonly due to hypersplenism or more rarely as a paraneoplastic effect (1-3). Mechanisms contributing to anemia include shortened red cell survival, decreased cell production due to chronic disease and bone marrow involvement by HD, as well as intensive radiotherapy and chemotherapy (3). Furthermore various immunologic abnormalities have been described in patients with HD including autoimmune hemolytic anemia (AIHA), neutropenia and immune thrombocytopenia (ITP) (2-12). The purpose of this report is to describe two cases of autoimmune disorders associated with HD.

CASE REPORTS

The records of 191 HD patients at University of Ankara, School of Medicine, Department of Pediatric Hematology-Oncology were reviewed between 1964 and 1997. Two of our patients manifested autoimmune disorders such as ITP and AIHA.

Case 1: 13-year-old boy had enlarged right supraclavicular and right axillary lymph nodes, hepatosplenomegaly, weight loss, and intermittent fever without infection. Biopsy of the cervical mass revealed mixed cellular HD (Fig. 1). Abdominal and thoracic computed tomography (CT) and ultrasound demonstrated enlarged periaortic, mesenteric lymph nodes, and hepatosplenomegaly. The bone marrow aspirate was normal. The patient was staged as IV-B HD and received two treatment courses of OPPA (Vincristine, Prednisone, Procarbazine, and Adriamycine), followed by mantle and splenic area radiotherapy (3000 cGy). Subsequently four courses C-MOOP (Cyclophosphamide, vincristine, procarbazine, and prednisone) chemotherapy was performed. The patient achieved complete remission after nine months. He was further admitted to our hospital because of thrombocytopenic purpura the following year. The platelet count was 20.000/mm3. The bone marrow aspirate was diagnos-

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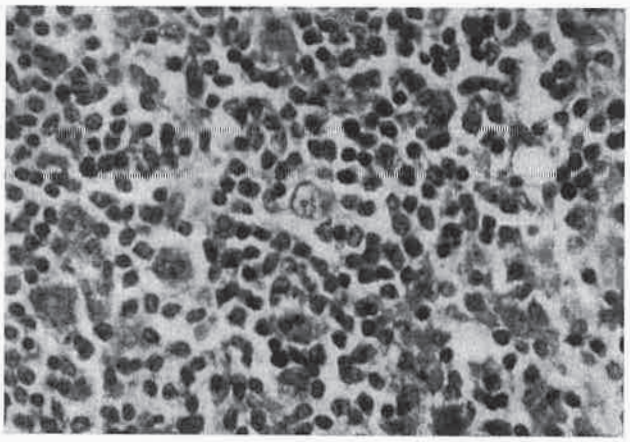

Fig 1. Mixed cellular type Hodgkin's disease (patient IG).

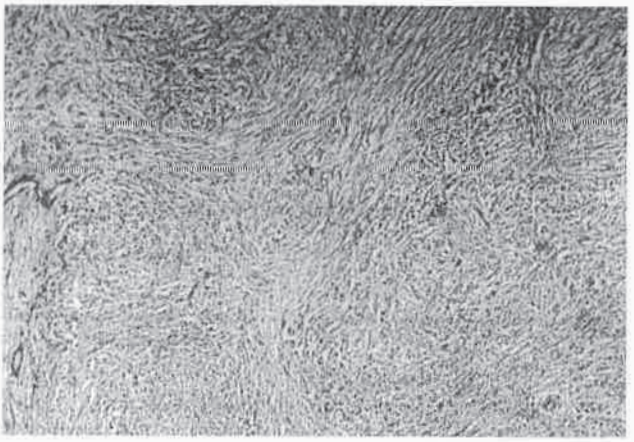

Fig 2. Nodular sclerosis type Hodgkin's disease (patient RK).

tic for ITP. Abdominal and thoracic CT were normal. The patient was treated with prednisone for 12 weeks (3 weeks 1 mg/kg per day, 9 weeks 2 mg/kg per day). He responded to prednisone and the platelet count became normal. Two years after the diagnosis of ITP, he was readmitted with abdominal pain, weight loss, and splenomegaly. Abdominal ultrasound demonstrated enlarged retroperitoneal lymph nodes, and splenomegaly. The bone marrow aspirate was normal. At staging laparatomy, a splenectomy was performed which showed splenic involvement. The patient was considered as relapsed HD and received three courses of CEM (CCNU, VP16-213, Methotrexate) chemotherapy.

Case 2: An 8-year-old boy had diffuse lymphadenopathy, hepatosplenomegaly, weight loss and fever without infection. Biopsy of cervical lymph nodes revealed HD of nodular sclerosis type (Fig. 2) An abdominal ultrasound demonstrated enlarged periaortic and celiac nodes, and hepatosplenomegaly. The patient was staged as IV-B HD and received two courses of C-MOPP chemotherapy. During the second course of chemotherapy jaundice developed. The diagnosis of AIHA was based on a strongly positive Coomb's test, elevated reticulocyte count, LDH and bilirubin. Other causes, such as drug-induced hemolysis or systemic lupus erythematosus were excluded. The dose of prednisone was increased to 2 mg/kg/day. There was a gradual response to anemia, Coomb's test became negative, reticulocyte count and bilirubin level normalized. However the patient died after the second course of chemotherapy by HD progression.

DISCUSSION

Immune thrombocytopenia and AIHA are uncommon causes of cytopenia in HD(3,5-7,9,12). In some series of patients with HD the occurrence of ITP was noted in only between 0.6 % and 2% of the patients (2,3,5,13,14). The presence of a positive direct Coomb's test in patients with HD has been noted. The association of AIHA with HD is reported to be 0.2 - 2.7 % (3,6,9,12). In our series, ITP and AIHA were found in 2 (1 %) patients.

ITP is characterised by documented destruction of platelets and the presence of normal or increased numbers of megakaryocytes in the bone marrow. The following generally accepted criteria for the diagnosis of ITP in HD was used (2,3): 1) Increased platelet destruction as manifested by thrombocytopenia (Platelet counts < 50.0 x 10^9 /l) and abundance of megacaryocytes in the bone marrow. 2) No evidence of the other clinical disorders associated with thrombocytopenia such as disseminated intravascular coagulation, sepsis, bone marrow invasion with HD and systemic lupus erythematosus. 3) No previous drugs known to be associated with the development of thrombocytopenia. 4) Absence of splenomegaly.

Hodgkin's disease is associated with a complex deficiency in cellular immunity. The panoply of alterations includes impairment of delayed cutaneous hypersensitivity, enhanced immunoglobulin production, high levels of circulating immune complexes, production of antilymphocyte and anti-Ia antibodies, decreased natural killer cell cytotoxicity, enhanced sensitivity to suppressor monocytes and suppressor T cells,

and a variety of other disorders of serum factors, including high levels of circulating IL-2 receptors (1,10,11). It is possible that autoimmune thrombocytopenia and AIHA in HD is, in some cases, the result of impaired immunoregulatory roles of T lymphocytes which have been reported to occur in this disease. Platelet destruction may be mediated by immunoglobulins which are specifically directed toward platelets. Alternatively, HD may be associated with the presence of excessive amounts of immune complexes which could be adsorbent onto platelets, thereby facilitating platelet destruction (4,10,15,16). Another hypothesis is that, tumor cells partially differentiate and release antigenic material directly into blood stream, then stimulates rejection of normal tissues (13). Some authors showed that, Reed-Sternberg Cells (R-SC) are capable of modulating their environment by producing a number of cytokines, including interleukin-6 (IL-6) (11). Autoimmune manifestations in HD could be related to a complex cascade of cytokine signals, in particular IL-6, orchestrated by R-SC, with the functional properties of these cells not yet elucidated (11).

Nodular sclerosis and mixed cellularity types HD are considered the most frequent histologic subtypes in patients with ITP or AIHA (2,3,5-9,12-14). The histologic subtypes were mixed cellularity in our one patient with ITP, nodular sclerosis in one patient with AIHA in accordance with the literature.

ITP occasionally accompanies clinically evident HD but it is more likely to appear after splenectomy and prolonged remission (5,7,8). AIHA usually preceded the diagnosis or a recurrence of the disease (6,9,12). The presence of a positive Coomb's test in patients with HD implies active and extensive disease and should be investigated accordingly (3,7). Further the Coomb's test should be used as a parameter of disease activity in those patients who are Coomb's positive at initial diagnosis. This contrasts to ITP occurring in patients with HD which appears to be unrelated to activity of underlying disease. In our one patient with HD, ITP was manifested in remission for HD. In the other patient with HD staged IV-B, AIHA appeared during the chemotherapy. AIHA resolved with prednisone treatment but progression occurred in HD. However, in spite of extensive disease finding of a positive Coomb's test does not necessarily imply a poor prognosis (6,7).

Each patient with HD and an autoimmune disorder represents a complex management problem. In the treatment of ITP with a history of HD, conventional therapeutic modalities may be employed: corticosteroids, splenectomy and perhaps immunosuppresive treatment (8,15). ITP associated with HD responds to corticosteroid treatment or splenectomy in the some way as does primary ITP. In contrast, the response rate of ITP with HD to cytostatics seems to be better than in patients with primary ITP (3). Splenectomy seems more efficient than corticosteroids but may be recommended for older patients, because the high incidence of post-splenectomy infections (13). However some authors suggested that the combination of corticosteroids and immunosupressive drugs may be indicated at the outset in patients with HD who develop ITP following splenectomy (5).

The patient with a history of HD who develops a Coomb's positive hemolytic anemia must undergo a carefully evaluation for evidence of active lymphoma. If active HD is not found, high-dose corticosteroids for 1-2 weeks followed by splenectomy is suggested. If active lymphoma is apparent at the onset of AIHA, therapy must be directed at both entities. Definitive therapy for the underlying HD, including the use of morrow-suppressive agents, appears to be necessary to successfully treat the immune hemolytic anemia that may be seen in this setting (7,8).

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INTRA CARDIAC THROMBOSIS ASSOCIATED WITH FACTOR V (1691 G-A) MUTATION (A CASE REPORT)

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Gülendam Koçak*

SUMMARY

Right heart thrombi occur rarely in the pediatric age group. We present a child with a right ventricular thrombus, diagnosed by cross sectional echocardiography who inherited FV 1691 G-A mutation . To our knowledge, intracardiac thrombosis due to the Factor V gene 1691 G-A mutation has not been reported yet. Therefore, this mutation should be investigated in all cases of intracardiac thrombosis.

Key words: *Right ventricular thrombosis, Factor V gene, 1691 G-A mutation.*

Intracardiac thrombi occur rarely in the pediatric age group (1-5). Right heart thrombus occurs in association with the presence of central venous catheters, vegetations due to endocarditis and thrombogenesis with dilated cardiomyopathy (1,5).

A mutation in the Factor V gene (1691 G-A in exon 10) was identified that formed the molecular explanation for the phenotype of Activated Protein C resistance in the large majority of affected individuals (6). This mutation, which is associated with significant increase in thrombotic risk, has been found in 30-50 % of selected families with thrombophilia, in 20% of consecutive patients with venous thrombosis (7,8).

To our knowledge,intracardiac thrombosis due to a mutation in the Factor V gene has not been reported yet.

We present a child, who inherited FV 1691 G-A mutation with a right ventricular thrombus, diagnosed by cross-sectional echocardiography.

CASE REPORT

A 4- year old girl was referred to Ankara University Pediatric Cardiology Unit with dyspnea, cyanosis and edema of lower extremities. The patient was previously treated in a district hospital with digoxin and diuretic where dilated cardiomyopathy has been diagnosed.

At initial examination, she was an ill-looking child with cyanosis, tachycardia, tachypnea, hepatomegaly and edema. A second degree pansystolic murmur was heard over the left sternal border. A gallop rhythm was present down the left sternal border and the 2nd heart sound was closely split and loud. The chest X-ray revealed enlarged heart and pulmonary edema; the ECG showed sinus tachycardia, reduced QRS complex voltage, 1 st-degree atrioventricular block and right QRS axis, right ventricular dominance and T wave abnormalities.

At that time; the routine laboratory findings revealed: hemoglobin 9,9 gr / dl, WBC 9,7 x10^9/l, platelets 203 x 10^9/l, with 50% polymorphonuclear leukocytes, 47% lymphocytes; 3% monocytes. Results of coagulation studies were: PT 19s (range: 20 - 40 s), antithrombin III activity 29 ng/dl (normal: 22 - 39 ng/dl). Protein S (%110) and Protein C (% 90) activity were within normal limits.

At echocardiographic examination, left ventricular function was found to be depressed (ejection fraction 38% and shortening fraction 18%). Because of pulmonary hypertension, right atrium and right ventricle were enlarged. There was a heavy ventricular trabecula-

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tion in the apex of the right ventricle. Color Doppler echocardiography revealed moderate tricuspid insufficiency.

An echocardiogram confirmed a dense, 10x12,5 mm in size, non-mobile round mass indicating a thrombus in the trabeculations of right ventricle with apparent attachments (Fig 1).

Since her

Protein C, Protein S and AT III activities were within normal limits we decided to study FV 1691 G-A mutation which was known to have a significant role in the foormation of thrombus. DNA was extracted by conventional methods and polymerase chain reaction of exon 10 of the Factor V gene was performed according to previously described method using primers 5'TCAGGCAGGAACAACACC3'and5'GTTACTTCAAGGACAAAATACCTGTAAAGCT3'. Amplification was performed for 35 cycles with annealing temperature of 58°C (Ericomp, USA). Amplified DNA was digested with Hind III enzyme (Promega, USA) at 37°C and subjected to 2% agarose gel electrophoresis (9) (Fig 2).

She was admitted to the hospital and started on intravenous heparin therapy to prevent further clot formation. Digoxin and diuretic therapy were continued on and captopril was started. Unfortunately on the eight day of admission, she died due to an uncontrolable ventricular tachycardia.

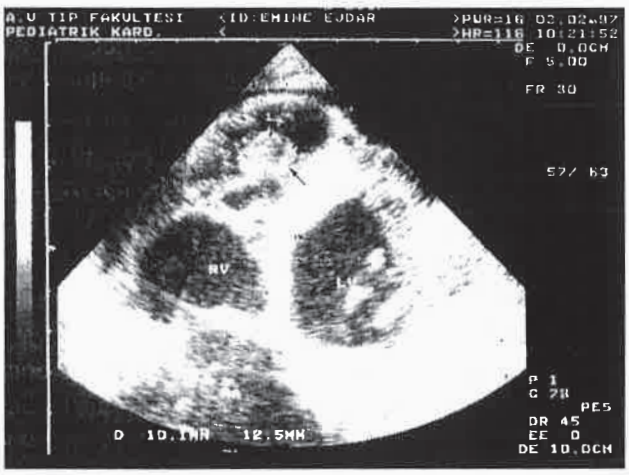

Fig 1. Two-dimensional echocardiogram. (Modified four-chamber view). Demonstrating right ventricular thrombus.
RV: right ventricle
RA: right atrium
LV: left ventricle

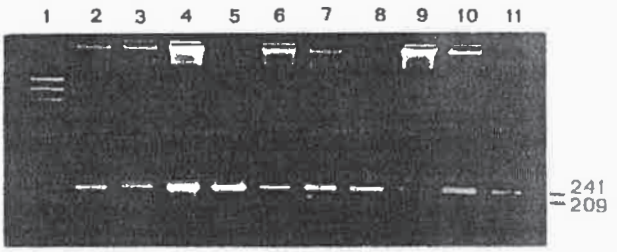

Fig. 2. Detection of FV 1691 G-A mutation

Lane 1 : X - 174 - Hae III (marker)
Lane 2 : Uncut PCR product (241 bp)
Lane 3 : Index patient heterozygote for FV 1691 G-A mutation (241 and 209 bp)
Lane 4, 7 : heterozygote for FV 1691 G-A mutation (241 and 209 bp) (controls)
Lane 5,6,8,9 : homozygote normal for FV 1691 G-G (241 bp)

DISCUSSION

Right heart thrombus is a life- threatening disorder and rare in the pediatric age group (5). Stasis of blood flow and abnormal hypercoagu able states are the principal causes of heart thrombi. Right heart thrombi frequently results in pulmonary embolism and may be responsible for cerebrovascular accidents in certain forms of congenital heart disease (10).

Patients with dilated cardiomyopathy often have pathologic evidence of ventricular thrombi at autopsy (11). In these patients, thrombi were located in a dyskinetic area of myocardium. The prevalance of thrombi has been reported to be 11% - 44% in two different studies in patients with dilated cardiomyopathy (12,13).

In this patient, according to echocardiographic findings dilated cardiomyopathy with severe pulmonary hypertension was diagnosed. The localization of thrombi were frequently reported in the left ventricle of the patient with dilated cardiomyopathy which was different from our patient (11 - 13). Although this patient had dilated cardiomyopathy, the right ventricular localizations of the thrombus, may well be explained by the severe pulmonary hypertension. The thrombus was not very large and non-mobile, we decided to treat the patient with anticoagulants because of high risk of operative therapy.

In this patient, most severe left ventricular dysfunction may be the cause of thrombus formation. Recently, arterial, venous and intracardiac thrombi due

to Protein C and S deficiencies have been reported (14 - 17).

But our patient had normal protein C and S levels. For that reason, we decided to study factor V gene 1691 G-A mutation which is known to have a significant role in the formation of thrombosis. Moreover, we found Factor V 1691 G-A mutation in healthy Anatolian population as 10% which was one of the highest in Western world (18). We think that factor V gene mutation may enhance the formation of thrombosis.

In conclusion, up to now intracardiac thrombus with Factor V gene mutation has not been reported. So we believe that Factor V gene 1691 G-A mutation should be investigated in all cases of intracardiac thrombosis and cross-sectional echocardiography should be performed in all patients with Factor V mutation because of the possibility of intracardiac thrombus.

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CORRECTIONS

* Aşkar İ, Sevin K, Saray A, et al. The effect of etodolac on the microvascular patency rates. J Ankara Med School 1999; 21:81-84

Figure 3 is printed as the correct size for the detail. We regret to produce the figure in a small size.

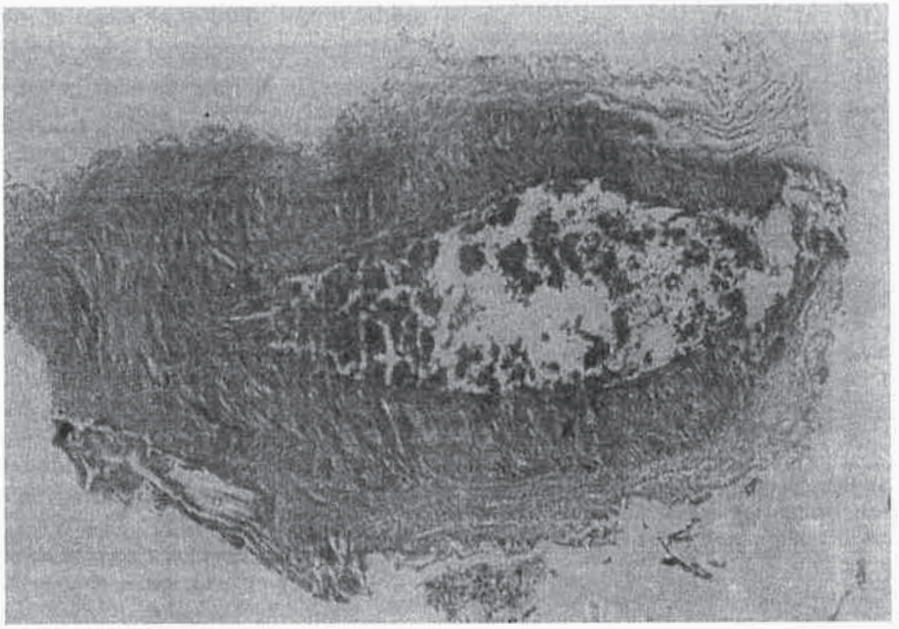

Figure 3. In the etodolac group, minimal endothelial damage in intima, focal hemorrhages in media, and minimal inflammation in adventitia were observed (HE, x25).

* Gökçora İH, Gözdaşoğlu S, Can B, et al. Cause and effect of treatment in lymphoedema of the left lower extremity: A case report J Ankara Med School 1999; 21:109-112.

Figures 1 and 2 should have appeared on the right figure legends. The correct version of Figure 1 and 2 are shown below. We regret the error.

Fig 1. Preoperative photograph of the child: note extremely small pelvis and apparent difference between the lower extremities

Fig 2. Postoperative apperance of the lower extremities.