Journal of Ankara Medical School

ISSN 1300–5464

Vol 19, No 2, 1997

Journal of Ankara Medical School

Vol 19, No 2, 1997

CONTENTS

Journal of Ankara Medical School

All the authors stated in the published paper are kindly requested to be a subscriber to the Journal. Subscription price for the teaching staff members is 600.000 TL; 50% reductions for rescarch fellows, practioners, etc.; 75% reductions for students, Subscription for the foreign countries: 40 $ or 60 DM.

Editorial Office:
A.Ü. Tıp Fakültesi Yayın Komisyonu Başkanlığı Sıhhıye-ANKARA ISNN 1300 - 5464

Typesetting • Design • Print: Öncü Limited, Tel: (0.312) 231 20 26 • Fax: (0.312) 230 73 52

Journal of Ankara Medical School

Published Quarterly by
ANKARA UNIVERSITY MEDICAL SCHOOL

INTRUCTIONS TO AUTHORS:

Journal of Ankara Medical School publishes original articles of research on clinical and basic sciences and concise case reports.

The language of the Journal is English.

All material should be addressed to the Editor, (Ankara Ünivversitesi Tıp Fakültesi Yayın Komisyonluğu Başkanlığı, 06100-Ankara, Turkey), in three copies and a floppy disk, ideally **Microsoft Word 6.0 or 2.0**. An introductory letter identifying the authors (s), their telephone and fax numbers and their address (s) should accompany the manuscript.

Journal accepts the contributions with the understanding that neither the article nor any part of its essential results has been published or submitted for publication elsewhere prior to its appearance in this Journal. Work already presented in a congress or published as an abstract within the context of congress or scientific meetings may be accepted for publication provided that this fact is mentioned.

All materials including text, figures, tables, references and glossy prints of figures should not exceed ten pages. The upperlimit for case presentation is three pages.

Title of the Paper: Must not exceed 80 spaces. If title exceeds 80 letter space a "running title" fewer than 40 letter spaces should be prepared in order to be placed on top of odd numbered pages.

The names (s) of author(s), including first name (s) must be written below the title. The academic degree(s) of author(s) can be stated as a foot-note with an asterix placed on surname(s) of the author(s). The name and address of Correspondent author should be stated.

Summary in a foreign language: An abstract not more then 200 words must be written in English.

Key Words not more than five should be added below the summary in alphabethical order.

Form: Article submitted must be double-spaced typewritten on standard size paper (21x30 cm). margins 3 cm to the left and 2 cm to the right should be left.

Illustrations: Photographs, graphics, and all other illustrations must be numbered according to consecutive appearence order. Graphics and figures should be made on glossy paper, preferably with china ink. Photogarphs should be made on glossy paper, black and white, with sufficient contrast. A small legend must accompany each figure numbered letters.

The legends must be written on a separate sheet of paper, in the order of appearance within the article.

Figures and photographs must be presented in an envelope. Title of the article and author(s) must be written at the back of the samples with a light pencil.

The place where the illustrations are desired to appear within the text should be indicated by numbering it on left margin.

Tables: The tables must be typewritten double-spaced on a separate sheet of paper numbered with Arabic numerals. The contents of the table must be clearly expressed with a short title.

The results of the work must be stated either by table or by explanation within the text. Duplication of the above should be avoided.

The desired place for the tables should be indicated on the left margin of the written text.

References: Must be numbered in parenthesis on the same level the manuscript line. In papers representing a research work only those references which deal with the research should be mentioned. References should not exceed 25 in research and 10 in case reports. References should be arranged sequentially as they appear in the text. Example references are given below:

- **Gozal D, Tiser A, Shupak A, et al. Necrotizing fasciitis. Arch Surg 1986; 121: 233-5.**
- **Moon RE, Gorman DF. Treatment of the decompression disorders. In: Bennett BP, Eliot DH, eds. The Physiology and Medicine of Diving. 4th ed. Philadelphia: W.B. Saunders, 1993: 454-80.**

Reprints are available at prices determined by article length and quantity.

JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 61-64

PLATELET FUNCTION DURING PHYSICAL EXERCISE AND RECOVERY IN MEN

Gülriz Ersöz* • Ali Murat Zergeroğlu** • Sema Yavuzer*

SUMMARY

Twelve healthy, sedentary males (aged 18-24) performed 15 minutes of cycling exercise with a load corresponding to 75% of maximal heart rate. ADP and collagen-induced platelet aggregation, ATP release and platelet count were measured before, immediately and 60 minutes after the exercise.

Both ADP and collagen-induced platelet aggregation increased significantly immediately after the exercise. Sixty minutes after the exercise platelet aggregation tend to decrease and approximated to the resting level. ATP release and platelet count did not change by exercise.

Our results indicate that acute severe exercise causes a transient increase of platelet aggregation.

Key Words: *Exercise, platelet aggregation, platelet function*

It is postulated that physical activity has protective effect against cardiovascular disease (1). Platelets are known to play an important role in the pathogenesis of cardiovascular disease (2, 3). So the response of platelets to physical exercise is one of the considerable pathophysiological interests.

The researches have mainly focused on the aggregatory response of the platelet. Agonist-induced aggregation in platelet rich plasma (PRP) have been used generally to estimate platelet function. But it is indicated that the results using this method have been conflicting. It is reported that the use of whole blood aggregation avoids the preparation artefacts of PRP and allows platelet function to be studied in the presence of other blood cells (4).

Since several granule contents of platelets are the major regulators of the microcirculation, platelet release response seems to be important. There is very few data about the effect of exercise on platelet release response (5)

In the presented study it is aimed to investigate the effects of acute exercise on platelet aggregation and ATP release in sedentary volunteers. An exercise protocol in intensity of 75 % of the each subject's maximal heart rate was performed that has been recommended for an improvement of aerobic power (6, 7). Al so it is planned to observe the platelet behavior after 60 minutes of a recovery period.

MATERIAL AND METHODS

Twelve healthy and sedentary male volunteers (aged 18-24) were participated the study. The subject was judged to be healthy with a medical examination that included a resting ECG and respiratory function test. All the subjects were non-smokers and none had taken medication known to affect the platelet functions within the preceding two weeks.

The maximal aerobic capacities (VO_2max) of the subjects were estimated according to indirect method of Astrand and Rhyming (8). The subjects performed work corresponding to 75% of the maximal heart rate for 15 minutes by bicycle ergometer (Monark 814E) approximately two days after the estimation of VO_2max.

The subjects were rested for 15 minutes before the exercise. Blood was collected by venipucture before, immediately and 60 minutes after the exercise. Blood samples were placed into siliconized test tubes

* University of Ankara, Faculty of Medicine, Department of Physiology
** University of Ankara, Faculty of Medicine, Department of Sports Medicine

Received: Nov 6, 1996 Accepted: Mar 7, 1997

Table 1: Maximal intensity of platelet aggregation, amount of ATP release induced by ADP and collagen, and platelet count before, immediately and 60 minutes after the exercise (n:12). Values are means±SE.

		Before	Immediately after	60 minutes after
Maximal Intensity (Ohm)	ADP	10.54±1.86	18.68±3.12*	13.93±1.61
	Collagen	14.52±3.13	26.17±4.38*	16.54±3.9
ATP Release (nM)	ADP	1.55±0.24	2.09±0.29	1.35±0.29
	Collagen	1.49±0.28	2.24±0.3	1.59±0.24
Platelet Count (x1000/mm^3)		313.7±18.4	321.9±14.8	317.9±15.4

containing 3.8% sodium citrate in ratio of 1:9 and taken in EDTA for analyses of platelet count and hematocrit.

Whole blood aggregation in impedance system was performed using a Chronolog Whole Blood Lumiaggregometer 560 (Chronolog Coorp., Hawertown, PA) (9). Maximal intensity of the aggregation (maximal increase in impedance) was estimated on the aggregation curve. Platelet ATP release was measured by bioluminescence technique (9). Luciferin-luciferase reagent was obtained from Chronolog Coorporation. The luminescence channel of the aggregometer was calibrated with 2nM of ATP (Chronolog Coorp, Hawertown, PA). ADP (10 µM, Chronolog Coorp, Hawertown PA) and collagen (2 µg/ml, Chronolog Coorp,Hawertown, PA) were used for platelet induction.

Platelet count was analyzed by Medonic Cell-Analyzer 610.

The results were evaluated by Wilcoxon's test statistically. Significant was taken to represent $p<0.05$.

RESULTS

Maximal intensity of collagen and ADP-induced platelet aggregation increase immediately after the exercise ($p<0.05$) (Figure 1). 60 minutes after the exercise platelet aggregation in response to both agents tend to decrease and approximated to the resting level (Figure 1).

There was no significant difference in maximal intensity between that measured 60 minutes after the exercise and that measured before the exercise. No significant difference was found between postexercise and recovery levels. Platelet ATP release, platelet count did not alter in relation with exercise.

The results are summarized in Table 1.

DISCUSSION

In the presented study it was observed that ADP and collagen-induced platelet aggregation increased after 15 minutes of exercise performed at 75% of maximal heart rate. 60 minutes after the exercise, maximal intensity of both ADP and collagen-induced aggregation decreased and approximated to the resting level. The increases in both ADP and collagen-induced ATP release by the exercise were not significant statistically.

It is difficult to compare and interpret most of the previous results about the effect of exercise as different intensities and durations of exercise have been used. It is generally suggested that the effects of exercise on platelet response to several agonists are due to the intensity and duration of the exercise (5, 10). It seems that moderate exercise does not cause any alteration in platelet function while strenuous or prolonged exercise increases the sensitivity of platelets. Wang et al (5) reported that in 10 healthy sedentary subjects, platelet adhesiveness and ADP-induced aggregation were increased by strenuous exercise and depressed by moderate exercise.

There is very few and conflicting data on the effect of exercise on platelet secretory response. Generally platelet factor 4 (PF4) and β-Thromboglobuline ((TG) have been used as the markers of secretory response. Drygas (10) showed that repeated bouts of

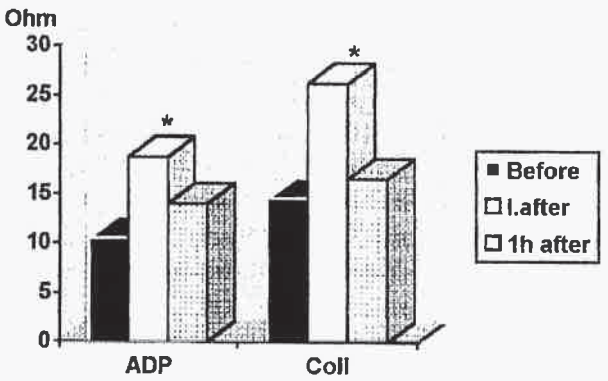

Fig 1. Maximal intensity of ADP and collagen-induced platelet aggregation before, immediately and 60 minutes after the exercise (n=12). *Significant difference before and immediately after the exercise, $p<0.05$.

maximal exercise caused a significant increase in platelet aggregation and PF4 release but the 60 minutes of exercise performed at 85% did not alter platelet aggregation (circulating platelet aggregates were estimated in EDTA-formaline in PRP) and PF4 release. Wang et al (5) reported that plasma levels of PF4 and β-TG increased with exhaustive exercise and no significant change caused by submaximal exercise (50-55% VO_2 max) was found. We observed an increase of platelet ATP release after the exercise performed at 75% VO_2max but this increase was not significant statistically and Our results indicate that the exercise, recommended for improvement of aerobic power, increase both platelet aggregation and secretion. The aggregation response seems to be more sensitive than secretory response.

Moreover the exact mechanisms in exercise-induced platelet activation have not been completely understood. Increase in plasma levels of catecholamines, endothelial damage due to shear stress and oxidative stress, alteration in tromboxane A2(TxA_2)/prostacyclin(PGI_2) ratio are thought to involve the platelet activation during physical activity (10, 11, 12).

It is known that physical activity increases the production of free oxygen radicals that promote platelet aggregation (13, 14). Also physical exercise alters antioxidant atatus.It was reproted that erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities decreased by submaximal exercise (15). This data has showed an inadequacy in detoxification of the radicals.

The most effective free radical-dependent reaction that involved in platelet activation is lipid peroxidation. Free radical-induced lipid peroxidation results in increase of arachidonic acid release and metabolism so the production of main metabolite TxA_2 increases (16). Inhibition of membrane dependent enzymes, such as Na^+-K^+ ATPase, Ca^{2+}-ATPase, results in increase of intracellular Ca^{+2} (17, 18). Free radicals inhibit the synthesis of PGI_2 in endothelial cell so TxA_2/PGI_2 imbalance occurs (19). All these alterations may involve in platelet activation during physical exercise.

Platelet aggregatory response tend to decrease after an hour of recovery. It is known that O_2 consumption decreases to rest values during recovery period, so production of free radicals decreases (19). In addition a rebound increase in antioxidant system has been observed in recovery period (20). Exercise induced platelet aggregatory response likely relates partly with increase in production of free radicals. Platelet aggregation seems to decrease while the production of free radicals decreases and the effectiveness of the antioxidant system increases. In conclusion, the physical exercise in intensity of 75% VO2max increases the rate of platelet aggregation. It is thought that the role of exercise-induced oxidative stress in postexercise transient increase of platelet aggregation must be investigated.

REFERENCES

1. Shapper AG, Wannamethee G. Physical activity and ischemic heart disease in middle-aged British men. Br Heart J 191; 66:384-94.
2. Davies MJ, Thomas AC, Knapman PA, Hangartner JR. Intramyocardial platelet aggregation in patients with unstable angina suffering sudden iscemic cardiac death. Circulation 1986; 73: 418-27.
3. Fitzgerald DJ, Roy L, Catella F, Fitzgerald GA. Platelet activation in unstable coronary disease. N Engl J Med 1986; 315: 983-6.
4. Hendra TJ, Oughton J, Smith CCT, Bertteridge DJ, Yudkin JS. Exercise induced changes in platelet aggregation: a comparison of whole blood and platelet rich plasma techniques. Thromb Res 1988; 52: 433-51.
5. Wang JS, Jen CJ, Kung HC, Lin LJ, Hsiue TR, Chen HI. Differnt effects of strenuous exercise and moderate exercise on platelet function in men. Circulation 1994; 90 (6), 2877-85
6. Astrand PO, Rodahl K. Physical training. In Astrand, P.O., Rodahl K., eds. Textbook of Work Physiology. 3rd ED. New York: Mc Graw-Hill Book Company, 1986: 413-522.
7. Adams WC. Exercise physiology. In Adams, W.C. ed. Foundations of Physical Education, Exercise, and Sports Sciences. Philedelphia: Lea and Febiger, 1991: 80-121.
8. Astrand PO, Rhyming I. A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during submaximal work. J Appl Physiol 1954; 7:218.
9. Wojensky C, Smith JB, Silver MJ. Evaluation of electrical aggregometer: comparison with optical aggregometer, secretion of ATP and accumulation of radio-labeled platelets. J Lab Clin Med 1983; 101: 44-52.
10. Drygas WK. Changes in blood platelet function, coagulation, and fibrinolytic activity, in response to moderate, exhaustive, and prolonged exercise. Int J Sports Med 1988; 9: 67-72.
11. Bottechia D, Bordin D, Fantin GP, Martino R. Response of platelets to prolonged physical exercise. J Sports Med 1987; 27: 276-84.
12. Winther K, Hillegass W, Geoffrey H, Tofler MB. Effects on platelet aggregation and fibrinolytic activity during upright posture and exercise in healthy men. Am J Cardiol 1992; 70: 1051-5.

13. Alessio HM, Goldfarb AH. Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. J Appl Physio 1988; 64 (4), 1333-6.
14. Fıçıcılar H. Sedanterlerde ve antrenmanlı bireylerde submaksimal egzersizin eritrosit süperoksit dismutaz ve katalaz enzim aktivitileri üzerine etkisi. The Journal of the Faculty of Medicine University of Ankara 1993; 46(2): 287-300.
15. Davies KL, Quinthanilha G, Brooks GL, Packer: Free radicals and tissue damage produced by exercise. Biochem Biophys Res Comm 1982; 107: 1198-1205.
16. Salvemini D, Botting R. Modulation of platelet function by free radicals and free-radical scavengers. Trends Pharmacol Sci 1993; 14 (2): 36-42.
17. Sahlin K, Ekberg K, Cizinsky S. Changes in plasma hypoxanthine and free radical markers during exercise in man. Acta Physiol Scand 1991; 142, 275-81.
18. Del Principe D, Menichelli A, De Matties M. Hydrogen peroxide has a role in the aggregation of human platelets. FEBS Lett 1985; 185 (1): 142-5.
19. Ohyaskiki T, Masayuki K, Katsuhiko M. Oxygen-radical-mediated lipid peroxidation and inhibition of ADP-induced platelet aggregation. Arch Biochem Biophys 1991; 288 (1): 282-6.
20. Gohil K, Viguie C, Stanley WC, et al. Blood glutathione oxidation during human exercise. J Appl Physiol 1988; 64(1):115-9.

JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 65-67

THE EFFECT OF ENDURANCE TRAINING ON PLATELET FUNCTION IN MEN*

Gülriz Ersöz** • Ali Murat Zergeroğlu*** • Sema Yavuzer**

SUMMARY

10 healthy, sedentary males were performed 30 minutes of cycling with a load corresponding to 75 % of maximal heart rate, 3 times a week, during 6 weeks. ADP and collagen-induced platelet aggregation, ATP release and platelet count were estimated before and after the first exercise and, the exercises that performed at the 3rd and 6th weeks of the training.

The ADP and collagen-induced platelet aggregation increased significantly after the first exercise. The aggregation responses to ADP and collagen did not alter in relation with the exercise at the 3rd and 6th weeks. No alteration was found in ATP release of platelets and platelet count during training. The platelet aggregation increased at rest by the training program.

Our findings indicate that regular physical exercise results in an adaptation of platelet function.

Key Words: *Exercise, training, platelet aggregation, platelet function*

It is suggested that regular physical exercise reduce the risk of thrombotic events and atherosclerotic cardiovascular disease. A relative risk of about 1.9 for coronary heart disease (CHD) was reported for sedentary life style compared with active life style. Many CHD patients are taking part in exercise programs for rehabilitation (1, 2). But the intensity and duration of activity required for protective and beneficial effects are not conclusive.

Since platelets are known to involve in pathogenesis and progression of coronary disease (3, 4), the effect of regular physical exercise on platelet activation gets one of the considerable subjects. The prospective studies about the effect of training program on platelet function are rare (5).

In the presented study it is aimed to investigate the effects of endurance training on ADP and collagen-induced platelet aggregation and ATP release.

MATERIAL AND METHODS

10 healthy, sedentary male volunteers, aged between 18-22, were participated the study. All were non-smoking and none had taken any medication at least 2 weeks before the training program.

Maximal oxygen consumption (VO_2max) of the subjects were measured by using the method of Astrand et al (6). Based on the results of these tests, the participants were performed 30 minutes of cycling with a load corresponding to 75% of maximal heart rate, 3 times a week during 6 weeks. VO_2max of the subjects were re-estimated at the 3rd and the 6th weeks and the loads were readjusted at the 3rd week.

Blood samples were collected in plastic syringes containing 3.8% trisodium citrate before and 3 minutes after the first exercise. Blood samples were also obtained before and after the exercise at the 3rd and the 6th week of the training.

Platelet aggregation and ATP release induced by ADP (10μM) and collagen (2μg/ml) were determined by Chronolog Whole Blood Lumiaggregometer 560 (Chronolog Coorp,Havertown, PA). The platelet aggregation was assesed by the method of electrical impedance (7). The maximum rate (tangent of the maximum slope) and maximum intensity (maximum change of impedance) were computed. ATP release from the

* The study was presented at XXVth FIMS World Congress in 1994 in Atina/Greece

** Ankara University, Faculty of Medicine, Department of Physiology

*** Ankara University, Faculty of Medicine, Department of Sports Medicine

Received: Nov 6, 1996 Accepted: May 7, 1997

platelet was measured by the method of bioluminescence (7). Luminescence channel was calibrated with 2nM of ATP. ADP, collagen, luciferin-luciferase reagent and ATP were obtained from Chronolog Coorp.

Analyses of platelet count were performed on Medonic Cell-Analyser 610.

Wilcoxon's test was used to evaluate the results. Significance was taken to represent $p<0.05$.

RESULTS

Maximal intensities and maximal rates of ADP and collagen-induced platelet aggregation increased significantly after the first exercise ($p<0.05$, $p<0.05$). There was no alteration in aggregation response of the platelet to ADP and collagen in relation with the exercise at the 3rd and 6th week of the training. ADP and collagen-induced ATP release of platelet and platelet count did not change during the training program in relation with exercise.

Maximum intensity and rate of ADP-induced aggregation ($p<0.05$, $p<0.05$) and maximum intensity of collagen-induced platelet aggregation ($p<0.05$) increased significantly at rest by training (Figure 2, 3). Basal ATP release did not change during the training program.

The mean VO_2max of the subjects was 3.2±0.15ml/min before the training program. At the 3rd week it increased to 3.8±0.27ml/min ($p<0.01$). At the 6th week of the the training program it was measured as 3.98±0.78 and significantly different from that measured before the training ($p<0.001$).

The results are summarized in Table 1.

DISCUSSION

Our data indicated that ADP and collagen-induced platelet aggregation increased by acute exercise (75% VO_2max). ATP release in response to both ADP and collagen increased but was not significant statistically. No alteration was observed in platelet aggregation and ATP release by the exercise at the end of the training program.

It is suggested that exercise protocols in different intensities affect platelet function differently. Short duration and moderate intensity of exercise seems not to affect platelet functions while intensive exercise is increasing platelet aggregability (8,9). Wang et al (9) reported that exhaustive exercise increased platelet adhesiveness and aggregation while moderate exercise (50-55% VO_2max) depressed platelet activation in healthy, sedentary subjects.

There is very few data about the effect of acute exercise on platelet secretory response. The effect of exercise on platelet release reaction also seems to relate with the intensity of the exercise (8, 9). We observed a postexercise increase of ATP release but this was not significant statistically. Buczynski et al (10) observed that submaximal physical exercise (75% VO2max) decreased the concentrations of ADP and ATP in platelets and the decrease was probably caused by the stimulation of secretory process.

It is suggested that sedentary and physically active subjects respond differently to the same exercise protocol and the effects of acute exercise tend to be more pronounced in sedentary than in active (9, 11, 12). But the prospective studies about the effect of training programs have not performed very often (5). In the presented study, the effect of the training program, that has been recommended for initiating aerobic training effects, on platelet function was estimated (12, 13).

Our results suggest that an adaptation of platelets to exercise occur as the training program proceeds. The reason for the effect of training on platelet function is also debated. Sinzinger and Fitscha (14) observed a decrease of ADP-induced platelet aggregation at rest and after the exercise by training accompanied by an increase of platelet sensitivity to PGI_2

Table 1: Maximal intensity and maximal rate of platelet aggregation, amount of ATP release induced by ADP and collagen, and platelet count before and after the exercise at 1st, 3rd and 6th week of the training. (n=10). Values are means and SD.

		Max. Int (Ω)		Max.Rate(Ω/m)		ATP Rel. (nM)		Pl.Count
		ADP	Collagen	ADP	Collagen	ADP	Collagen	($x10^3/mm^3$)
1st week	Before	11.4±4	24.1±7	5.3±2.8	10.1±3	1.31±1.2	1.47±1	144.1±50.2
	After	18.2±11*	31.6±9*	8.2±4.3*	12.3±4*	1.63±1.1	1.53±0.8	153.3±53.6
3rd week	Before	16.8±6.4	27.1±8	8.0±5.0	11.7±4	1.07±1	1.1±0.5	155.6±51.9
	After	19.8±11.2	28.5±9	9.2±4.8	12.5±4	1.34±0.5	1.1±0.5	176.7±57.7
6th week	Before	17.1±6.3=	32.1±10=	8.4±5.4=	12±6	1.2±1.1	1.2±0.5	136.9±51.9
	After	16.1±5.1	27.9±8	8.4±7.3	12.4±5	1.21±1.1	1.2±0.6	150±62.9

* Significantly different from measured before the first exercise, $p<.05$

= Significantly different from basal levels that measured before the training, $p<0.05$.

(both at rest and after the exercise). They indicated the important role of platelet sensitivity to PGI_2 in platelet activation .

In our previous study we observed a postexercise increase of antioxidant activity (erythrocyte superoxide dismutase activity) at the 3rd and 6th week of a similar training program (15). Kedziora et al (16) reported a postexercise increase in superoxide dismutase (SOD), catalase (CAT) and gluthation peroxidase (GPx) activities in blood platelets accompanied with decrease in platelet malondialdehyte (MDA) and thromboxane A2 (TxA_2) concentration. Antioxidants are known to be regulators of platelet function and it has been reported that they decreased the sensitivity of platelets to several agonists (17). PGI_2 synthetase is stimulated by antioxidants while TxA_2 synthesis in platelet is inhibited by radical scavengers (17). It is thought that the adaptation of platelets partly relate with the adaptation to oxidative stress that is occured with increase in antioxidant activity.

Moreover we observed that the aggregability of platelet in response to ADP and collagen increased significantly at rest by training. Our unpublished data also indicated that maximal intensity of ADP-induced platelet aggregation in basketball players was higher than of sedentary subjects. Our results were contradictory to previous reports. The reason for the increase in platelet aggregation at rest by training can not be elucidated. Further research is needed to explain the increase in platelet aggregability of active subjects. Significant increase in VO_2max of the subjects at the 3rd and the 6th weeks of the training indicated the adequate intensity and duration of the program for improving the aerobic capacity.

REFERENCES

1. Morris JN, Clayton DG, Everitt, MG, Semmens AM, Burgess, EH. Exercise in leisure time: coronary attack and death rates. Br Heart J 1990; 63:325-54.
2. Shapper AG, Wannamethee G. Physical activity and ishemic heart disease in middle-aged british men. Br Heart J 1991; 66: 384-94.
3. Davies M, Thomas AC, Knapman PA, Hangartner JR. Intramyocardial platelet aggregation in patients with unstable angine suffering sudden ischemic cardiac death. Circulation 1986; 73: 418-27.
4. Fitzgerald DJ, Roy L, Catella F, Fitzgerald GA. Platelet activation in unstable coronary disease. N Engl J Med 1986; 315: 983-6.
5. Wang JS, Jen CJ, Chen HI. Effects of exercise training and deconditioning on platelet function in men. Arterioscler Thromb Vasc Biol 1995; 15 (10): 1668-74.
6. Astrand PO, Rhyming I. A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during submaximal work. J Appl Physiol 1954; 7:218.
7. Wojensky C, Smith J., Silver MJ. Evaluation of electrical aggregometer: Comparison with optical aggregometer, secretion of ATP and accumulation of radio-labeled platelets. J Lab Clin Med 1983; 101: 44-52.
8. Drygas WK. Changes in platelet function, coagulation, and fibrinolytic activity, in response to moderate, exhaustive, and prolonged exercise. Int J Sports Med 1988; 9: 67-72.
9. Wang JS, Jen CJ Kung, HC, Lin LJ, Hsiue TR, Chen HI. Different effects of strenuous exercise and moderate exercise on platelet function in men. Circulation 1994; 90 (6), 2877-85.
10. Buczynsky A, Kedziora Kornatowska K, Kedziora J, Wachowicz B. Effects of submaximal physical exercise and immobilization in bed on adenin nucleotides concentration in human blood platelets. J Physiol Pharmacol 1995; 46(2): 213-9.
11. Kestin AS, Ellis PA, Barnard MR, Errichetti A, Rosner BA, Michelson AD. Effect of strenuous exercise on platelet activation state and reactivity. Circulation 1993; 88 (PT 1) 1502-11.
12. Sellier P, Corona P, Audouin P, Payen B, Plat F, Ourbak P. Influence of training on lipids and coagulation. Eur Heart J 1988; 9 (Suppl M): 32-6.
13. Astrand PO, Rodahl K. Physical Training . In: Astrand, P.O., Rodahl, K.eds. Textbook of Work Physiology. 3rd Ed. New York: Mc-Graw-Hill Book Company, 1986: 413-522.
14. Adams WC. Exercise Physiology. In: Adams, W.C. ed. Foundations of Physical Education, Exercise, and Sports Sciences. Philedelphia: Lea and Febiger, 1991:80-121.
15. Sinzinger H, Fitscha P. Jogging causes a significant increase in platelet sensitivity to prostacyclin. Int J Sports Med 1986; 338-41.
16. Zergeroğlu AM, Ersöz G, Yavuzer S. Submaximal exercise and erythrocyte superoxide dismutase and catalase enzyme activities. XXVth FIMS World Congress Athens-Greece, Abstract Book 1994; p129.
17. Kedziora J, Buczynski A, Kedziora-Kornatowska K. Effect of physical exercise on antioxidative enzymatic defense in blood platelets from healthy men. Int J Occup Med Environ Health 8 (1): 33-9, 1995.
18. Salonen JT. Antioxidants and platelets. Ann Medicine 1989; 21: 59-62.

JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 69-72

LEARNING AND NITRIC OXIDE*

Banu Ocakçıoğlu • Sema Yavuzer****

SUMMARY

Neurons in the mammalian central nervous system are able to alter the strength and patterns of their synaptic connections. This activity that is dependent on synaptic plasticity plays an important role in learning and memory.

Recent evidence suggests that nitric oxide (NO) has been implicated in long term potentiation (LTP) which is a cellular model of learning and memory. Intensive studies about this subject has been continuing but the results of different investigations are contradictory and the mechanism is not yet clear.

In this study it was aimed to investigate the effects of NO synthase inhibition on learning and memory in Swiss Albino rats that were administered intraperitoneally L-NAME (Nw-Nitro-L-Arginine Methyl Ester) at a dose of 20 mg/kg one hour before the test of swimming to a platform in a water tank.

It was observed that, in the L-NAME administered group learning was disrupted significantly ($p<0.01$) in comparison with the serum physiologique administered control group and it was concluded that NO might play a role in short term memory.

Key Words: *Long term potentiation (LTP), L-NAME, NOS inhibition, spatial learning*

Neurons in the mammalian central nervous system are able to alter the strength and patterns of their synaptic connections. The activity dependent plasticity is one possible mechanism by which animals learn and remember (1).

Induction of long term potentiation (LTP) requires a postsynaptic event (activation of NMDA receptors and Ca^{2+} influx) and maintenance of LTP involves a presynaptic event (increase in transmitter release). A retrograde messenger such as NO that is released from the dentritic spines of the active postsynaptic cell diffuses to the presynaptic terminals to activate one or more second messengers that act to enhance trammitter release and thereby maintain LTP. Two gases that diffuse readily from cell to cell, nitric oxide (NO) and carbon monoxide (CO) have properties that have made them interesting candidates acting either alone or jointly with other molecules, for the retrograde messenger of LTP (2).

In the CNS, NO is produced in some neurons following activation of excitatory amino acid receptors, particularly NMDA receptors. NO is synthetized from L-arginine by the cytoplasmic enzyme nitric oxide synthase (NOS) which is a calcium dependent enzyme. Activation of the NMDA receptor results in the elevation of intracellular calcium (Ca^{2+}); which in turn activates NOS via the calcium calmodulin complex. NO is not a classical neurotransmitter in the CNS since it is not released by exocytosis and does not interact with a receptor protein but rather diffuses rapidly across the membrane (3).

In the recent years many investigations have been made about the effects of NO on learning and memory. However the effects and the probable mechanisms have not yet been clearly unterstood. Some of the investigations suggest that NOS inhibition disrupts LTP and learning and the others claim that NOS inhibition doesn't prevent LTP induction and doesnít disrupt learning (4,5).

The present study was performed with the aim of investigating the effects of NO on learning and memory by the method of swimming to a platform in a water tank after administration of a NOS inhibitor, L-NAME (Nw-Nitro-L-Arginine Methyl Ester).

MATERIALS AND METHODS

In the present study, 16 male Swiss Albino rats weighting 100-180 gr which were brought up in seasonal light and dark cycle under normal laboratory

* Presented at the 5th Marmara Medical Days, September 16-19, 1996 İstanbul

** Ankara University, Medical School, Department of Physiology

Received: Nov 12, 1996 Accepted: May 7, 1997

conditions were used. They had free access to food and water. Performance of swim to platform was tested in C.H. Vanderwolf's water tank which was modified by adding 1.5 kg milk to make water opaque (6).

The apparatus used in the swimming to platform task was a rectangular aquarium measuring 43x90 cm and 45 cm depth, filled with water at 20°C to a depth of 25 cm. A wire mesh platform 21.5x18.5 cm was placed in the center of the aquarium with its long axis parallel to the long axis of the aquarium and 1 cm below the water surface.

Training consisted of 10 trials for each animal and the animals were placed sequentially in one of the four corners of the water tank facing that corner of the aquarium. On each trial, rats were allowed to swim until they climbed onto the platform (the escape latency). The escape latency was determined by a chronometer. Animals were guided to the platform if they had not found it in 60 seconds. Several prominent room cues were visible from the pool. Intertrial interval was 30 sn. The test was repeated after 24 hr (retention test). One hour before the test L-NAME at a dose of 20 mg/kg was injected intraperitoneally and to control animals same amount of 0.9% saline was administered.

When the performed method of the study is considered regarding the primary and secondary memories, the period between the attempts conforms with the period of the primary memory. The first trial of the retention test was accepted as recall test and it was thought that learning was continued in the rest of the trials.

Evaluation: On the first day of the control groups' mean escape latency was found as 7.9 sec so the escape latencies over 10 sn were considered as error (a trial in which more than 10 sec was taken to climb up was considered to be an error). The escape latency has been determined by taking the average of swimming to platform periods defined during the ten trial that have been applied separately for each animal. Later on the mean value of escape latency has been determined by taking the average of escape latency values for every subject in the group.

In the control and test groups, mean error numbers, mean value of escape latency, number of failing to find the platform were compared and evaluated by Wilcoxon test and Mann-Whitney U test.

RESULTS

FIRST DAY: The results of the first day are presented in Table 1.

1. The L-NAME administered group's mean number of error in 10 trials was 4.50 and that of the control group was 2.63. The test group's first day error number was significantly higher with respect to the control group ($p<0.01$) (Figure 1).

2. On the first day, the number of trials in which the platform couldn't be found was zero in the control group and three in the L-NAME injected group.

3. Escape latency was 13.9 sec in the L-NAME administered group and 7.9 sec in the control group.

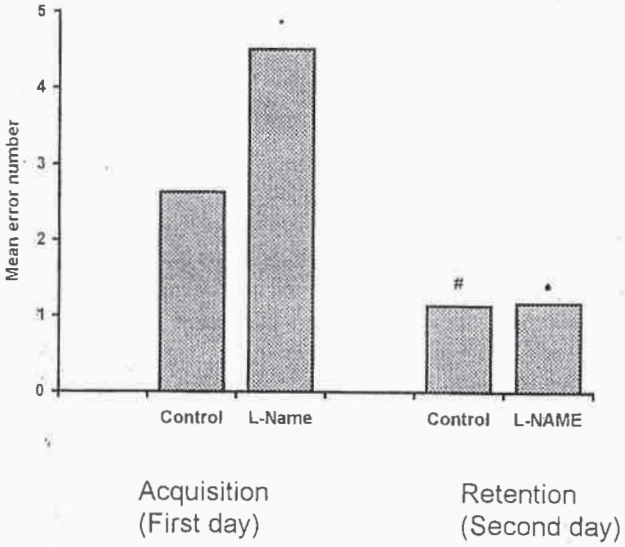

Fig 1: Comparison of mean error numbers of the control and the L-NAME administered groups in acquisition and retention tests.
* $P<0.01$ differs from the control group.
# $P<0.05$ differs from the first day.
• $p<0.05$ differs from the first day.

Table 1: Mean latency and mean error numbers of the control and the L-NAME (20 mg/kg) administered groups in acquisition and retention tests (Values are mean ± SD for 8 animals per group. n for each shown in brackets.)

	First Day (Acquisition)		Second Day (Retention)	
	Mean error number	Mean latency (Sec)	Mean error number	Mean latency (Sec)
Control Group [8]	2.63± 0.52	7.9138±1.3078	1.14±1.21	4.8893±2.0585
Test Group [8] (L-NAME 20 mg/kg)	4.50±1.51	13.9200±6.3992	1.17±1.60	5.9367±6.2600
P	<0.01	<0.01	>0.05	>0.05

Escape latency was significantly high in the test group ($p<0.01$) (Figure 2).

SECOND DAY: Results of the second day are presented in table 1. Second day results of the L-NAME group were similar to those of the control group.

COMPARISON OF THE FIRST AND THE SECOND DAY FINDINGS

1. In the control group performance of the rats on the second day was higher with respect to the first day. Mean error number on the second day (1.14) was lower significantly with respect to the first day ($p<0.05$) (Figure 1).

2. In the L-NAME group mean error number on the second day (1.17) was lower with respect to the first day ($P<0.05$).

3. In the control group escape latency on the second day (4.88) was lower significantly with respect to the first day ($p<0.05$) (Figure 2).

4. In both groups when mean value of escape latency on the first day (control 7.9 sec, test 13.9) was compared with the latency of the first trial on the second day (control 4.2, test 3.6) a significant difference was found ($p<0.05$) and it was considered that this showed retrieval.

DISCUSSION

In the present study, administration of L-NAME at a dose of 20 mg/kg retarded but not prevented the eventual learning in the place navigation test. This finding is concordant with the literature data. Bannerman et al reported that a single i.p. injection of 10 mg/kg L-NAME resulted in only a partial blockade of enzyme activity (percentage inhibition in hippocampus 68,2; cortex 67.3; cerebellum 52.6). However, a single injection of 75 mg/kg L-NAME resulted in a near complete inhibition of brain NO synthase (percentage inhibition of synthase activity in hippocampus 92.9; cortex 93; cerebellum 89). Chronic i.p. injections of 75 mg/kg L-NAME resulted in slightly greater inhibition of NO synthase (hippocampus 96.6; certex 96.6; cerebellum, 95.1) (4). Yamada et al reported that NOS activity in the cerebral cortex, the hippocampus and the striatum of the 60 mg/kg L-NAME treated rats were reduced to 11.2%, 9.2% and 11.9% of the control values respectively (7).

In their study it was observed that daily administration of L-NAME (10-60 mg/kg) resulted in a dose dependent impairment of performance during the acquisition of the radial arm maze task while it failed to affect performance in those rats that had previously acquired the task (7). Although the NOS activity was not determined in our study, according to the literature data the L-NAME dose used in our study might have caused NOS inhibition approximately over 60% and the disruption of spatial learning probably depends on this inhibition. Estall et al reported that in a spatial learning test when L-NAME was administered at a dose of 10 and 20 mg/kg chronically, it disrupted learning in a dose dependent manner (8). According to our results and literature data NO mediates spatial learning and inhibition of its synthesis disrupts learning. Similarly, Böhme et al reported that systemic administration of NOS inhibitor disrupts spatial learning and blocks induction of LTP in the hippocampal tissue (9).

Chapman et al found that NOS inhibition produced by administrating L-NAME at doses of 10 mg/kg and 75 mg/kg disrupted acquisition of spatial learning behavior but retention wasn't affected. In the same study coadministration of NO precursor L-arginine reversed the disruption of learning (1).

In the present study the findings of the test group on the second day supported the finding that NOS inhibition didn't affect retention. Although error number of the test group on the first day was higher significantly with respect to the control group, on the second day error numbers were similar in L-NAME administered group (1.17) and the control group (1.14). On the other hand, there is partial similarity between the results of investigations in which NOS inhibitor L-NAME and NMDA receptor antagonists were administered.

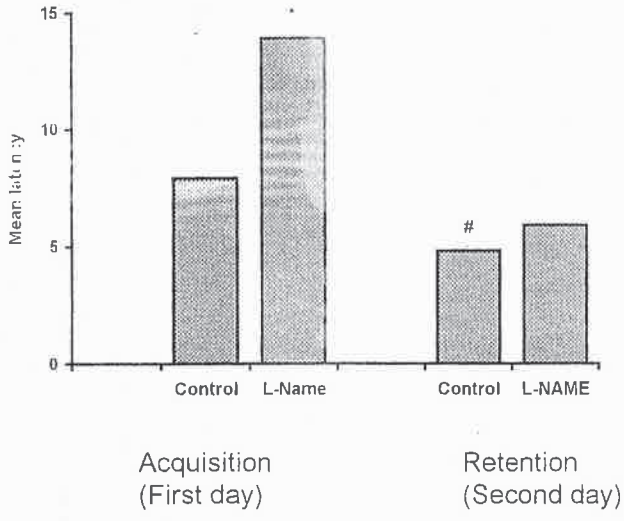

Fig 2. Comparison of mean latency of the control and the L-NAME administered groups in acquisition and retention tests.
*P<0.01 differs from the control group.
#P<0.05 differs from the first day.

Morris et al observed that NMDA antagonist D, L-AP5 disrupted performance of spatial learning but didn't affect retention of spatial information when administered intracerebrovascularly (i.c.v.) (10).

Mondadori et al found that NMDA receptor antagonist MK 801 (i.p.) decreased learning, but it had no effect when administered after the task (11).

These experiments suggest that a NMDA receptor mechanism in the hippocampus perhaps LTP, is involved in spatial learning (2).

It was determined that i.c.v. administered AP5 at sufficient doses blocked LTP totally (10). Harris et al reported that NMDA receptors are ubiquitous in the mammalian central nervous system but are not distributed homogeneously. LTP occurs in regions which have relatively high concentrations of NMDA receptors. NMDA receptors are key elements for triggering synaptically evoked LTP in area CA1 (12).

It was suggested that NO was responsible for the acquisition of information rather than its maintenance or long term storage (10). The results of our investigation also support this view. On the other hand, it appears that NO mediates NMDA receptor activated LTP induction (13).

Böhme et al showed that NOS inhibitor L-NOARG (L-Nw-nitroarginin) administration blocked induction of LTP and this effect was reversed by L-arginine (9). It appears that NO production is necessary only during LTP induction, since NOS inhibitors applied 20-30 min after high frequency stimulation do not reverse established LTP (13).

It was also reported that acquisition of conditioned eyeblink reflex in the rabbit (1) and a passive avoidance task in the chick was disrupted after the injection of NOS inhibitors (14).

On the other hand, Bannerman et al reported that inhibition of NOS failed to block dendate LTP in vivo. They have put forward three possible explanations for the absence of a blockade of LTP with systemic administration of a NOS inhibitor. First, residual NOS activity may be sufficient to support normal LTP. Second, NO may play a threshold modulatory role in LTP induction as the other messengers. Third, NO may have no role in the induction of LTP in vivo (4).

But the results of the present study, show clearly that administration of NOS inhibitor disrupt spatial learning but doesn't effect retention. According to the literature data LTP in CA1 region is accompanied by a substantial increase in the number of synapses formed on the dendrite shafts of pyramidal cells and on spines (13). It is known that synaptogenesis has role in production of secondary memory.

In conclusion it is suggested that NO has a role in primary memory.

REFERENCES

1. Chapman PF, Atkins CM, Allen MT, Haley JE, Steinmetz JE. Inhibition of Nitric Oxide Synthesis Impairs Two Different Forms Of Learning. Neuroreport 1992; 3: 567-570.
2. Kandel ER, Schwartz JH, Jessel TM. Cellular Mechanisms of Learning and Memory. Essentials of Neural Science, Prentice Hall International Inc.,1995; 629-692.
3. Ch S. The Generation of Nitric Oxide and its Roles in Neurotransmission and Neurotoxicity. Keio Journal of Medicine 1995; 44(2): 53-61.
4. Bannerman DM, Chapman PF, Kelly PAT, Butcher SP, Morris RGM. Inhibition of Nitric Oxide Synthase Does Not Prevent the Induction of Long Term Potentiation in vivo.The Journal of Neuroscience, 1994; 14(12): 7415-7425.
5. Bannerman DM, Chapman PF, Kelly PAT, Butcher SP, Morris RGM. Inhibition of Nitric Oxide Synthase Does Not Impair Spatial Learning.The Journal of Neuroscience, 1994; 14 (12): 7404-7414.
6. Vanderwolf CH. Near-total loss of Learning and Memory as a Result of Combined Cholinergic and serotonergic Blockade in the Rat. Behavioural Brain Research. 1987; 23.43-57.
7. Yamada K, Noda Y, Nakayama S, Komori Y, Sugihara H, Hasegawa T, Nabeshima T. Role of Nitric Oxide in Learning and Memory and in Monoamine Metabolism in the rat Brain. British Journal of Pharmacology 1995; 115: 852-858.
8. Estall LB, Grant SJ, Cycala GA. Inhibition of Nitric Oxide (NO) Production Selectively Impairs Learning and Memory in the Rat. Pharmacology Biochemistry and Behavior. 1993; Vol.46: 959-962.
9. Böhme GA, Bon C, Lemaire M, Reybaud M, Piot O, Stutzmann JM, Doble A, Blanchard JC. Altered Synaptic Plasticity and Memory Formation in Nitric Oxide Synthase Inhibitor - treated Rats.Proc.Natl. Acad.Sci. USA, 1993; Vol. 90: 9191-9194.
10. Morris RGM. Synaptic Plasticity and Learning: Selective Impairment of learning in Rats and Blockade of Long Term Potentiation in vivo by the N-Methyl-D-Aspartate Receptor Antagonist AP5.The Journal of Neuroscience, 1989; 9(9): 3040-3057.
11. Mondadori C, Weiskrantz H, Buerki H, Petschke F, Fagg GE. NMDA Receptor Antagonists can enhance or Impair Learning Performance in Animals. Exp. Brain Res. 1989; 75: 449-456.
12. Harris EW, Ganong AH, Cotman CW. Long Term Potentiation in The Hippocampus Involves Activation of N-methyl-D-aspartate receptors. Brain Research, 1984; 323: 132-137.
13. Schuman EM, Madison DV. Nitric Oxide and Synaptic Function.Annu.Rev. Neuroscience, 1994; 17: 153-183.
14. Hölscher C, Rose SPR. Inhibiting Synthesis of the Putative Retrograde Messenger Nitric Oxide Results in Amnesia in a Passive Avoidance Task in the chick.Brain Research, 1993; 619: 189-194.

JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 73-75

ANTIOXIDANT ENZYMES AND PLATELET FUNCTION IN MALIGNANT MESOTHELIOMA*

Şinasi Yavuzer** • Gülriz Ersöz*** • Hakan Fıçıcılar*** • Nezih Özdemir**
Metin Baştuğ*** • Sema Yavuzer***

SUMMARY

The presented study has been carried out to investigate the activity of free radical-scavenging enzymes; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and platelet functions in 12 malignant mesothelioma (MM) patients and 12 healthy persons. Although, in MM patients SOD and GPx enzyme activities were lower than the controls, this was not statistically significant. On the other hand CAT activity was markedly lower in the MM patients ($p<0.001$). Maximal rates of ADP and collagen-induced platelet aggregation that measured in MM group were higher than those measured in the controls ($p<0.05$). Platelet counts were in normal limits in both two groups. The presented data has indicated that the oxidative stress can play a role in the pathogenesis of MM. There are several potential mechanisms by which the oxidants can participate in carcinogenic process. These include direct and indirect interaction with DNA, activation of oncogenes and pertubation of cellular antioxidant defenses. Meanwhile reactive oxygen species (ROS) can also activate platelets and aggravate the platelet functions.

Key Words: *Malignant mesothelioma, oxidative stress, antioxidant defense, platelet aggregation*

Exposure to asbest fiber can cause deposition in lung tissue and induces mesothelioma after a long latency period. Due to fibrous structure of some minerals, phagocytosis is incomplete and macrophages can be chronically activated, thus releasing a number of inflamatory mediator and reactive oxygen species (1). Proliferative changes occur in lungs following inhalation of those mineral dusts, presumably by oxidant dependent mechanism. ROS damage a number of macromolecules including proteins, lipids and DNA. In addition, they alter genetic process affecting the expression of various genes. On the other hand, thromboembolism has been frequently reported in malign mesothelioma, but prevalance studies are lacking. An association between MM and thrombocytosis has been also observed by various authors (2).

The presented study has been carried out to investigate the activity of ROS scavenging enzymes; SOD, CAT, GPx and platelet function in the MM patients and healthy persons.

MATERIAL AND METHODS

Investigation was carried out in twelve hospitalized malign pleural mesothelioma patients in Department of Thoracic Surgery. An aged-matched group of twelve normal healthy subjects volunteered as the control group.

Venous blood samples were taken from fasting patients and controls and placed into siliconized tubes containing trisodium citrate (for evaluating platelet aggregation) and into heparinized tubes (for measuring antioxidant enzyme activities).

Erythrocyte SOD, CAT and GPx activities were determined spectrofotometrically (3,4,5). The chemicals were obtained from Sigma Co. and Hitachi Model 100-20 spectrofotometer was used for determining enzyme activities.

ADP (10μM, Chronolog Reagent) and collagen (2 μg/ml, Chronolog Reagent) induced platelet aggregation were measured by electrical impedance technique by using Chronolog 560 WB Aggregometer (6). Maximal aggregation rate was calculated on the aggregation curve. Platelet counts were performed on Medonic Cell Analyser 610.

RESULTS

Erythrocyte CAT activity of the MM patients was lower than of the controls ($p<0.001$). Copper-Zinc

* The study was presented at VIII Biennial Meeting International Society for Free Radical Research, Barcelona-Spain, on October 1996

** Ankara University, Medical School , Department of Thoracic Surgery
*** Ankara University, Medical School, Deparment of Physiology

Received: Nov 6, 1996 Accepted: May 7, 1997

superoxide dismutase (Cu-Zn SOD) and GPx activities were lower in the MM patients than in the controls but the differences were not statistically significant. (Table 1).

Platelet aggregation responses to ADP and collagen were significantly increased in the MM group ($p<0.05$) however, platelet counts were in normal limits (Table 1, Figure 1)

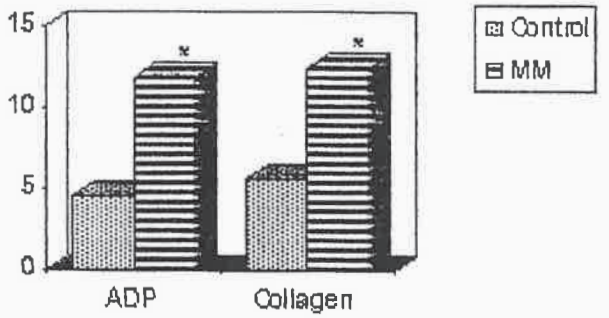

Fig. 1. Maximal rate (ohm/min) of ADP and collagen-induced platelet aggregation in the control (n=12) and the MM groups (n=12).

DISCUSSION

In this study it was observed that the erythrocyte antioxidant enzyme activities of MM patients were lower than of the controls. The difference between CAT activities of the two groups was significant ($p<0.001$). The rate of platelet aggregation responses to ADP and collagen were significantly high in the patient group ($p<0.05$).

It is known that generation of ROS increases in MM. On the other hand, it was observed that antioxidant capacities of the patients were lower than of the healthy individuals (1). This data is pointed the inadequacy in the detoxification of ROS. Namely, the patients are under oxidative stress. Another evidence for this opinion is increased tendency of platelets to aggregation in MM. It is known that ROS activate platelets and aggrevate platelet functions (7,8).

Manzini et al (2) reported thrombocytosis in MM cases. Nevertheless in our study, it was observed that there was no significant difference between platelet counts of the patients and the controls. This result indicates that the increase in the rate of platelet aggregation in MM is not related with platelet count. It is thought that this increase is due to a factor (or factors) that affects platelets directly and this factor is probably ROS which the generation increases.

Therefore, these results confirm the role of oxidants in the pathogenesis of MM. There are several potential mechanisms by which the oxidants can participate in carcinogenic process. These include direct and indirect interaction with DNA, activation of oncogenes and pertubation of cellular antioxidant defences (9,10,11). It is reported that exposure to mineral dusts causes spesific alterations of some components of the antioxidant defense system. It is also suggested that even increase in antioxidant capacity, it be insufficient in preventing MM (1).

On the other hand, an association between MM and thromboembolism has been frequently reported(2). ROS-induced platelets may play a major role in thromboembolic complications. Pacchiarini and collegues (12) showed that mesothelioma tumor cells possesed proaggregating and procoagulant properties in vitro. They reported that the proaggregating activity was a peculiar feature of the malignant cells, thus normal mesothelial cells did not induce platelet aggregation.

In conclusion it was determined that there was increased tendency of platelets to aggregation in MM patients. On the base of previous studies and our results, it is thought that the activated platelets may play an essential role in thromboembolic complication in MM patients.

Table 1: Erythrocyte SOD, CAT and GPx activities, maximal rate (max. agg. rate) of ADP and collagen-induced platelet aggregation, platelet count in the controls and the MM patients.

		Control (X±SD)	MM (X±SD)
SOD	(U/gHb)	2859.9±717.3	2535.5±1014.4
CAT	(k/gHb)	300.0±49	219.7±43.0**
GPx	(U/gHb)	3.3±1.4	2.9±1.0
Max. agg.	ADP	4.6±2.2	11.9±3.3*
rate (ohm)	Collagen	5.6±2.1	12.4±3.0*
Platelet	Count	273±48	291±50
(1000x	/mm3)		

* $p<0.05$
**$p<0.001$

REFERENCES

1. Jannsen YM., Borm PJA, Marsh JP, et al. The role of active oxygen species in lung toxicity induced by mineral fibers and particulates. In:Environmental Oxidants. Nriagu, J.O., Simmons, M.S. (Eds.), John Wiley and Sons, Inc, 1994; 445-458.
2. Manzini VP. Thrombocytosis in malignant pleural mesothelioma. Tumori. 1990, 76: 576-578.
3. Winterbourn CC. The estimation of red cell superoxide dismutase activity. J Lab Clin Med.1975; 85: 337-341.
4. Aebi H. Catalase in vitro. Methods in Enzymology. 1984, 105,121-126.
5. Beutler E. Gluthatione Peroxidase. In: Red Cell Metabolism: A Manual of Biochemical Methods 3rd Ed. Grune and Stratton, NY, 1984: 66-68.
6. Wojenski C, Silver MJ. A quick method for screening platelet dysfunctions using whole blood lumiaggregometer. Thromb Haemostas. 1984; 5(2), 154-156.
7. Salvemini D, Nucci G, Sneddon JM et al. Superoxide anions enhance platelet adhesion and aggregation. Br J Pharmacol. 1989; 97, 1145-1150.
8. Ohyashiki T, Kobayashi M, Matsui K. Oxygen-radical-mediated lipid peroxidation and inhibition of ADP-induced platelet aggregation. Arch Biochem Biophys 1991; 288(1), 282-286.
9. Walker C, Everitt J, Barrett JC. Possible cellular and molecular mechanisms for asbestos carcinogenicity. Am J Indust Med 1992; 21, 253-273.
10. Sahu SC. Role of oxygen radicals in carcinogenesis. In vitro Toxicol. 1990, 3, 161-171.
11. Byczkowski J. Asbestosis and oxidative stress. In: Environmental Oxidants. Nriagu, J.O., Simmons, M.S. (Eds.), John Wiley and Sons Inc, 1994; 445-458.
12. Pacchiarini L. Proaggregating and procoagulant activities of human mesothelioma tumor cells at different stages of in vitro culture. Haemotologica. 1991; 76(5), 392-397.

THE ROLE OF DEFECTIVE ANTIOXIDANT DEFENSE IN PRIMARY FIBROMYALGIA SYNDROME*

Sema Yavuzer** • Hakan Fıçıcılar** • Güneş Yavuzer** • Metin Baştuğ**
Meltem Dalyan*** • Gülay Dinçer***

SUMMARY

Primary fibromyalgia syndrome (PFS) is a pathological condition involving generalized muscular pain, multiple tender points, fatigue, abnormal muscle metabolism and sleep disturbance. Despite comprehensive academic interest and studies, the etiology of this condition is not completely understood. Besides, there are similar muscular findings among PFS and equine postanaesthetic myositis in which free radicals may play a significant role in the pathogenesis. The presented study has been performed to investigate the capacity of intracellular antioxidant defense in PFS. To achieve this goal, erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities in 22 patients with PFS and 20 healthy persons (control group) were determined. In the PFS group, SOD and CAT activities were significantly lower than control ($p<0.001$). This data that is figured out for the first time supports the hypothesis that in PFS cases the intracellular antioxidant defense is defective and insufficiently detoxified free oxygen radicals may be the cause of all symptoms in such patients.

Key Words: *Primary fibromyalgia syndrome, oxidant stress, antioxidant enzyme activities*

Primary fibromyalgia syndrome (PFS) is a common musculoskeletal disease characterized by widespread pain, multiple tender points, fatigue, sleep disturbance and morning stiffness. In addition, most of the patients complain of reduced physical performance, endurance, and effort dispnea. Despite comprehensive academic interest and studies, the etiology of this condition is not completely understood (1). It has been reported that there were functional and metabolic disturbances in the painful muscles of the PFS patients (2, 3, 4, 5). Some of these disturbances are insufficiency of relaxation, decreased blood flow, decreased oxidative metabolism, reduced level of ATP, ADP and phosphocreatine, accumulation of lipofuscin, mild structural pathology in mitochondria and contractile proteins. Equine postanaesthetic myositis is a similar condition in which muscles become hard, intracompartmental muscle pressure increases, local capillary blood flow decreases and lactate concentration increases in the blood draining from affected muscle which indicates local hypoxia and anaerobic metabolism. These muscular findings could be either localized in a particular muscle group or generalized (6). Moreover, muscle spasm due to pain and stiffness can cause disruption of regional blood flow and results in increased generation of free radicals.

This study was performed to investigate the capacity of intracellular antioxidant defence in PFS. To achieve this goal, erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities were determined in PFS patients in comparison to healty controls.

MATERIALS AND METHODS

Twenty-two patients (17 female, 5 male) satisfying American College of Rheumatology (ACR) 1990 classifications for PFS (7) were included into the study, after having a preliminary evaluation consisting of a detailed history, physical examination and labratory assesments. Patients with a history of traumatic, neurologic, muscular, infectious, osseous, endocrine or other rheumatic conditions were excluded. Tender point examination was performed by exertion of uniform amount of manual finger pressure, until the fingernail blanches, on each of nine paired anatomic locations which are insertion of suboccipital muscle, lower sternomastoid muscle, second costochondral

* The study was presented at VIIIth Biennial Meeting International Society for Free Radical Research, 1-5 October 1996, Barcelona, Spain.

** University of Ankara, Faculty of Medicine, Department of Physiology
*** University of Ankara, Faculty of Medicine, Department of Physical Medicine and Rehabilitation

Received: Nov 28, 1996 Accepted: April 2, 1997

junction, 2cm. distal to lateral epicondyle, mid-upper trapezius muscle, origin of the supraspinatus muscle, upper-outer quadrant of the buttock, prominence of greater trochanter and the medial fat pad of the knee. The sites were graded as: 0=not tender or mildly tender, 1=moderately tender, 2= severly tender. A tender point score was computed by the addition of these graded points. Pain, sleep disturbance and fatigue was evaluated by 0-10 visuel analogue scale in which 0=none, 10=unbearable (8). Age-matched twenty healthy controls (10 male, 10 female) were also examined.

Erythrocyte SOD and CAT activities were determined in the heparinised blood samples obtained from the subjects. After separating the plasma from the blood samples, erythrocytes were washed twicely with saline and were hemolized by adding 1.5 volume distilled water. Then the etanol-chloroform extracts were prepared, and the enzyme activities were spectrophotometrically determined (9, 10). 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 student's t test was used with an alpha level of 0.05 for the significance.

RESULTS

Mean age of PFS patients was 35 years and mean duration of PF symptoms was 3.3 years. Mean tender point score, pain, sleep disturbance, fatigue scores were found to be as 19.3, 7, 6.5 and 8 respectively.

In the PFS group, SOD and CAT activities were significantly lower than control group($p<0.001$) (Figure 1). The SOD activity (mean ± S.d.) of control group was 3309 ± 291 U/grHb, while in PFS group it was 2134 ± 875 U/grHb. The CAT activity was measured as 300 ± 49 k/grHb in the control group and 171 ± 89 k/grHb in the PFS group.

DISCUSSION

This data that is figured out for the first time is consistent with following hypothesis.

1-In the PFS cases the intracellular antioxidant defence is defective. Therefore, the organism is under the effect of oxidant stress. Accumulation of lipofuscin in the affected muscles further supports this hypothesis (11).

2-The insufficiently detoxified free oxygen radicals may be the cause of almost all symptoms of PFS (12, 13, 14, 15, 16, 17).

Reactive oxygen species that can not be scavenged sufficiently may cause a decrease in ATP level by affecting aerobic and anaerobic energy systems. In the cells that exposed to oxidants, glycolytic pathway diminishes due to both inhibition of glyceraldehyde 3-phosphate dehydrogenase and decreased levels of nicotinamide adenine dinucleotide. Moreover, oxidative pathway reduced by the inhibition of ATP synthetase (18, 19).

Also free radicals cause a decrease in activity of Na^{+}-K^{+} ATPase and Ca^{+2}- ATPase by oxidation of thiol groups (19, 20). Thus, intra and extracellular ionic imbalance occurs. Both decreased Ca^{+2}-ATPase activity and reduced high-energy phosphate levels can cause relaxation insufficiency in affected muscle. Insufficient relaxation of affected muscles, mainly diaphragma, and reduced ATP, phosphocreatin levels in the painful muscles of primary fibromyalgia patients have been reported (3) (Figure 2).

Intra and extracellular ionic imbalance and oxidation of membrane components that caused by free radicals result in increased membrane permeability. Additionally, oxidative stress destroys the synthesis of gamma-aminobutyric acid (GABA) by decreasing glutamic acid decarboxylase (GAD) activity (19, 21). Insufficient inhibitory synaptic transmission may increase neuronal excitability. In addition, GABA and serotonin are the transmitters that related to non-REM

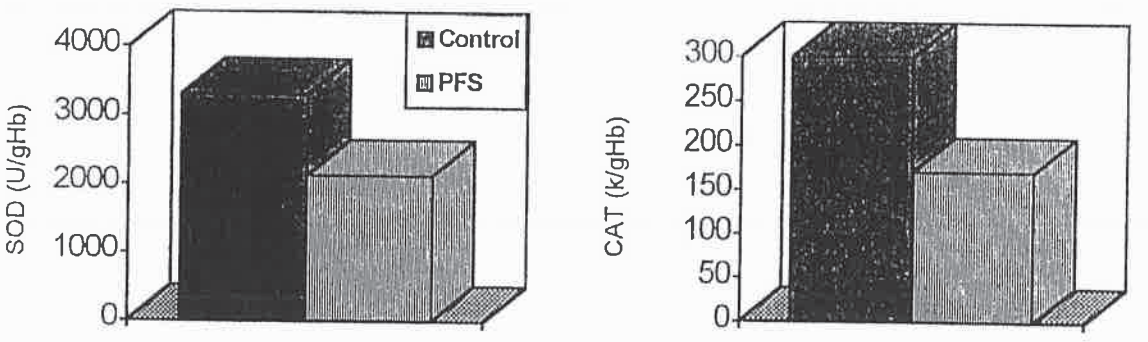

Fig. 1. Erythrocyte SOD and CAT activities in the control and PFS groups (mean ± S.d.)

sleep which is disturbed in PFS (12, 14, 15, 16) (Figure 3). Thus, the symptoms that observed in PFS, such as sleep disorders, anxiety and chronic fatigue, seem to be related with oxidative stress (Figure 4). In conclusion, it is suggested that oxidative stress play a primary role in the pathogenesis of PFS.

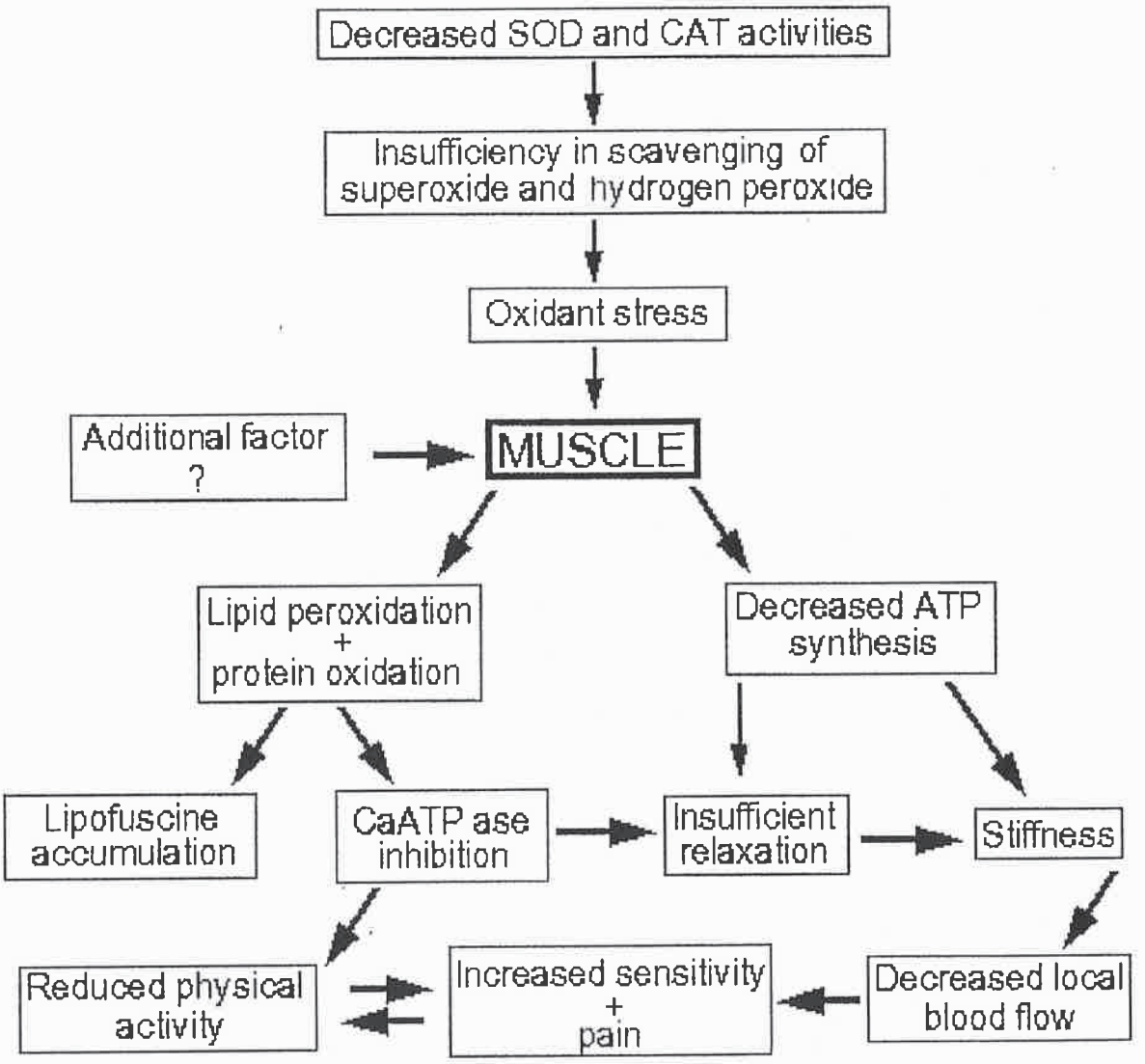

Fig. 2. The relationship between oxidative stress and some biochemical and physiological disturbances of affected muscle in PFS.

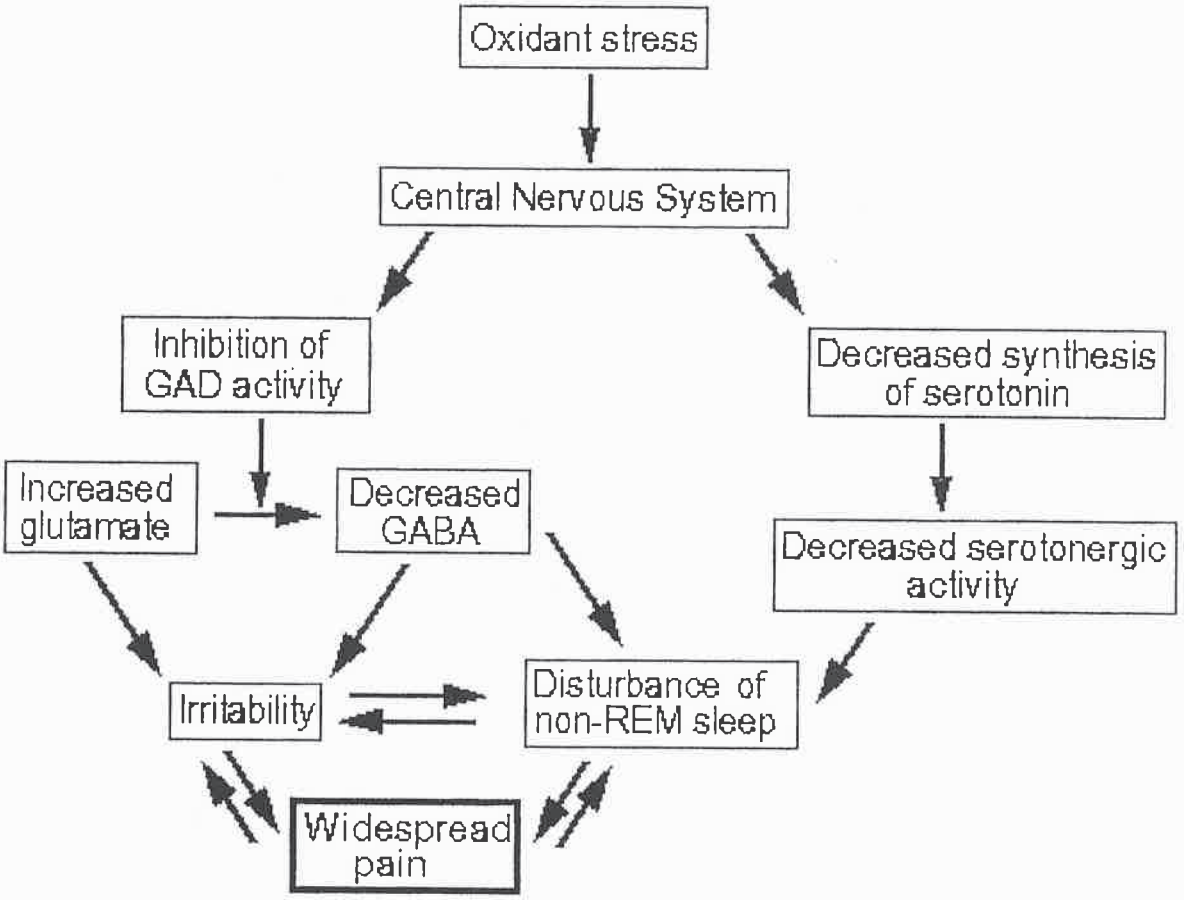

Fig. 3. The relationship between oxidative stress and non-REM sleep disturbance and irritability in PFS cases.

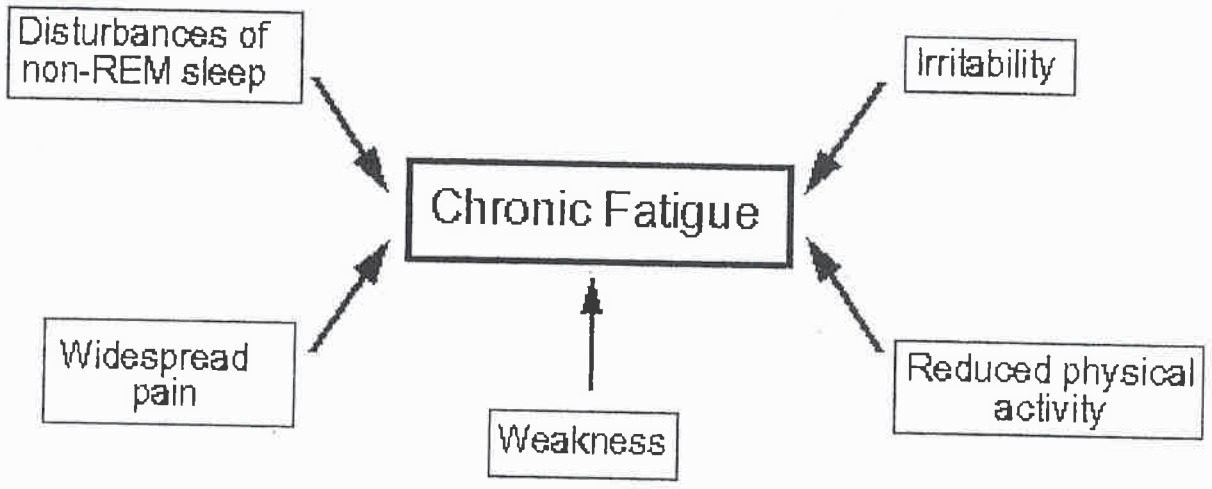

Fig. 4. The various disturbances caused by oxidative stress may be reason for chronic fatigue in PFS.

REFERENCES

1. Yunus MB, Ahles TA, Aldag JC, et al. Relationship of clinical features with psychological status in primary fibromyalgia. Arthritis Rheum 34: 15-21, 1991.
2. Bäckman E, Bengtsson A, Bengtsson M, et al. Skeletal muscle function in primary fibromyalgia. Effect of regional sympathetic blockade with guanethidin. Acta Neurol Scand 77: 187-191, 1988.
3. Bengtsson A, Henriksson KG, Larsson J: Reduced high-energy phosphate levels in the painful muscles of patients with primary fibromyalgia. Arthritis Rheum 29: 817-821, 1986
4. Bennett RM: Muscle physiology and cold reactivity in the fibromyalgia syndrome. Rheum Dis Clin North Am 15: 135-147, 1989.
5. Yunus MB, Kalyan-Raman UP: Muscle biopsy findings in primary fibromyalgia and other forms of nonarticular rheumatism. Rheum Dis Clin North Am 15: 115-134, 1989.
6. Sertevn D, Mottart E, Deby C, et al. Equine postanaesthetic myositis: a possible role for free radical generation and membrane lipoperoxidation. Res Vet Sci 48: 42-46, 1990.
7. Wolfe F, Smythe HA, Yunus MB, et al. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. Arthritis Rheum 33: 160-172, 1990.
8. Littlejohn GO: A database for fibromyalgia. Rheum Dis Clin North Am 21: 481-557, 1995.
9. Aebi H: Catalase in vitro. Methods Enzymol 105: 121-126, 1984.
10. Winterbourn CC, Hawkins RE, Brian M, et al. The estimation of red cell superoxide dismutase activity. J Lab Clin Med 85: 337-341, 1975.
11. Drewes AM, Andreasen A, Schroder HD, et al. Pathology of skeletal muscle in fibromyalgia: a histo-immunochemical and ultrastructural study. Br J Rheumatol 32: 479-483, 1993.
12. Cazevieille C, Muller A, Meynier F, et al. Superoxide and nitric oxide cooperation in hypoxia/reoxygenation-induced neuron injury. Free Radic Biol Med 14: 389-395, 1993.
13. Crofford LJ, Demitrack MA: Evidence that abnormalities of central neurohormonal systems are key to understanding fibromyalgia and chronic fatigue syndrome. Rheum Dis Clin North Am 22: 267-284, 1996.
14. Gilman SC, Bonner MJ, Pellmar TC: Peroxide effects on [3H]L-glutamate release by synaptosomes isolated from the cerebral cortex. Neurosci Lett 140: 157-160, 1992.
15. Hirata H, Ladenheim B, Rothman RB, et al. Methamphetamine-induced serotonin neurotoxicity is mediated by superoxide radicals. Brain Res 677: 345-347, 1995.
16. Jimenez Del Rio M, Velez Pardo C, Pinxteren J, et al. Binding of serotonin and dopamine to serotonin binding proteins in bovine frontal cortex: evidence for iron-induced oxidative mechanisms. Eur J Pharmacol 247: 11-21, 1993.
17. Russell IJ, Bowden CL, Michalek J: Platelet 3H-imipramine uptake receptor density & serum serotonin levels in patients with fibromyalgia/fibrositis syndrome. J Rheumatol 19: 104-109, 1992.
18. Halliwell B: Oxidants and central nervous system: Some fundamental questions. Acta Neurol Scand 126: 23-33, 1989.
19. Weiss SJ: Oxygen, ischemia and inflammation. Acta Physiol Scand Suppl. 548: 9-37, 1986.
20. Keher JP: Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol 23: 21-48, 1993.
21. Bondy SC, Lebel CP: The relationship between excitotoxicity and oxidative stress in the central nervous system. Free Radic Biol Med 14: 633-642, 1993.

JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 83-88

AN EVALUATION OF THE INHALANT RELATED DISORDERS IN A UNIVERSITY PSYCHIATRY CLINIC*

Aykut Özden** • Saynur Canat***

SUMMARY

Objective: Although the exact prevalence of inhalant related disorders are generally unknown in Turkey, there are strong suggestions that it is increasing steadily. As being a major health problem, we wanted to evaluate the characteristics of patients with inhalant related disorders and the outcome of the treatments they received. Method: We evaluated 20 patients admitted consecutively to our hospital with inhalant misuse. Results: The results showed that almost every patient came from low socioeconomic status and had intact family structure that seemed to enable their admission to psychiatry. They were mainly worker adolescent boys with predominantly adhesive and thinner abuse. They were followed on an individual outpatient basis and the outcome mostly depended on patient characteristics. Conclusion: A structured team work, and preferably a separate unit is needed for the treatment and follow up of these patients. On the other hand, there must be some interventions done for the patients who do not or could not admit to psychiatry, as they might be the majority of inhalant misusers.

Key Words: *Inhalant Related Disorders, Volatile Substance Abuse, Adolescence*

Inhalant related disorders (IRD) include various clinical conditions (dependence, abuse, intoxication, delirium, etc.) that are associated with inhalant use (1). The literature has other synonyms for these disorders, like; volatile abuse, solvent abuse (2), but in this paper only IRD and "inhalant misuse" will be used, interchangeably. IRD is mainly a problem of last two or three decades, and it can be speculated that; it is one of the major health problems of adolescents and young adults of today, especially in the low socio-economical levels (3).

Since the cost of these substances is relatively low and their availability is rather easy, they seem to get more popular among children and adolescents everyday (4). It is estimated that about 16.5% of persons aged 18 to 25 misuse inhalants at least once (5). Their high potency of dependence, high risk of numerous physical and psychiatric problems and sometimes sudden death, highlights its importance for the clinicians and other health care providers (6).

There are countless products that can be inhaled and have a potencial of misuse; adhesives, aerosoles, gasoline, paint thinner, nail paints, other paints, anesthethic gases, dry cleaning liquids, etc (2). They contain various chemical substances like; iso-butane, n-hexan, toluene, xylene, ethyl acetate, chloroform, etc (2). Their preferences by the "consumers" vary along time, for example in England, adhesives had been used by 80% of all inhalant misusers in 1982, but in 1987 they dropped to 8%, while butane rose to 42% and aerosoles to 23% (7).

The potential risks of inhalant substances are also countless; ranging from mild forgetfulness to death from toxication, asphyxia or trauma (2, 4). They are probably more fatal than alcohol and nicotine in the short term. Moreover, about 10% of the inhalant misusers developes other, more heavy substance dependence (e.g. heroin) after a few years (8), which underlines another public health problem.

IRD is known to occur in Turkey for about a decade or so, but it came to public attention for the last two or three years. This is particularly because of the growing number of misusers in the society. Nowadays, even lay people witness them in the parks

* Poster presentation, Xth World Congress of Psychiatry, Madrid, Spain, 1996.

** Psychiatrist

*** Professor of Psychiatry

Received: May 14, 1997 Accepted: May 21, 1997

and backstreets of Istanbul and Ankara, inhaling glue from a nylon bag. We, as the clinicians of the adolescent unit of the psychiatry clinic of university of Ankara, have also started to face with a flow of inhalant misuser patients brought by their anxious families. This made us think about the problem and we decided to intervene in a more systematic way. We want to perform interventions not only in the secondary prevention area, but also in the primary prevention, which must be obligatory in dealing with the IRD problem (9).

As a start, we planned to investigate the general characteristics of the IRD patients admitted to our hospital, with this study. After this step, we plan to evaluate the efficacy of our treatment modalities and then on the primary prevention area we plan to investigate the prevalance of IRD, risk group characteristics and the efficacy of various prevention programs.

METHOD

This study is performed as a cross-sectional analysis. We have included 20 consecutive cases of IRD, admitted to our hospital in the second half of 1995. These patients are referred to us either from other units of our psychiatry clinic (e.g. inpatient wards) or they come to our adolescent psychiatry unit directly, for treatment purposes. We did not include previous cases of IRD admitted to our hospital.

We used a questionnaire prepared in the light of relevant literature, but we believe that almost every question of this questionnaire is a part of their routine evaluation process. So it can be concluded that this study includes the main characteristics of the patients with IRD. The questionnaire was filled by the interviewer after the patients were informed about the nature study and were asked to give consent, of which neither turned down.

RESULTS AND DISCUSSION

The socio-demographical characteristics of the patients are evaluated and 95% (n=19) is found to be male and 45% (n=9) is worker, while 30% (n=6) is student and 25% (n=5) is unemployed. To be male is an expected finding, as a body of literature reports high prevalance of IRD among males (3, 6, 10). These worker and unemployed boys come from rather poor families and previous papers underlined the risk of IRD in this population (3, 11). Various occupational sites are found to increase the risk of IRD (2) and our 9 worker patients come from those workplaces, too; polyestere production, car repairement, wall and industrial painting and furniture production. Nevertheless, with a percentage of 30, students can not be overlooked and may be taken as a signal of spread of the disorder to the students.

Mean age of the patients is 17.1 years, and the age of first intake 14.9 years (Mean age - mean duration: 17.1 - 2.2 = 14.9). There are reports in the literature indicating that the mean age of the onset of IRD is getting down, even as low as 13 years (7). Although we could not find a previous pervasive study on this subject in Turkey, we seem to follow this trend. We do not suppose that adolescents of Turkey aged 15 years, were aware of the effects of inhalants 20 years ago, let alone misusing them. Our patients' education level is 7 years, which is compatable with age 13, while their mean age is 17 years, which indicates year loss in the school. It is not clear that their academic failure has resulted from their inhalant misuse or not, but we suppose that low educational level is prevalent in this group, which may be a difficulty in the therapeutic process.

When family characteristics are investigated (Table - 1). They mostly live with their families of origin (85%), has a low - moderate economical status (85%) and live in the suburbs of Ankara (65%). Low socio-economical status has been reported to be a risk factor for IRD in the literature (3). However, the reason beneath this discrepancy may be the different treatment or misuse preferences of the "rich" and "poor" patients. Patients from high socio-economical levels might be referring to private practice psychiatrists, or they may be preferring other, more expensive substances. Another finding of this study is the high percentage of patients living with their family of origin. It would not be surprising if they had been living with relatives or in single parent families, since, as Ash noted (2), broken families constitute a risk factor for all substance dependences, but Turkey seems to have a privilege on this area, divorce rate is still relatively low compared to western countries. Nevertheless, it can be speculated that, although their parents were not divorced, they could not provide enough supervising to their child. Low socio-economical status may have played a role in this.

On the other hand, psychiatric problems in their families are not as low as the number of broken families. This may constitute a risk for these patients, by both psychological and genetic means (12, 13). Although intact family structure may be of benefit, having family members with psychiatric disorders may not.

Table 1: Family Characteristics of the Patients

	n	%
LIVING WITH ...		
Family	17	85
In Orphanage	2	10
Friends	1	5
RESIDENCY		
Ankara - Suburb	13	65
Ankara - Downtown	4	20
Outside of Ankara	3	15
ECONOMICAL STATUS OF THE FAMILY		
Low (Poor)	8	40
Moderate	9	45
High (Rich)	3	15
PSYCHIATRIC PROBLEMS IN THE FAMILY		
None	14	70
Psychosis	2	10
Alcohol Dependence	3	15
Personality Disorders	1	5
BROKEN FAMILY	2	10

Table 2: Various Characteristics of the Inhalant Misuse in the Patients

	n	%
MOTIVES OF ONSET*		
Experimentation	13	65
Relief From Problems	5	25
Both	2	10
CAUSES OF CONTINUATION*		
Psychedelic Effects (Euphoria, etc.)	9	45
Relief From Problems	8	40
Both	3	15
FROM WHOM IT IS LEARNED		
Friends	17	85
Television	3	15
FROM WHERE IT IS OBTAINED		
Small Markets	16	80
Stationary (Writing Material Stores)	4	20
WITH WHOM IT IS USED		
Friends	12	65
Alone	8	35
PREVIOUS TREATMENTS		
None	17	85
Present	3	15
AWARENESS LEVEL ON ITS HAZARDS		
Low	7	35
Moderate	7	35
High	6	30

* Exceeds total number of patients because of multiple causes.

All patients are misusing adhesives, with three (%15) of them taking paint thinner additionally. Adhesives are cheap and easy to find in Turkey, and maybe in other countries as well. They are even cheaper than cigarette. Although there are about 6 different trademark names of adhesives, our patients are found to misuse two of them. Namely; U-4® and Bally®. This seemed to be in relation with their toluen content. On the other hand, 3 patients who were misusing paint thinner are found to be workers, who use paint thinner in their daily work. We think that working in that place has an important impact on their misuse. Some of them, even, may have been dependent by passively inhaling paint thinner while working using it.

It is also interesting to find such high percentages of cigarette (n=19, 95%) and alcohol (n=14, 70%) use in these patients. These substances might have opened the door to inhalant misuse (14). On the other hand, 25% (n=5) have already started to use other substances, like cannabis and biperiden (6).

We have found that the duration since the onset of misuse has been 2.2 years, which can not be taken as short for such dangerous substances. Although their frequency of use were hard to evaluate, we learned that, generally, they were using it almost everyday for a few months and then decreasing it to twice or thrice a week. For the doses, we have asked the number of "tubes of adhesive", which is a common way of expressing the dose in their jargon. The mean is a lot higher than we expected: 18.6 tubes / day (about 130 ml of adhesive). Even, some of them were using more than 20 - 30 tubes /day, and one of them reported of using 80 tubes / day (his parents confirmed the dose) ! These findings imply the importance of a prevention program, before it hurts more people.

It is also interesting to find that neither aerosoles nor other inhalants, except for adhesives and paint thinner, had been misused. We know that a wide range of substances are being misused in other countries (7). We call this finding positive for now, but we must not forget that our adolescents could start on them, too.

The other characteristics are showed in Table - 2. The leading motive of first inhalation seemed to be experimantation, mostly because of a friend's insistance (65%+10%). There are reports in the literature supporting the hypothesis that peer cluster effect is the leading motive in the onset of substance related disorders (15, 16). Three patients reported that they have first seen it on television and after that they had an enormous interest on these substances. Akdemir et al., previously reported a similar finding in their inhalant

misuser patients from another hospital in Ankara (17). These findings underlines the danger of unguided and causal television programs on such issues. On the other hand, in 25% of patients the first motive was to get relief from problems of life. When questioned about the cause of continuation, 40% reported relief from problems and 45% reported that they liked the euphoria, disinhibition and sometimes hallucinations and other perceptional symptoms and 15% both. It is of interest that the number of misusers who take it for relief is increased. After the first one or two experimentations most of the users give it up (18), but having various problems of life, like family conflicts, economical problems workplace problems might be inducing some of them to keep on misusing, as a self medication, as Vaillant noted previously (19).

Other findings on the characteristics of inhalant misuse are; mostly small markets sell the adhesives to these patients (and generally, even though it is known that they are misusers), with higher prices than usual; the patients mostly use it as a group, by exchanging the nylon bag full of adhesive. Except for three of them, none of the patients received treatment before. Finally, the awareness level of the patients on the hazards of these substance is found to be relatively inadequate. It is evaluated by questionning their level knowledge on the dependence potential and adverse effects of the substances. General public might be less aware, but these patients, with a 2 years of inhalation history and with various psychiatric and other problems resulted from inhalant misuse, should have been more aware. For example, only 6 of them knew that these substances could cause dependence and sometimes death. These findings imply the importance of educational programs on both patient and public basis. Low awareness has been found in IRD patients in other countries, too (20).

When asked about the psychiatric, physical and legal problems they experienced (not the ones we found) in relation to inhalant misuse, 90% (n=18) reported psychiatric problems; irritability (70%), forgetfulness (55%) and withdrawal from people (20%) as the leading ones. Fifty percent reported physical problems like; dispnea (25%), fatigue (20%) and pain (15%). Forgetfulness and fatigue has already been reported in the literature (21), but irritability and dispnea, generally, has not. Since the number of our patients is low, we can not make big conclusions from this, but these symptoms may be taken for the warning of an unidentified IRD, along with others. For the legal problems, 65% had one or more trouble with police, mostly because of being caught on taking inhalant or in an intoxicated state. Although two patients reported that they had been caught stealing in order to get more adhesive, most of these legal problems are hard to differentiate as resulting from IRD or conduct disorder (50% had this diagnosis). Lockhart and Lennox (22) has also found anti social acts as more prevalent in these patients, but these findings should not be taken, like, all IRD patients are anti social, since prejudice prevents effective intervention.

Table - 3 outlines the evaluation and the management of these patients. Although there are several diagnosis of IRD in DSM-IV, like, inhalant related intoxication, delirium, demans, psychosis, affective disorder and anxiety disorder, our patients had neither of them. They had mainly inhalant dependence and abuse. Although we have observed comorbid psychiatric disorders, like, major depression in two and panic disorder in one patient, we could not find a direct relation with the inhalant misuse that warrants an IRD. Conduct disorder is found to be highly prevalent among our patients (50%). This may partly result from the high rate of any substance abuse in conduct disorder patients. Half of our patients did not have any conduct problems but did misuse inhalants. This shows us that being a "good boy" does not necessarily prevent from substances.

We also found out that 12 patients with inhalant misuse were hospitalized, 13 patients given medication, 3 patients detoxified with benzodiazepines and 7 patients were offered only psychotherapy. Since inhalant misuse does not show significant withdrawal

Table 3: Results of the Psychiatric Evaluation and Management

	n	%
TYPE OF IRD		
Abuse	9	45
Dependence	11	55
COMORBID PSYCHIATRIC DISORDER*		
None	8	40
Conduct Disorder	10	50
Others**	5	25
TREATMENT GIVEN*		
Hospitalization	12	60
Medication	13	65
Detoxification	3	15
Psychotherapy alone	7	35
OUTCOME FOR THE FOLLOWING THREE MONTHS		
High	5	25
Moderate	5	25
Low	10	50

* Exceeds total number of patients because of dual-diagnosis and multi-treatment

** Major Depression, panic disorder, borderline personality dis., mental retardation

symptoms, detoxification rate is low (18). It is done in patients consuming high doses of inhalants. The medications given were mostly low doses of antipsychotics or antidepressants, while two patients with significant major depression received full dose anti depressant therapy. Hospitalization seemed necessary for 60% of these patients because of various reasons, like, to perform a detailed psychiatric and physical examination, detoxification and to take them away from their inhalant misuse inducing environment.

Psychotherapy was performed in an individual supportive therapy status, as it is offered in the literature (23, 24). Establishing a treatment alliance, providing relief for the emotional stress, problem solving techniques and information on the effects of these substances have been the first goals of such a therapy. However, enhancing their self-esteem was the major goal of their therapy, since it is consistently found low in other studies (15, 25).

However, treatment outcome does not seem promising, even for the first three months after treatment, with only 25% of the patients reaching total abstinence. Since the number of patients is low, this finding may be misleading. Nevertheless, we observed that patients with low to moderate outcome were the ones with conduct disorder or low to moderate levels of awareness on the hazards of inhalant misuse. Other factors (including the treatment given) did not seem to differentiate patients with poor and good outcome, but as the number of patients increase we will be able to perform more reliable and statistically proved conclusions.

CONCLUSION

We can conclude that; our patients with IRD are generally males with low socio-economical and education levels, who live with their family of origin and are mostly industrial workers or are unemployed. They have a mean age of 17 years and began misusing inhalants approximately 15 years of age. Adhesive misuse is very predominant, followed by paint thinner.

These preliminary findings may be outlining the general characteristics of a risk group for IRD. Thus, a prevention program has to include adolescents who work in industrial areas, but nevertheless, should not let out students. It also has to include information on the hazards of the inhalants, since none of our patients seemed to be aware of them when they first took the substance, and moreover, 70% of them has still inadequate awareness. The program should include problem solving and maybe relaxation techniques, since a majority reported that they were continuing to misuse mainly for emotional relief. Also, a prevention has to deal with the availability of the substance, too. Our patients were obtaining the adhesives from the small markets. Making legistations on the selling of volatile substances to IRD patients may prevent some of the misuse, as it was done in other countries previously (9).

We also need more systematic studies evaluating the effects of prevention intervention. Moreover, a structured team work, and preferably a separate unit is needed for the treatment and follow up of these patients. On the other hand, there must be some interventions done for the patients who do not or could not admit to psychiatry, as they may be the majority of inhalant misusers.

REFERENCES

1. American Psychiatry Association. Dignostic and Statistical Manual of Mental Disorders, Fourth Edition. Washington DC, APA Press, 1994.
2. Ash CH. Solvent abuse, little progress after 20 years. Br J Med 1990; 300, 135-136.
3. Edling C, Lindberg A, Ulfberg J. Occupational exposure to organic solvents as a cause of sleep apnoea. Br J Industrial Med 1993; 50, 276-279.
4. Richardson H. Volatile substance abuse: evaluation and treatment. Human Toxicology 1989; 8, 319-322.
5. Oetting ER, Beauvais R. Peer cluster theory, socialization characteristics and adolescent drug use: a path analysis. J Counseling Psychol 1987; 34, 205-213.
6. Liss BI. Government, trade and industry and other preventative responses to volatile substance abuse. Human Toxicology 1989; 8, 327-330.
7. Billington AC. Volatile substance abuse, the role of agencies in the community in prevention and counselling. Human Toxicology 1989; 8, 323-325.
8. Davies B, Thorley A, O'Connor D. Progression of addiction careers in young adult solvent misusers. Br J Med 1985; 290, 109-110.
9. Lockhart WH, Lennox M. The extent of solvent abuse in a regional secure unit sample. J Adolescence 1983; 6, 43-55.
10. Dinwiddle SH, Reich T, Cloninger CR. The relation of solvent use to other substance. Am J Drug Alcohol Abuse 1991; 17, 2, 173-186.
11. Ramsey J, Anderson HR, Bloor K et al. An introduction to the practice, prevalence and chemical toxicology of substance abuse. Human Toxicology 1989; 8, 261-269.
12. Donovan JE, Jessor R. Structure of problem behavior in adolescence and young adulthood. J Consulting Clin Psychol 1985; 58, 890-904.
13. Millman RB. General principles of diagnosis and treatment. APA's Annual Review of Psychiatry Vol 5 (Ed.: Frances AJ, Hales RE) Washington DC, APA Press, 1986; 122-136.

14. Nicholi MA. The adolescent. The New Harvard Guide to Psychiatry (Ed.: Nicholi MA) Cambridge MA, Belknap Press, 1988; 647-649.
15. Newcomb MD, Bentler PM. Impact of adolescent drug use and social support on problem of young adults. J Abn Psychol 1988; 97, 525-531.
16. Oral G, Ziyalar A, Tanman Ç, İlkay E. Volatile substance use and the "Paint Thinner Sniffer Boys". Syndrome 1995; 7, 9, 78-83.
17. Akdemir A, Türkçapar H, Kılıç EÖ et al. Characteristics of inhalant misuser adolescents admitted to the psychiatry clinic. Turkish J Psychiatr 1994; 5, 3, 213-216.
18. Vaillant GE. The alcohol dependent person and drug dependent person. The New Harvard Guide to Psychiatry (Ed.: Nicholi MA) Cambridge MA, Belknap Press, 1988; 700-713.
19. Wright JD, Pearl L. Knowledge and experience of young people regarding drug abuse, 1969-1989. Br J Med 1990; 300, 99-103.
20. Edeh J. Volatile substance abuse in relation to alcohol and illicit drugs: psychosocial perspectives. Human Toxicology 1989; 1, 321-329.
21. Khantzian EJ. A contemporary psychodynamic approach to drug abuse treatment. Am J Drug Alcohol Abuse 1986; 12, 213-222.
22. McDermott D. The relationships of parental drug use and parents' attitudes concerning adolescent drug use to adolescent drug use. Adolescence 1984; 19, 89-97.
23. Kaplan HI, Sadock BJ, Grebb JA. Substance Related Disorders. Synopsis of Psychiatry (7. Basım) Baltimore, Williams and Wilkins, 1994; 383-456.
24. Schilling RF, McAlister AL. Preventing drug use in adolescents through media interventions. J Consulting Clin Psychol 1990; 58, 4, 416-424.
25. Schuckitt MA. Drug and Alcohol Abuse. (3. Basım) New York, Plenum Press, 1989.

JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 89-94

EDUCATIONAL NEEDS OF PSYCHOTIC AND DEPRESSIVE INPATIENTS FROM TURKEY*

Aykut Özden** • Işık Sayıl*** • Hüseyin Özsan**** • Duru Kuzugüdenlioğlu*****
Handan Tuğcu******

SUMMARY

Objective: *It is well known that psychiatric patients benefit from learning about mental illness and how to cope with it, but the specific interests of these patients are somewhat unclear. We have been conducting psycho-education sessions in our clinic's inpatient wards for a long time, and we wanted to shape our sessions according to the needs of the "consumers". Thus, the aim of this study is to investigate the specific educational needs of the patients.* ***Method:*** *We conducted the study as a comparison between psychotic and depressive inpatients, and included 30 patients from each group. A questionnaire was prepared and used to determine the needs, in the light of literature, Turkish culture and our previous experiences.* ***Results:*** *The results showed that; the issue that most of the patients reported strong interest in learning more about was the possibility of relapses and prevention from it. Psychotic patients generally reported interest in issues like medications and side effects, while depressive patients reported learning more about strategies to cope with common problems.* ***Conclusion:*** *We concluded that investigating the educational needs of patients must not be overlooked since it can effect the topics of the psycho-education sessions and if possible separate sessions for depressive patients may be useful as their needs are somewhat different.*

Key Words: *Psychoeducation, schizophrenia, depression, rehabilitation*

There is a huge body of knowledge on the efficacy of psychiatric medications and psychotherapeutic interventions, but we have not reached a totally satisfying outcome for any psychiatric disorder, yet. Almost every disorder in psychiatry still has a relapse rate that can not be ignored. Our experience made us believe that most of these relapses could be prevented by an active program, which includes pharmacotherapy, family counseling, regular appointments, and skills training. However, every one of them needs an active participation on the side of patients. The patients can not be forced to take medication while at home, or to comply with the regular appointments.

Compliance seems to be one of the major keys for success in psychiatry. Even a depressive patient who could be well, if he/she used your prescribed medication, may not remit soon because of noncompliance. It is found that only one third of all patients comply with treatment, one third sometimes comply, and one third never comply (1). It can be speculated that it is even worse in psychiatry, where medications have multiple side-effects and patients often hesitate believing in their illness, which again lower compliance with medications. We believe that a leading reason for patients showing non-compliance with the treatment in psychiatry is the lack of enough knowledge on the treatment and illness.

One of the major approaches to help the patients is, then, to provide the basic information about their psychiatric disorders (2). Diagnosis, signs and symptoms, treatment approaches, side-effects of medication and course of illness are the main informations given to the patients, among others.

Psychoeducation (PE) programs for the mentally ill became widespread during the 1980's as a mean of providing a forum for the relevant education and mutual support of participants (3). A body of research has been conducted between late 1970's and 90's on the effectiveness of PE on delaying the recurrence of a psychiatric disorder episode (4). For instance, Akiskal

* Poster presentation, Xth World Congress of Psychiatry, Madrid, Spain, 1996.

** Psychiatrist MD.
*** Professor MD.
**** Associate Professor MD.
***** Psychiatry Resident MD.
***** Psychologist PhD.

Received: May 14, 1997 Accepted: May 21, 1997

(5), Glick et al. (6) and Honig et al. (7) reported the effectiveness of PE in affective disorders. Wallace (8) and Foote et al. (9) showed the importance of PE in the management of substance abusers. Miller et al. (10) used PE in borderline personality disorders. The results showed consistency from the USA to Japan and from Italy to China (11, 12).

Naturally, schizophrenia has been one of the major areas of research on the effectiveness of PE. Miller (13) and McFarlane (14) showed the benefits of PE in schizophrenia. Hogarty et al. (15) even reported a 50% reduction in relapse rates in schizophrenics living in high expressed emotion households with only PE, and its effects persisted for 24 months, while the effects of social skills training were lost. PE, also helps patients to understand the medications (16), to attribute the causes of illness to relevant issues, which is frequently found distorted in both the relatives and patients (17), both of which may enhance patients' compliance.

Second generation studies, as Goldstein noted (4), tested more specific hypothesis concerning the most efficient format for delivery of such programs, such as; relatives-only, single family unit, multiple family groups, etc., and found no clear-cut differences among them. As Birchwood et al. concluded (18), delivery of the information, rather than the mode of delivery, is the crucial element in the intervention. However, McFarlane and colleagues found that (19) psychoeducational multiple family groups are not only more effective in extending remission, but also more cost-effective with a cost-benefit ratio of 1 : 34.

Despite the widespread clinical practice of educating the patients and relatives about psychiatric illness, relatively little is known about the specific educational needs of these patients (2). The curriculum of most educational programs, including ours, has been established by treatment providers, based on their beliefs as to what information about the illness and its treatment, patients and families needs to know, rather than on objective data collected from the patients and relatives themselves. It appears to be a major flaw of the educational programs.

In the Psychiatry Clinic of University of Ankara, Medical School (UAMS), we conduct patient PE programs, for years. Although the importance of PE for patients has long been known, a structured PE, which considers the different educational needs of different patient groups, has not been established until now. As a matter of fact, we decided to establish a structured and continuous PE program, and as mentioned above, wanted to base it on the objective needs of the "consumers".

Thus, the aim of this study is to evaluate the specific educational needs of schizophrenic and depressive patients, the two most prevalent diagnosis in our inpatient wards, and probably the ones who most needed PE. We also wanted to learn the modes of PE presentation that they prefer most, since the way we use for years (lectures) might not be the most suitable one.

METHOD

The study is conducted in the Psychiatry Clinic of UAMS, in the first half of 1995. The subjects for this study comprised 60 inpatient chronic schizophrenics and major depressive patients, 30 from each group, admitted to our clinic's inpatient wards for male and female psychotics. There were equal number of male and female patients, 30 from each. To be included in the survey, patients had to have an education of at least 5 years.

Since the number depressive patients admitted to our wards is relatively low, it took about half a year to complete the study. We tried to include all of the admitted patients for not to be biased. Very few patient declined to complete the survey, but about 10% had to be replaced by new subjects, because of incomplete fulfilling of the questionnaires.

A questionnaire was developed by the authors to assess the educational needs. Fifty items pertaining to different areas of educational need were generated by a group of five professionals, and questions from previous surveys (2) were used but with the addition of new items relevant to Turkish culture. They rated their interest in learning more about each item on a five point Likert-type scale, with 1 denoting "not interested" and 5 denoting "very interested". Patients were also encouraged to write additional topics if necessary. Finally, patients were asked to present their preferences on the mode of getting this education. They ranked 4 items; Lectures (one hour a week), booklets, group sessions (including discussion and sharing of experience, once or twice a week), courses (a week long course twice or three times a year). They were also encouraged to write down any other mode that is not mentioned.

Since the aim of the study was to investigate the nature of the educational needs of the patients of schizophrenia and major depression, no statistical analysis is performed in order to compare the groups.

Another part of this study is the evaluation of their relatives' educational needs with the same questionnaire, which is presented in another paper (20).

RESULTS AND DISCUSSION

Tables 1 and 2 outlines the socio-demographycal features of the patients. They are in their early thirties, with schizophrenics being a little younger (30.5 years). For the education levels of the subjects, schizophrenics appeared to be less educated (9.9 years) than the depressive patients (11 years), which may be a result of their ongoing debilitating illness. Nevertheless, both results are not lower than the general educational level of Turkish people.

A high percent of patients is found to be married (60% of total), and this finding is more apparent in the depression group (70%). When we evaluate the occupational status of the subjects, we find a high rate of unemployment, which is more prevalent in schizophrenic patients than depressives. Unemployment may be high because of the number of housewives or girls, who are very prevalent in Turkey. Another cause of unemployment in the schizophrenics may be a direct consequence of their illness.

Table - 1 also outlines the duration of the illness and the number of hospitalizations for both groups. Schizophrenics are found to be obviously more chronic than the depressive patients, but the difference between the mean number of hospitalizations is not that apparent, which may imply similar severity despite different chronicity.

In Table - 3, the rank order of educational topics made by major depressive patients is outlined. "Coping with sadness, hopelessness and pessimism" is the most rated item in the list which indicates a

Table 1: Age, Education Status, Duration of Illness and the Number of Hospitalizations

	DEPRESSIVE GROUP (n=30)	SCHIZOPHRENIC GROUP (n=30)
AGE	33.5 ± 9.6	30.5 ± 10.1
EDUCATION	11.0 ± 3.7	9.9 ± 3.1
DURATION OF ILLNESS	1.7 ± 0.8	6.1 ± 3.7
NUMBER OF HOSPITALIZATIONS	1.2 ± 0.4	2.0 ± 1.1

Table 2: Marital Status and Occupations of the Patients

	DEPRESSIVE GROUP (n=30)		SCHIZOPHRENIC GROUP (n=30)	
	n	%	n	%
MARRIED	21	70.0	15	50.0
SINGLE	7	23.3	12	40.0
DIVORCED / WIDOW	2	6.7	3	10.0
UNEMPLOYED	11	36.7	14	46.7
EMPLOYED	19	63.3	16	53.3

Table 3: Educational Topic Ratings of Depressive Patients

RANK	ITEM	MEAN
1	Coping with sadness, hopelessness and pessimism	4.46
2	Strategies for solving problems	4.36
3	Early warning signs of relapse	4.33
4	The prevalance and etiology of the disorder	4.30
5	Coping with social isolation and withdrawal	4.23
6	Making plans for the future	4.20
7	Lack of interest and motivation	4.20
8	Stress reduction	4.16
9	Improving independent living skills	4.10
10	Psychiatric medications	4.07

healthy concern with a major symptom of depression. It is followed by "Strategies for solving problems" and "Early warning signs of relapse". These three items and the items between #5 and #9 revealed an interest in coping skills with the major problems that they face. We would not be surprised if the "side-effects of medications" or "psychiatric medication" had ended up in the top five, but the patients did not appear to be as much concerned as we have expected them to be. This is an important finding because our PE hours mainly emphasize education on medication. Maybe we should shift the balance towards coping skills more than we did before, especially when we are giving PE to major depressive patients.

The first three ratings have some similarity with the Mueser et al.'s 1992 survey (2), except that they found "Ways of managing stress more effectively" as the number one rated item, instead of "Coping with sadness...". "Side-effects" became number two and "Early warning signs of relapse" number three, which is also at #3 in our survey.

In Table - 4, the rank order of educational topics made by schizophrenics is showed. "Early warning signs of relapse" became the number one rated item, followed by "Psychiatric medications" and "Side-effects of medications". These top three items also appeared in the top ten of Mueser et al.'s list (1992), which may be indicating same needs in different cultures. They found "Getting what you need from the mental health system" as the most rated item, followed by "Relapse signs" and "Psychiatric Medication". Our patients seemed to know how to get their needs from the mental health system, (they ranked it at #9) maybe because of the long duration of their illness. Other items in our list revealed an interest in the coping skills, just as depressive patients, mostly for negative symptoms of schizophrenia. Their relatives also reported great interest in the coping skills for negative symptoms (20). It is interesting not to have symptoms, such as hallucinations or delusions in the top twenty, but it may be related with the symptomatology of schizophrenics included in the study.

There are also several differences between the two diagnostic groups' ratings. Depressive patients were more interested in coping skills, while schizophrenics reported a need to learn more about medications and side-effects. These results may be due to the nature of patients' condition. Schizophrenic patients may be experiencing side effects of medications more than the depressives, and also they might have learned that they had to take medication for a longer time than the depressive patients.

Table 4: Educational Topic Ratings of Chronic Schizophrenic Patients

RANK	ITEM	MEAN
1	Early warning signs of relapse	4.33
2	Psychiatric medications	4.30
3	Side-effects of the psychiatric medication	4.26
4	Improving independent living skills	4.23
5	Making plans for the future	4.23
6	Coping with lack of interest and motivation	4.23
7	Coping with anhedonia	4.20
8	Coping with sadness, hopelessness and pessimism	4.16
9	How and where to get help for the mental illness	4.07
10	Stress reduction	4.07

Table 5: Patients' Preferences on the Mode of Education Presentation*

	DEPRESSIVE GROUP (n=30)	SCHIZOPHRENIC GROUP (n=30)
LECTURE	2.2 ± 1.2	1.8 ± 0.6
BOOKLETS	1.9 ± 0.9	2.3 ± 1.1
GROUP SESSIONS	2.5 ± 1.1	2.5 ± 1.2
COURSES	3.2 ± 1.0	3.1 ± 1.1

* Since it is a rank order, from 1 to 4, low mean indicates high preference.

On the other hand, Özsan et al. (20) found several contrasting results with the patients; relatives of schizophrenics rated coping skills, while relatives of depressives rated information on medications and side effects higher. Nevertheless, the number of similar results surpasses the contrasting results, for example, all of the groups are interested in relapse prevention and coping with symptoms.

Table - 5 shows the rank order of educational modes preferred by the patients. Schizophrenic group rated "lectures" and depressives "booklets" on top. "Groups" became third and "Courses" fourth in both patient groups. Their relatives revealed similar results, except that "booklets" became second in the relatives of depressive patients and "group sessions" in the relatives of schizophrenics. Lectures are the usual mode of education presentation of our PE programs, but reports from other authorities indicate the usefulness of group sessions (14). However, our subjects did not prefer groups as much as lectures. It may be the result of unfamiliarity of Turkish people group sessions. Lectures, however, have the potential of passifizing the listeners, which may end up with a decrease in their problem solving capabilities. A PE program should not overlook this potential danger, and whatever the mode of education is, it should foster their problem solving sources.

Group sessions are still present in our arsenal, and they should be kept going for the compensation of "emotional" part of the experiences of the patients. They may find soothing answers for their questions in a PE hour, but may not find the universality, altruism, instillation of hope and most importantly, ventilation and catharsis, that a group session can provide.

Courses are favored by neither of the groups, which can be understandable since they are difficult to attend for people who have a regular job, and since they are not as frequent as lectures or groups. Booklets, however, ranked high in our study, especially in the depressive group. They are practical sources of information, because they can be carried to anywhere with the holder. Our department had released a booklet for schizophrenics previously, which was welcomed by both the patients and relatives. However, booklets can not answer all questions and carry the disadvantage of misunderstanding, which can be solved easily in a face to face modality.

CONCLUSION

This survey of patients' educational needs emphasizes the importance of assessing the specific needs of the different diagnostic groups. Patients and relatives are capable of identifying their educational needs, and these perceptions need to be accommodated in order for providers to overcome the dissatisfaction of mental health consumers with current treatments. If we are to promote the dialogue between patients, families and mental health professionals, we would be advised to design educational programs based on objective data collected from the mental health consumers (2). Finally, as Walsh (3) noted, these PE programs have to be evaluated regularly for their effectiveness, and we should build knowledge from each preceding class and try to be interesting, relevant and fun (21).

REFERENCES

1. Kaplan HI, Sadock BJ, Grebb JA. Synopsis of Psychiatry. Seventh Edition. Baltimore, Williams and Wilkins. 1994; pp 11-12.
2. Mueser KT, Bellack AS, Wade JH, Sayers SL, Rosenthal CK. An assessment of the educational needs of chronic psychiatric patients and their relatives. Br J Psychiatry. 1992; 160; 674-680.
3. Walsh J. Methods of psychoeducational program evaluation in mental health settings. Patient Educ Couns. 1992; 19: 205-218.
4. Goldstein MJ. Psychoeducation and relapse prevention. Int Clin Psychopharmacol. 1995; 9 (Suppl 5); 59-69.
5. Akiskal HS. Chronic depression. Bull Menninger Clin. 1991; 55 (2); 156-171.
6. Glick ID, Burti L, Okonogi K, Sacks M. Effectiveness in psychiatric care - 3. Br J Psychiatry. 1994; 164: 104-106.
7. Honig A, Hofman A, Hilwig M, Noorthoom E, Ponds R. Psychoeducation and expressed emotion in bipolar disorder: preliminary findings. Psychiatry Res. 1995; 56: 299-301.
8. Wallace BC. Treating crack cocaine dependence: the critical role of relapse prevention. J Psychoactive Drugs. 1990; 22: 149-158.
9. Foote J, Seligman M, Magura S, Handelsman L, Rosenblum A, Lovejoy M, Arrington K, Stimmel B. An enhanced positive reinforcement model for the severely impaired cocaine abuser. J Subst Abuse Treat. 1994; 11: 525-39.
10. Miller CR, Eisner W, Allport C. Creative coping: a cognitive-behavioral group for borderline personality disorder. Arch Psychiatr Nurs. 1994; 8: 280-285.
11. Glick ID, Burti L, Suzuki K, Sacks M. Effectiveness in psychiatric care - 4. Psychopharmacol Bull 1992; 28: 257-259.
12. Xiang M, Ran M, Li S A controlled evaluation of psychoeducational family intervention in a rural Chinese community. Br J Psychiatry. 1992; 165 (4); 544-548.
13. Miller TW. Group sociotherapy: a psychoeducative model for schizophrenic patients and their families. Perspect Psychiatr Care. 1989; 25: 5-9.
14. McFarlane WR. Multiple family groups and psychoeducation in the treatment of schizophrenia. New Dir Ment Health Serv. 1994; 13-22.

15. Hogarty GE, Anderson CM, Reiss DJ, Kornblith SJ, Greenwald DP, Ulrich RF, Carter M. Family psychoeducation, social skills training, and maintenance chemotherapy in the aftercare treatment of schizophrenia - 2. Arch Gen Psychiatry. 1991; 48: 340-347.
16. Goulet J, Landone P, Lavoie G, et al. Effect of patient education on young psychotic patients. Can J Psychiat. 1993; 38: 571-573.
17. Özsan H, Sayıl, Özden A, Tu cu H, O uz T. The causes and treatment of psychiatric disorders as seen by patients. Poster presented at the XVth World Congress of Social Psychiatry, September 1995, Rome, Italy, 1995.
18. Birchwood M, Smith J, Cochrane R. Specific and non-specific effects of educational intervention for families living with schizophrenia. A comparison of three methods. Br J Psychiatry. 1992; 160; 806-814.
19. McFarlane WR, Lukens E, Link B, Dushay R, Deakins SA, Newmark M, Dunne EJ, Horen B, Toran J. Multiple-family groups and psychoeducation in the treatment of schizophrenia. Arch Gen Psychiatry. 1995; 52: 679-687.
20. Özsan H, Özden A, Sayıl I, Yıldız D. Educational needs of the relatives of psychotic and depressive inpatients from Turkey. Poster presentation, Xth World Congress of Psychiatry, August 1996, Madrid, Spain, 1996.
21. Mohr WK. A nurse-led educational program in psychiatric settings. developing a curriculum. J Psychosoc Nurs Ment Health Serv. 1993; 31: 34-38.

7JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 95-98

A RETROSPECTIVE ANALYSIS OF PSYCHIATRIC PATIENTS EVALUATED BY BRAIN PERFUSION SPECT*

Aykut Özden** • Hakan Kumbasar** • Erkan İbiş*** • Özlem Küçük*** • Gülseren Aras***

SUMMARY

Objective: *Brain Perfusion Single Photon Emulsion Computed Tomography (BP-SPECT) is an important but rather expensive method for evaluating brain function and its use in psychiatry is increasing. The aim of this study is to investigate the motives of psychiatrists who recommended BP-SPECT be performed, the results of these evaluations and the implications of those results.* ***Method:*** *We investigated the files of all 60 psychiatric patients who were evaluated with BP-SPECT.* ***Results and Conclusion:*** *The results of this study showed that BP-SPECT can be a useful diagnostic method if used properly and we found out that psychiatrists had to revise their knowledge about BP-SPECT, since there were not as many evaluations as it would be, and in an unnegligible number of patients the results were not even recorded. An interdisciplinary approach and more education could resolve this problem and make BP-SPECT gain its real value.*

Key Words: *Brain Perfusion SPECT, brain Imaging, psychiatry*

Brain imaging procedures have made a great contribution to both the understanding and the treatment of psychiatric disorders in this century (1). They came a long way from the basic and rather simple instruments, such as, x-ray graphy, to the highly sophisticated and equally expensive positron emission tomography (PET) of recent times. BP-SPECT is one of the relatively new procedures that has a wide spectrum of application in neuropsychiatry (2). PET is obviously an elegant technique superior than BP-SPECT, but it requires an on-site cyclotron, a costly imaging device, and a sophisticated technical operating staff, which limits its application to research area (3, 4).

The application areas of BP-SPECT in psychiatry range from psychoses to neuroses and from childhood disorders to dementias and substance related disorders (5). In schizophrenia, BP-SPECT and PET evaluations helped clinicians to understand the roots of this illness, revealing the hypofrontality and metabolic underactivity in basal ganglia (6, 7, 8). Similar studies are conducted in our department as well, with a consistent finding of lower frontal / occipital ratios and frontal / whole slice ratios (9) and decreased activity in basal ganglia (10) in schizophrenics with prominent auditory hallucinations. Other areas of BP-SPECT in psychiatry has been; depression (5, 11), alcohol dependence (12, 13), substance dependence (14), obsessive compulsive disorder (15, 16), panic disorder (5) and Tourette syndrome (17, 18).

One of the major areas of interest in BP-SPECT studies is the evaluation of dementias. The usefulness of BP-SPECT in dementias is gaining greater acceptance, especially in the early and differential diagnosis of the different types of dementias (5). In patients with clinical diagnosis of probable Alzheimer's disease (AD), reported sensitivities of BP-SPECT in comparison with normal controls ranged from 70% to 100% (5). As Bonte et al. noted (4); with high resolution BP-SPECT, regional cerebral blood flow studies of these patients would aid in separating patients with untreatable ADs from those patients with treatable causes of dementias. Moreover, it can be used to screen individuals who are at risk to the familial form of AD (4) and it is of equal value in differential diagnosis of dementias with PET (3).

* Poster presentation at the 10th World Congress of Psychiatry, Madrid, Spain, 1996.

** University of Ankara, Medical School, Department of Psychiatry
*** University of Ankara, Medical School, Department of Nuclear Medicine

Received: May 14, 1997 Accepted: Mar 21, 1997

BP-SPECT can also demonstrate abnormalities in the subcortical structures and the cerebral cortex in patients with AIDS-dementia-complex, even when magnetic resonance imaging (MRI) is negative and the patient is relatively asymptomatic (19). Wang et al. (20), demonstrated the usefulness of BP-SPECT in demented parkinsonian and hemiparkinsonian patients, as well as idiopathic parkinson's disease, and Messa et al. (5) reviewed the usefulness of BP-SPECT in other neurological disorders or conditions, such as cerebro vascular disease, migraine, intracranial neoplasms, postirradiation necrosis, Huntingtonís disease and brain death.

Another important area of evaluation with BP-SPECT in neuropsychiatry is epilepsy. The sensitivity of BP-SPECT in defecting functional abnormalities in partial epilepsy during the interictal phase is approximately 70% (5). Duncan et al. (21) reported that HMPAO-SPECT is useful in detecting lateralising abnormalities in temporal lobe epilepsy (TLE), even with a 75% accuracy. However, the main objective of radionuclide functional imaging in epilepsy is the identification of epileptic foci before surgery, by which high cost and invasive investigations will be minimized (5). It is a reliable technique for the presurgical localization of complex partial seizures of temporal lobe origin, and one study showed no disagreement with electro encephalographic (EEG) findings (22). Uvebrant et al. demonstrated that (23), BP-SPECT yielded relevant information in 79% of pediatric cases examined for epileptic surgery, while MRI and computerized tomography (CT) revealed 49% and 36% respectively.

In summary, BP-SPECT is a very useful neuroimaging instrument for psychiatrists and other clinicians. The question here is "How do we use it?". As Messa et al. (5) documented; "psychiatrists, neurologists, neurosurgeons and general practitioners have a poor level of knowledge about radionuclide techniques." This may be an over-generalization, but our clinical experiences make us believe that clinicians frequently consider diagnostic tools, such as; EEG, CT and even sometimes simple blood analysis, as a panacea for their diagnostic problems. Recommending an EEG, for example, gives a break for the clinician to think about the patient, or forget about the problems and relieve the anxiety, that the patient's condition induced in him or her. Moreover, sometimes asking a CT or even an x-ray graphy helps the clinician to save his/her face when asked about whether he/she considered organic etiologies, but he/she may not even have performed a physical questioning and examination ...

Given the abuse of various diagnostic instruments, BP-SPECT is no exception in facing such a fate. It may evoke a "magic-tool-that-shows-everything" reaction in the clinicians, just as MRI once evoked. Overuse of BP-SPECT is, however, a great waste of money and time. It is far more expensive than other imaging procedures. On the other hand, a consequent disillusionment could cause a counter-reaction, like not evaluating patients with BP-SPECT, though it is indeed necessary.

Here, we wanted to evaluate the referral patterns of psychiatrists for BP-SPECT imaging. The aim of this study is to investigate the motives of psychiatrists, the results of the evaluation and the implications of those results. The findings may improve our knowledge on BP-SPECT and the ways to use it in a more constructive way.

METHOD

We investigated the files of all psychiatric patients who were evaluated with BP-SPECT, in the Nuclear Medicine Department (NMD) of University of Ankara, Medical School (UAMS) between 1991 and 1995. Only patients referred from Psychiatry Department (PD) of UAMS are sincluded in the study. There were 60 cases as such, but we might have lost several patients who were evaluated by BP-SPECT, but unrecorded in the files. However, we suppose them to be minority.

Patients' files are investigated for the socio-demographic variables, psychiatric diagnoses, the objectives of BP-SPECT evaluation and the other diagnostic evaluations such as EEG, CT, MRI, etc. We also evaluated the impact of BP-SPECT findings on the patientís management. We tried to find out what has changed or confirmed after the results of BP-SPECT arrived. The results of BP-SPECT are obtained both from the files of PD and the NMD, where it is performed.

Since it is a descriptive study on the patients evaluated with BP-SPECT, no statistical analysis is performed. The names of the patients and the clinicians kept anonymous.

RESULTS AND DISCUSSION

When the socio-demographical characteristics of the psychiatry patients, evaluated with BP-SPECT between 1991 and 1995 are investigated, we see that most of the patients are; female (58.3%) , married (61.7%) , middle aged (20-60 years: 81.7%), high school graduate (41.7%), employed (41.7%) and living in Ankara (58.3%). These results are not surprising, except that unemployed persons are found to be 38% which is high for such a costly investigation, but most of these unemployed persons are housewives or girls whose husbands or parents pay the costs.

Table - 1 outlines the primary psychiatric and neurological diagnoses of the patients. As it can be seen from the table, although most of the patients had psychiatric diagnoses, a high number of patients (30%) had primarily neurological diagnoses. The high number of neurological patients might be explained as comorbidity (e.g. mild depression and epilepsy) or patients mistakenly referring first to psychiatry, although they primarily have neurological disorders (e.g. undiagnosed Huntington's disorder patients referring with paranoid symptoms or depression).

Nevertheless, the majority has a psychiatric disorder, of which depression leads, followed by dementias and psychotic disorders. There are 12 dementia patients, which makes 3 persons per year. Given the importance of BP-SPECT in differential diagnosis and evaluation of dementias, there might have been more dementia patients.

The main objective of BP-SPECT evaluation is found to be "confirmation of an organic condition" with 78.4%. Unfortunately, most of the objectives were not recorded in their files, but with tracing some clues and excluding the previously diagnosed dementia patients, we considered them to be "confirmation of an organic cause." Clinicians unsure about the diagnoses of their patients might have recommended BP-SPECT be performed. Evaluation of dementias was the objective in 12 dementia patients (20%), and one patient, who has Tourette's syndrome, evaluated in order to see the treatment response (1.6%), but he had not have a previous BP-SPECT. In fact, no patient seemed to have a control BP-SPECT, although it can provide important insights on the progress of treatment (19).

Table - 2 outlines the positive findings of neuroimaging evaluations of the patients. BP-SPECT findings are investigated from both NMD files and their psychiatry files. We found out that results of only 42% of patients were recorded in their files. This is an unexpected finding for us, because we believe that BP-SPECT is not an ordinary investigation that can be "forgotten" to record. However, we obtained the missing results from the NMD and found out that 53.3% of patients had a positive finding on BP-SPECT. They mostly had hypoperfusion in basal ganglia and/or parietal lobes. It can be speculated that these results indicate careful selection of patients for BP-SPECT investigation.

Another important investigation is the MRI, which is not performed or performed but not recorded in their files, in 88.3% of the patients. This is a far too less percentage for an important investigation like MRI. However, two thirds of the patients had a brain tomography before or after BP-SPECT and 26.7% of patients had a positive finding on CT, while most of them were non-significant, like minimal cerebral atrophy.

EEG was performed in 43.3% of the patients and 20% had a positive finding. No patient seemed to have a cranial x-ray graphy or maybe it is not recorded. Whatever the reason is, more x-ray and EEG should have been performed, or if it has been done, it should have been recorded.

The impact of BP-SPECT findings on patient management is assessed and we found out profound changes or confirmation of diagnoses in 23.3%, but again there was no documentation in half of the

Table 1: Primary Diagnoses of the Patients

(n=60)	n	%
PSYCHIATRIC DIAGNOSES	42	70.0
Depression	13	21.7
Dementia	12	20.0
Psychosis	11	18.3
Psychoneurosis	2	3.3
Other Psychiatric Disorders*	4	6.7
NEUROLOGICAL DIAGNOSES	18	30.0
Epilepsy	9	15.0
Movement disorders	6	10.0
Cerebrovascular disorders	3	5.0

* Tourette, mental retardation, borderline personality disorder, intermittant explosive disorder.

Table 2: Positive Findings in Neuroimaging Evaluations*

	n	%
BP-SPECT		
Positive	32	53.3
Negative	28	46.7
Not recorded in the psychiatry files	25	41.7
MRI		
Positive	4	6.7
Negative	3	5.0
Not performed or not recorded	53	88.3
BRAIN CT		
Positive	16	26.7
Negative	24	40.0
Not performed or not recorded	20	33.3
EEG		
Positive	12	20.0
Negative	14	23.3
Not performed or not recorded	34	56.7

* No cranial x-ray graphy is recorded in the files.

patients, which can also be speculated as "no effect", which is actually 26.7%, since it might have been recorded if it had revealed a significant change or confirmation.

CONCLUSION

BP-SPECT investigation has an important place in neuroimagery for a long time, but we believe that, as Hellman and Tikofsky stated (24), clinicians are not yet convinced that it has an impact on the management of their patients. Our clinicians are found to overlook the usefulness of BP-SPECT, since most of the results and other evaluations were not recorded and the number of BP-SPECT investigations is few (12 patients/year). Moreover, BP-SPECT is not an investigation for structural changes, so the findings must be confirmed with MRI or CT. Although these procedures can not be used interchagably, nor they are equal to BP-SPECT, they may be used in collaboration. We found patients of whom neither is performed. However, we could also say that the selection of patients for BP-SPECT is rather careful, but the fact that there is no control BP-SPECT for any patient may be another underevaluation.

An interdisciplinary collaboration and more education on BP-SPECT may be advisable, so that the value of such an important diagnostic improvement enhances.

REFERENCES

1. Abou-Saleh MT. Brain imaging in psychiatry. Br J Psychiat 1990; 157 (9);1.
2. Holman LB, Tumeh SS. SPECT: Applications and potential. JAMA 1990; 263; 561.
3. Cohen MB, Metter EJ, Graham LS. Differential diagnosis of dementias with "pure" I-123 iodoamphetamine and a clinical camera. J Nucl Med 1983; 24; 106.
4. Bonte FJ, Hom J, Tintner R, Weiner MF. Single photon tomography in Alzheimer's Disease and dementias. Seminars in Nucl Med. 1990; 20 (4); 342-352.
5. Messa C, Fazio F, Costa DC, Ell PJ. Clinical brain radionuclide imaging studies. Eur J Nucl Med. 1995; 25 (2); 11-143.
6. Berman KF, Weinberger DR. Cerebral blood flow studies in schizophrenia. In: Handbook of Schizophrenia. (Eds.: Nasrallah HA, Weinberger DR) Amsterdam, Elsevier. 1986; 277-307.
7. Delisi LE. The use of PET to image regional brain metabolism in schizophrenia and other psychiatric disorders In: Handbook of Schizophrenia (Eds.: Nasrallah HA, Weinberger DR) Amsterdam, Elsevier. 1986; 309-324.
8. Buchsbaum MS, Haler RJ. Functional and antomical brain imaging: impact on schizophrenia research. Schizophr Bull. 1987; 13; 115.
9. Erbaş B, Kumbasar H, Erbengi G, Bekdik C. Tc-99m HMPAO/SPECT determination of regional cerebral blood flow changes in schizophrenics. Clin Nucl Med. 1990; 15 (12); 904-907.
10. Erbaş B, Kumbasar H, Erbengi G, Aysev A, Bekdik C, İnlüoğlu G. Regional cerebral blood flow determination in schizophrenia using Tc-99 HMPAO SPECT. Paper presented at the 24th National Psychiatry and Neurological Sciences Congress, Ankara, Turkey, 1988.
11. Schlegel S, Aldenhoff JB, Eissner D, Lindner P, Nickel O. Regional cerebral blood flow in depression. J Affective Disord. 1989; 17; 211-218.
12. Kumbasar H, Erbaş B, Aytaç S, Doğan YB, Bekdik C, Erbengi G, Akyar S. Evaluation of regional cerebral blood flow changes of alcoholics with Tc-99m HMPAO/SPECT and comparison with computerized tomography (CT) parameters. Paper presented at the 25th National Psychiatry and Neurological Sciences Congress, October 1989, Mersin, Turkey, 1989.
13. Caspari D, Trabert W, Heinz G, Lion N, Henkes H, Huber G. The pattern of regional cerebral blood flow during alcohol withdrawal - a SPECT study with 99mTc-HMPAO. Acta Psychiatr Scand. 993; 87; 414-417.
14. Mathew RJ, Wilson WH. Substance abuse and cerebral blood flow. Am J Psychiatry. 1991; 48; 292-305.
15. Baxter LR, Phelps ME, Mazziotta JC, Guze BH, Schwartz JM, Selin CE. Local cerebral glucose metabolic rates in obsessive compulsive disorder. Arch Gen Psychiatry. 1987; 44; 211-218.
16. Baxter LR, Schwartz JM, Mazziotta JC. Cerebral glucose metabolic rates in non-depressed obsessive compulsives. Am J Psychiatry. 1988; 145; 1560-1563.
17. Chase TN, Geoffrey V, Gillespie M, Burrows GH. Structural and functional studies in Gilles de la Tourette syndrome Rev Neurol 1986; 142; 851-855.
18. Riddle MA, Rasmusson AM, Woods SW, Hoffner PB. SPECT imaging of cerebral blood flow in Tourette Syndrome. In: Advances In Neurology. Chase TN et al. (Eds.). New York, Raven Press. 1992; pp 207-211.
19. Kramer EL, Sanger JJ. Brain imaging in AIDS dementia complex. Seminars in Nucl Med. 1990; 20 (4); 353-363.
20. Wang SJ, Liu RS, Liu HC, Lin KN, Shan DE, Liao KK, Fuh JL, Lee LS. Tc-99m HMPAO/SPECT of the brain in early Parkinson's disease: correlation with dementia. Eur J Nucl Med. 1993; 20 (4); 339-344.
21. Duncan R, Patterson J, Hadley DM, Wyper DJ, McGeorge AP, Bone I. Tc99m HM-PAO SPECT in temporal lobe epilepsy. Acta Neurol Scan. 1990; 81; 287-293.
22. Duncan R, Patterson J, Roberts R, Hadley DM, Bone I. Ictal / postictal SPECT in the pre-surgical localisation of complex partial seizures. J Neurol Neurosurg Psychiatry. 1993; 56; 141-148.
23. Uvebrant P, Bjure J, Hedstr^m A, Ekholm S. Brain SPECT in neuropediatrics. Neuropediatrics. 1991; 22; 3-9.
24. Hellman RS, Tikofsky RS. An overview of the contribution of regional cerebral blood flow studies in cerebrovascular disease. Seminars in Nucl Med. 1990; 20 (4); 303-324.

JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 99-102

THE CHANGE OF FEELINGS AMONG GROUP PARTICIPANTS IN RELATION TO THEIR FIRST GROUP-SESSION EXPERIENCE AS RELATIVES OF PSYCHOTIC IN- PATIENTS

Gülören Ünlüoğlu* • Rüçhan Kartallar • Ladiper Gürakar*****

SUMMARY

This study was conducted with 98 relatives of psychotic in-patients. The goal was to evaluate the change in their feelings concerning group sessions held in the psychiatry department of Ankara Medical School for the family members of the psychotic patients. The data was collected in a six months period. Each subject was given a questionnaire following his/her first group sesion. The questions were related to how they felt just prior to group, during the group and following the group. The subjects had to check the most suitable state for himself/herself, the choices being "fine/comfortable", "does not matter" and "anxious/under pressure". The relationship between the answers for three various stages of group-session was examined using Goodman and Kruskalís Gamma and Spearman's correlation Coefficient. The changes found were statistically significant from baseline for some emotional states.

Key Words: *Relative Group, Change of Feelings*

When an individual is diagnosed as psychotic or schizophrenic the other members of his/her family are also effected emotionally in various degrees which can be detected by the evaluating physician. On the other hand over the last 20 years there has been a shift in the conceptualization of the role of social and family factors in schizophrenia. Some research workers have focused attention on the schizophrenic's home conditions and his/her wider environment trying to detect their effects on the onset and prognosis of the illness (Barrowclough and Tarrier 1984). Hogarty et al.(1979) found comparative measures of intrafamilial stress related to relaps. Vaughn and Leff (1976) worked on the influence of family and social factors on the course of psychiatric illness. So both the family members versus the patients influence and are influenced by each other. In a study by Ünlüoğlu and Kartallar (4), the family members participating in relative group sessions stated their satisfaction after the sessions. The authors reported a feeling of relief among participants which could be observed by therapists due to the participants body language. Also some of them verbalized their feelings more comfortably and openly after such sessions, during their progress interviews with members of therapy team. Mosher and Keith (5) suggest to make the relatives an active member of therapy process in a study they conducted in 1990. Ünlüoğlu and Sayıl (6) reported statistically significant decrease in the readmission rate of psychotic in-patients whose relatives participated in group work, in comparison to those whose relatives did not.

Since the group-work done with relatives of psychotic inpatients came close to two decades, the therapy team felt the need to get reliable answers to some questions they raised in relation to the feelings of the participating relatives concerning the group session (A). What were the relatives feelings (a) prior to, (b) during, and (c) following their first group session, in terms of being comfortable versus anxious? (B) Was the group session of any benefit to them? (C) Would they be interested in participating in such group sessions again while their patients are in the hospital? (D) Would anyone be interested in coming for such sessions at times, after his/her relative's discharge from the hospital?

METHOD

Setting

This study was conducted in the Psychiatry Department of the Medical School of Ankara

* Professor of Psychiatry, Department of Psychiatry in Medical School of Ankara University, Turkey
** Forensic Psychologist (PhD), Department of Psychiatry in Medical School of Ankara University, Turkey
*** Social Worker, Department of Psychiatry in Medical School of Ankara University, Turkey

Received: May 17, 1996 Accepted: Mar 21, 1997

University, in the first 6 monhts of 1990, with relatives of psychotic male and female patients who were admitted to the hospital where they were treated in two seperate wards. Female ward has 20 beds and male ward has 28 beds. On both wards the treatment aproach is multidimentional. One part of the general treatment program is the psychoeducational group sessions conducted by therapy team with patients relatives every week on each ward (a) to give them information about the illness (b) to understand the relatives toughts, feelings and difficulties concerning their patients and the ilness, and (c) to support them in various possible ways.

The relative or family member is invited to attend the relative groups during their first contact with the doctor of the ward. Each session is conducted following the visiting hours every week on both male and female wards. Twenty three sessions were held in 6 months in two seperate wards adding up to 46 sessions. In each session 5-14 relatives were present among whom 1-3 were new-comers.

Study Team

The study team consisted of a pshychiatrist, a psychologist and a social worker.

Subjects and Procedure

Subjects were selected among relatives who participated in the relative groups for the first time regardless of their age, sex marital status and education.

Forms which had two parts were prepared and printed. First part consisted of their demographic characteristics and second part included the questions which were prepared to find out the answers related to their feelings about this first group session.

After the relative group was over, the new members were asked to stay and fill out this form which did not need a long time to fill. Ninety-eight relatives attended the groups and filled out the forms during the study period.

The first question was related to their feelings "prior to", "during" and "following" the relative grup sesison in terms of being "fine/comfortable", "does not matter" and "anxious/under pressure". The second question was: "Does the participant feel that he/she benefited from the session" which had 4 choises for the answer, "much", "moderate", "little" and "not at all". The third question was: "Would he/she like to attend other groups while his/her relative is in the Hospital" with two choices as "yes" and "no". The fourth question being: "Would he/she like to attend some groups in future after his/her patient is discharged from the hospital" with the alternatives of "yes" and "no".

At the end of 6 months a total of 98 subjects participated in relative groups. The number of male subjects was 55 (56%) and females 43 (44%). Their age range was between 15-64. The accumulation was between ages 25-34 with a ratio of 28.6%. Fourtyfive to 54 age range followed with a ratio of 25.5%. The subjects educational level ranged from being "literate" to "university graduate". First row was taken by primary school graduates: (37 subjects= 37.7%), followed by high school graduates (34=34.4%). There was no unemployed subject. Majority of subjects were employed as civil servants (38=38.8%). Thirty (30.6%) of the subjects were house wives, taking the second row. Among women this had a rate of 69.8%. Eighty (81.6%) were married and 10 (10.2%) single. Two males were divorced and 6 females widowed.

Data Analysis

Data was processed and analyzed with SPSS/PC+ Ver. 4.0.

Since the subjects had to check the most suitable state for himself/herself, concerning his/her feelings about the group during three various stages in relation to their change of feelings concerning this group session; the relationship between the answers for 3 various stages of group sessions were examined using Goodman and Kruskal's Gamma and Spearman's Correlation coefficient. The answers to following 3 questions were evaluated with percentages.

FINDINGS

Majority of subjects was within 25-34 age group; their education level mostly "primary school,"; occupation "civil servants" and marital status "married".

Prior to Relative Group 59.18% of subjects felt "anxious/under pressure", during the group this dropped to 15.31% and following the group the percentage of discomfort was only 2.44%.

Those who felt comfortable prior to group was only 21.43% whereas at the end, this ratio raised to 83.67% demonstrating a great difference from baseline.

This finding shows statistically significant relationship between the findings of "prior to group" and "following the group," with Goodman and Kruskal's Gamma (G=0.471) and Spearman's Correlation Coefficient (r=0.234; p= 0.022). The relationship between feelings "prior to" and "during" the group session is linear. Yet one may believe that the feelings "prior to" relative group session, in a way may determine to a great extent, the level of feeling "fine/com-

Table 1: Feelings of Relatives for the Group Session

	Prior to		During		Following	
	n	%	n	%	n	%
Anxious/under pressure	58	59.18	15	15.31	2	2.44
Does not matter	19	19.39	3	3.0	14	14.29
Fine/comfortable	21	21.43	80	80.69	82	83.67
Total	98	100.00	98	100.00	98	100.00

fortable", "during" the group (G= 0.713, r= 0.254; p<0.02) However the comparison between, feelings "during" and "following" the group were not statistically significant (G=0.340, r=0.113); p>0.05).

Table 2: The Degree of Benefit From the Relative Group According to the Subjects

Degree of Benefit	n	%
Much	28	28.57
Moderate	53	54.08
Little	16	16.33
None	1	1.02
Total	98	100.00

According to table 2, 97 subject out of 98 benefited from relative group in various degrees. More than 82% benefited "moderately" and "much" passing the three/fourths of total subjects.

Table 3: Subjects Who Want to Participate in Such Sessions While Their patients Are in the Hospital

	n	%
Wants	94	95.92
Does not want	1	1.02
No Response	3	3.06
Total	98	100.00

Table 3 showes that 95.92% would like to participate in relative groups while his/her relative is in the hospital.

Table 4: Subjects Who Would Like to Paticipate in Such Sessions After Their Patients' Discharge

	n	%
Wants	92	93.88
Does not want	1	1.02
No Response	5	5.10
Total	98	100.00

Table 4 shows the interest relatives demonstrate in participating even after their relatives' discharges from the hospital.

DISCUSSION

Understanding the families and working with them have been an important issue since the last two decades (7). Not only treatment centers but also Schools of Social Work have changed their curriculum introducing family therapy and family services in order to teach their students during their training years (8). Since family stress is a well recognized concept, to work with families should be one of the goals of any treatment plan (9). The data in this study showes the importance of working with relatives of psychotic inpatients. Even one session could diminish the anxiety level (Table 1) and elicit change of feelings from being "uncomfortable" to being "fine".

It is interesting to note that all the subjects were literate in this study, whereas at an earlier study in the same setting there were illiterate relatives, participating in relative groups (10).

The degree of benefit from the relative group seems quite positive according to the report of the subjects in Table 2. More than a quarter benefited "much" (28.57). Fifty-three relatives (54.08%) stated their benefit level as "moderate" which seemed better than expected. Also most of the subjects wanted to continue not only while their patients are in the hospital but also after their discharge. Antoher earlier study showed the average number of participation as 5 sessions (4).

It is this team's strong suggestion to professionals who, yet have not been working with their patients' relatives or family members, to add into their treatment program a component that makes relatives of patients an active participant of the therapy team. This type of contact with therapy team not only gives a chance to them for ventilation but also it gives them some information about their patients' feelings and fears, and how they can handle or manage their patients better, thus influences them to feel closer to the hospital. In other words, they feel they are understood, their worries shared and guidance given to them (2). However there are some relatives who feel

rivalry or threat toward therapists, which must urge the therapist to be very careful in relating to the relatives of their psychotic patiens. Another very important advantage of such a study is setting a model for junior professionals who would be more informed to deal with such situations easier in the future.

REFERENCES

1. Barrowchough C, Tarrier N. Psychosocial in terventions with families and their effects on the ocurse of schizophsenia: a review. **Pychosocial Medicine,** London 1984; 14: 629-642.
2. Hogarty G, Schooler NR, Ulrich R., Mussare F, Ferro P, Herron E: Fluphenazine and social therapy in the aftercare of schizophrenic patients. **Archives of General Psychiatry** 1979; 36, 1283-1294.
3. Vaughn C, Leff JP. The influence of family and social factors on the course of psychiatric illness. **British Journal of Psychiatry,** 1976; 129: 125-137.
4. Ünlüoğlu G ve Kartallar R. Bir Ders Yılı Süresinde Yatan Erkek Hastaların Aileleriyle Yapılan Grup Çalışmalarının Değerlendirilmesi. Presentation in **XXIV. Ulusal Psikiyatri ve Nörolojik Bilimler Kongresi, Ankara;** 1989.
5. Mosher RL, KeithSJ. Psychosocial treatment: Individual, group, family and commumity support appraches. **Schizophrenia Bulletin:** 1980; 6: 10-41.
6. Ünlüoğlu G, Sayıl I. Effect of Group work with admitted psychotic patientsí Families on Their readmission, **Journal of Ankara,** Medical Shchool, 1991; 13(4): 331-337.
7. Bell JE. **Family Therapy.** New York, Jason Aronson inc., 1975.
8. Güran N. Aile Hizmetleri, **H.Ü. Sosyal hizmetler Yüksekokulu Dergisi,** Ankara, 1983, 1(1): 13-21.
9. Falloon RH. Family Stress and Schizophrenia. **Psychiatric Clinics of North America.** 1986; 9(1): 169-181.
10. Ünlüoğlu G, Tuncay V. Group Work With Psychotic Patients' Familes, **IVth. Mediterranian Congress of Social Psychiatry, Program and Abstracts 12-15 Oct. 1983, Ankara, Turkey,** Ankara, Ajans Turk Press, 1983, 51-52.

JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 103-107

HUMAN LEUCOCYTE ANTIGENS IN ISCHEMIC AND DILATED CARDIOMYOPATHY

Adalet Gürlek* • Olcay Aydıntuğ** • Selim Yalçın*** • Derviş Oral* • Hüseyin Tutkak**

SUMMARY

Several reports have suggested an association between some human leucocyte antigen(s) (HLA) and ischemic and idiopathic dilated cardiomyopathy (IC and IDC, respectively). In order to investigate a possible association between HLA frequency and the occurrence of IC and IDC in Turkish patients, we performed HLA class I and II typing in 24 patients with IDC and 34 patients with IC. Results were compared with those of 400 healthy controls.

In the IDC group the prevalences of HLA-B27 and -B7 were significantly higher ($p<0.05$), whereas HLA -B35, -Cw4 ($p<0.05$), and -Cw7 ($p<0.01$) were significantly less than in the controls. DQw2 antigen was present in 45% of the control group, whereas no positivity was found in the IDC ($p<0.01$). Similarly DQw7 positivity was 42% in the control group and 4.2% in the IDC group ($p<0.01$). In the IC patients HLA-B51(5), -B7 and -DR1 were more common than in the control group. ($p<0.05$).

We conclude that immunological mechanisms associated with HLA loci may play a role in the pathogenesis of IDC and IC.

Key Words: *Ischemic cardiomyopathy, dilated cardiomyopathy, human leucocyte antigens*

Idiopathic dilated cardiomyopathy (IDC) is a clinical entity of unknown and probably heterogenous etiology. Abnormalities in both cellular and humoral immunity have been described in human IDC which support the involvement of autoimmune mechanisms in the pathogenesis of this disorder. However this was not proven (1-13).

It is suggested that a viral infection may act as a triggering factor for further myocardial damage in the pathogenesis of IDC (4). Development of autoimmunity is the summation of diverse genetic traits that give rise to a genetic predisposition expressed both as an increased susceptibility to infectious agents and to organ specific autoimmune reactions (4). Similarly, the extent and frequency of autoimmune response in atherosclerotic heart disease is currently unknown (5). To examine the possible role of immune response factors in the pathogenesis of IDC and IC , we compared the frequency of HLA class I and II antigens in IDC and IC patients with those of healthy controls.

PATIENTS AND METHODS

We studied 24 IDC (14 men ,10 women, aged 32-72 years, mean 52) and 34 IC cases (32 men, 2 women, aged 40-75 years, mean 71). All patients had undergone routine clinical and haemodynamic evaluation including coronary angiography, cardiac catheterisation and echocardiography at the dept. of Cardiology , Ibn-i Sina Hospital. Patients were considered as IDC when underlying valve disease , congenital heart disease, hypertension and pericarditis were excluded. Patients with a history of heavy alcohol consumption were not included in the study. There were no diseases known to be associated with spesific HLA antigens (such as rheumatoid arthritis, myastenia gravis, multiple sclerosis, systemic lupus erythematosus, insulin dependent diabetes mellitus). An initial echocardiography was performed in each patient which revealed a mean ejection fraction (EF) under 30 %. Echocardiography was followed by ventriculography and coronary angiography which showed normal coronary arteries. Myocardial biopsy was performed in 8 of 24 IDC patients which showed compatible findings with IDC . In the remaining 16 patients biopsy could not be performed either because of inappropriate general condition or lack of consent for biopsy. Thirtytwo of 34 IC patients had old anterior and 2 had old inferior myocardial infarction on the

* Medical School of Ankara University Ibn'i Sina Hospital, Ankara. Departments of Cardiology
** Medical School of Ankara University Ibn'i Sina Hospital, Ankara. Immunology
*** Medical School of Ankara University Ibn'i Sina Hospital, Ankara. Division of Internal Medicine

Received: Oct 4, 1996 Accepted: May 21, 1997

Table 1: List of HLAs tested in the study

HLA class I :	A1, A2 , A3, A23(9) , A24(9) , A25(10) , A26(10) , A11, A28 A30+31(A19) B51(5) , B7, B8 , B44(12) , B13 , B14 , BW62(15) , B17 , B18 , B49(21), BW55(22) , B27 , B35 , B40 , BW73 , CW1 , CW2 , CW3 , CW4 , CW5 , CW7
HLA class II:	DR1 , DR2 , DR3 , DR4 , DR11(5) , DR7 , DRW52 , DRW53 , DQW1 , DQW2 , DQW7

electrocardiography. The mean EF was lower than 30 % by echocardiography. Coronary angiography and ventriculography showed 3 vessel disease in 30 and two vessel disease in 4 of 34 IC patients.

Tissue typing was performed at the Laboratory of the Dept. Of Immunology.

The control group (203 female, 197 male) consisted of 400 healthy individuals. List of HLAs tested is given in Table 1. All the patients and 400 controls were screened for Thirty HLA class I [two splits for A9(A23 and A24) and A10(A25 and A26)] and 9 HLA classII antigens. HLA-Cw7 , DQw2 ,DQw7 were investigated in all the patients but in only 100 normal controls. HLA class I and II typing were done by the microcytotoxicity method (16, 17). Briefly, lymphocytes for HLA typing were isolated from venous blood collected into heparinized vacutainer tubes. Mononuclear cells were separated by Ficoll-Hypaque (Sigma) density gradient sedimentation. T and B lymphocytes were further isolated by immunomagnetic seperation using Dynabeads (Dynal, Oslo). Dynabeads HLA class I rosetted T cells were used for HLA class I determination and Dynabeads HLA class II rosetted B cells were used for HLA class II determination. Terasaki plates previously dispensed with optimally diluted HLA antisera (Behring) were thawed immediately before use.. One microlitre of cell suspention was added to each well and the plates were incubated for 20 minutes at room temperature. Five microlitres of complement was added to each well and incubated for 30 minutes at room temperature for class I typing and 40 minutes for class II typing. One microlitre of acridine orange/ethidium bromide staining solution was added to each well and incubated for 15 minutes at room temperature. Terasaki plates were than read under fluorescence microscope for simultaneous assesment of viable (yellow) and dead (red) cells.

Chi-square test was used for the statistical evaluation of the results. Associations based on small numbers were tested by Fisher's exact test. Associations were regarded as significant if $p < 0.05$. Relative risks were calculated as suggested (18).

RESULTS

HLA frequencies for patients with dilated and ischemic cardiomyopathy and the control group are shown in Tables 2-5. HLA-B27, and -B7 were found more common in IDC cases ($p<0.05$) whereas HLA-B35, -Cw4 ($p<0.05$) and -Cw7 ($p<0.01$) antigens were less when compared with the controls. DQw2 antigen was present in 45% of the control group, whereas no positivity was found in the IDC patients ($p<0.01$). Similarly HLA-DQw7 positivity was 42% in the control group and 4.2% in the IDC group ($p<0.01$). Relative risk was 4.2 for B27 and 3.8 for B7 in the IDC.

In the IC cases HLA-B5(51), B7 and DR1 were more common than in the controls ($p<0.05$). Relative risk was 3.4 for B7, 2.1 for B51(5) and 2.1 for -DR1.

DISCUSSION

Idiopathic dilated cardiomyopathy is a heterogenous disease in which multiple elements such as infectious, toxic and genetic factors may be contributory.

Table 2: HLA-A antigens frequencies in patients with idiopathic dilated cardiomyopathy, ischemic cardiomyopathy and in controls

HLA type	IDC n=24			IC n=34			Controls n=400	
	n	%	* P	n	%	P	n	%
A1	6	25	ns	8	23.5	ns	83	20.7
A2	9	37.5	ns	12	35.2	ns	156	39
A3	5	20.8	ns	11	32.3	ns	86	21.5
A23(9)	0	0		0	0		30	7.5
A24(9)	6	25	ns	12	35.2	ns	123	30.7
A25(10)	1	4.1	ns	0	0		6	1.5
A26(10)	1	4.1	ns	5	14.7	ns	44	11
A11	2	2.3	ns	1	2.9	ns	39	9.7
A28	0	0		3	8.8	ns	30	7.5
A30+31(A19)4	16.6	ns	2	5.8	ns	37	9.2	

*P value : Patients vs controls ns: Non significant

Table 3: HLA B antigens frequencies in patients with idiopathic dilated cardiomyopathy ischemic cardiomyopathy and controls.

HLA type	IDC n= 24			IC n=34			Controls n=400	
	n	%	P *value	n	%	P value	n	%
B51 (5)	7	29	ns	12	35,2	p<0,05	79	19,7
B7	6	25	p<0,05	6	17,6	p<0,05	32	8
B27	4	16,6	p<0,05	2	8,3	ns	18	4,5
B8	2	8,3	ns	5	14,7	ns	30	7,5
B44(12)	4	16,6	//	5	14,7	//	58	14,5
B13	1	4,1	//	0	0	//	21	5,2
B14	1	4,1	//	1	2,9	//	14	3,5
Bw62	1	4,1	//	0	0	//	21	5,2
B17	2	8,3	//	3	8,8	//	21	5,2
B18	3	12,5	//	3	8,8	//	26	6,5
B49	1	4,1	//	2	5,8	//	33	8,2
B40	1	4,1	//	2	5,8	//	35	8,7
Bw55	3	12,5	//	0	0	//	25	6,2
B35	2	8,3	P<0.05	14	41,1	P<0.05	127	31,7
BW73	1	4,1		0	0		15	3,7

* P Value: Patients vs controls ns : Non significant

Table 4: HLA C frequencies in patients with idiopathic dilated cardiomyopathy ischemic cardiomyopathy and controls

HLA type	IDC n= 24			IC n=34			Controls n=400	
	n	%	P *value	n	%	P value	n	%
Cw1	4	16,6	ns	5	14,7	ns	100	21
Cw2	5	20,8	ns	2	5,8	ns	34	8,5
Cw3	1	4,1	ns	1	2,9	ns	55	13,7
Cw4	5	20,8	p<0,05	11	32,3	ns	127	31,7
Cw5	3	12,5	ns	3	8,8	ns	23	5,7
Cw7	0	0	p<0,01	4	11,7	ns	29**	29

* P Value: Patients vs controls ns : Non significant
** HLA Cw7 was examined in all patients but in 100 controls

Table 5: HLA DR and DQ frequencies in patients with idiopathic dilated cardiomyopathy, ischemic cardiomyopathy and controls

HLA type	IDC n= 24			IC n=34			Controls n=400		
	n	%	P *	n	%	P	n	%	P
DR1	2	8,3	ns	7	20,5	p<0,05	33	8,2	ns
DR2	8	33,3	ns	6	17,6	ns	102	25,5	ns
DR3	1	4,1	ns	4	11,7	ns	23	5,7	ns
DR4	7	29,1	ns	8	23,5	ns	92	23	ns
DR11(5)	9	37,5	ns	16	47	ns	142	35,5	ns
DR7	2	8,3	ns	2	5,8	ns	66	16,5	ns
DQw1	15	62,5	ns	18	52,9	ns	214	53,5	ns
DQw2	0	0		5	14,7	p<0,01	45	45 > 0.01	
DQw7	1	4,1	p<0,01	9	26,4	ns	42	42 >0.05	

ns : Non significant
** DQw2 and DQw7 were examined in all patients but in 100 control groups.

Some reports suggest that immune system may be involved in the pathogenesis of IDC and there is some experimental evidence to support this concept (1-15). The exact nature of the immunological abnormality is not clear, but some defects in in vitro supressor activity have been found in IDC patients (8, 18-20). Several groups have identified abnormalities of both humoral and cellular immunity in patients with IDC. These findings have included decreased T supressor cell function, decreased natural killer cell activity and a higher than expected incidence of heterophil antibodies against constitutes of the heart (9, 10, 13, 19-22). John et al. confirmed that the helper cell count is higher in patients with IDC giving a higher helper\supres-

sor ratio when compared with age matched normal subjects (6). In some reports of IDC cases a plausible role for autoimmunity has been suggested. This involves an initial cardiac damage (possibly virus induced) which exposes autoantigens, induction of abnormal expression of class II HLAs and presentation of autoantigens to T lymphocytes (4, 5, 23, 26). These may all contribute to further damage and sustained organ dysfunction. However, the rarity of cardiomyopathy despite widespread viral infection proposes that factors determining host immune response may be operative (4). This suggests that the regulation of the immune system might have been somehow disturbed. The HLAs are known for their participation in immune responses and in disease susceptibility for several disorders. There are reports suggesting some associations HLAs with IDC and IC. (3,4,5,7,8,26-31). Previous studies have suggested a significant association between HLA-DR4 and the occurrence of IDC (3,7,13). Limas et al. have further suggested that HLA-DR4 may be a genetic marker for the susceptibility to IDC (4). Carlquist et. al. have confirmed that HLA-DR4 was more common in IDC than in the controls (7). they have also reported that DQw4 frequency was increased and DRw6 was decreased in IDC. More recently Carlquist et. al. investigated major histocompatibility comlex classII gene frequencies by serologic and deoxyribonucleic acid genomic typing in IDC and found an increased frequency of DR4 in their patients. They, however , conclueded that a complex immune-related etiology might exist for IDC which can not be explained solely by the presence or absence of a single class II allele (32). We have found no discrepancy between DR4 frequencies of patients and the control group , whereas DR4 was found to be positive in 54 % of IDC patients versus 32 % of controls ($p<0.02$) in Anderson's study (13). They have emphasised however that no single HLA-A, -B or -DR type can account etiologically for most cases, but the presence of unevenly distributed some HLA might raise the question of genetic predisposition in at least some patients with IDC (13). Coughlin and et al. have underlined the role of racial differences in the frequency of HLA-DR4 allele (33). Our result related with HLA-DR4 can not be explained by different HLA-DR4 distribution of populations from different genetic heritage as the distribution DR4 in population controls of the foregoing studies is similar (ranging between 18-24 %) to DR4 distribution of our controls (23 %). Anderson et. al. have found that the haplotype frequency of HLA-B27 was significantly higher in IDC. HLA-B27 was present in 3% of the control subjects versus 29% of IDC cases and the relative risk for HLA-B27 was found 14 in their study. On the contrary, however Limas et al. did not find any significant difference for B27 antigen (4). Our result regarding the increased frequency of HLA-B27 in Turkish IDC patients is therefore in harmony with Anderson's study. We have also found an increase in HLA-B7 frequency in IDC patients which was also reported by Zerbe et. al. previously (34). We calculated a relative risk of 4.2 for B27 and 3.4 for B7. B27 and B7 are antigens in the HLA B7 CREG (18). The lower frequency of HLA-B35, -Cw4 and Cw7 in IDC is difficult to comment on but may be speculated as an implication of a preventive role of these alleles in IDC. The same speculation can be made for the absence of DQw2 and the very low positivity of DQw7 in the IDC group.

An immunological component has been suggested in the development of coronary artery disease as well. This was based primarily on the presence of lymphocytes and macrophages in the atherosclerotic lesions (27). Gown et al. have described a mixture of smooth muscle cells, macrophages and numerous lymphocytes - principally of CD8 and some CD4 (+) T cells beneath the fibrous cap (27). A substantial proportion of these cells are immunoactivated as indicated by the expression of HLA-DR and interleukin-2 receptor molecules (27-29).

Limas et al. have reported an overrepresentation of HLA-DRw6 in patients with ischemic heart disease (29). The presence of HLA-DRw6 correlated most closely with family history of heart disease and not with the other risk factors, such as lipid abnormalities, diabetes, smoking or hypertension. We could not search for HLA-DR6 in our study.

HLA-DR1 was increased in our IC patients. HLA-DR1 was found to be correlated with the presence of anti-beta receptor antibodies in IC (5).

Our results along with the related data from the literature suggest that in at least some patients immunological mechanisms associated with HLA loci may play a role in the pathogenesis of IDC and IC.On the contrary , however , Grant et. al. have reported that there is no HLA association with IDC or IC which is conflicting with the results of some previous reports (35). Further studies with larger patient numbers are necessary for understanding the full extent of HLA associations and pathogenetic mechanisms.

REFERENCES

1. Jeffrey L , John F C , Charles W: HLA-A, B, DR typing in idiopathic dilated cardiomyopathy: A search for immune response factors. Am J. Cardiol 1984; 53: 1326-1330
2. Hagaki T, Yamakawa K, Ono S, Yoshinaga T: Dilated cardiomyopathy associated with natural killer cell deficiency. Am. Heart J. 1988; 115: 1326-38
3. Limas C J , Limas C, Spencer H: Anti-beta receptor antibodies in human dilated cardiomyopathy and correlation with HLA-DR antigens. Am. J. Cardiol. 1990; 65:483- 7
4. Limas C J , Limas C. HLA antigens in idiopathic dilated cardiomyopathy. Br. Heart J 1989: 62: 378-83
5. Limas C J , Limas C : HLA-DR antigen linkage of anti-beta receptor antibodies in idiopathic dilated and ischemic cardiomyopathy. Br. Heart J. 1992; 67: 402-5
6. John E S, Davy K, David IH: T lymphocyte subsets in idiopathic dilated cardiomyopathy. Am. J. Cardiol 1985; 55: 755-58
7. Carlquist J F., Menlove RL, Murray MB: HLA class II (DR and DQ) antigen association in idiopathic dilated cardiomyopathy validation study and meta analysis of published HLA association studies. Circulation 1991; 83: 515-27
8. Stain H, Tamera JH, Newin W, Alan S: Immune function in patients with chronic stable congestive heart failure. Am Heart J. 1993; 125: 1651- 58.
9. Fowles RE, Bieber CP, Stinson EB: Defective in vitro supressor cell function in idiopathic congestive cardiomyopathy. Circulation 1979; 59: 483-91
10. Eckstein R, Mempel W, Bolte HD: Reduced supressor cell activity in congestive cardiomyopathy and myocarditis. Circulation 1982; 65: 1224-1229
11. Neumann DA, Burek CL, Baughman AL, Rose NR: Circulating heart- reactive antibodies in patients with myocarditis or cardiomyopathy. J. Am. Coll. Cardiol. 1990; 16:839-46
12. Coforia AL , Bonifacio E , Stewart ST: Organ spesific circulating cardiac autoantibodies in dilated cardiomyopathy. J. Am. Coll. Cardiol. 1990; 115:27-34
13. Anderson JL, Carlquist JF, Lutz JR: HLA-A, B, DR typing in idiopathic dilated cardiomyopathy : A search for immune response factors. Am. J. Cardiol. 1984; 53: 1326-30
14. Kuhl U, Schulthelss HP : Immunohistogical characterization of infiltrating lymphocytes in biopsies of patients with clinically suspected dilated cardiomyopathy. Eur Heart J 1994 ; 15: 62-7
15. Limas C J , Limas C : Immune- mediated modulation of sarcoplasmic reticulum function in human dilated cardiomyopathy Basic Res Cardiol 1992; 87 269-76
16. Erasaki PI, Bernaco D, Park MS, Ozturk G. Microdroplet testing for HLA A, B , C and D antigens. A. J. Clin. Pathol. 1978; 69: 103-120
17. Vartdal F, Bratlie A, Gaudernack G, Funderal S. Microcytotoxic HLA typing of cells directly isolated from blood by means of antibody coated microspheres. Transplantation proceedings 1987; 1: 655-57
18. Schwartz BD. The human major histocompatibility, human leucocyte antigen (HLA) complex In: Basic and Clinical Immunology, Stites DP, Terr AI (eds). Lange Medical Publications, Lebanon, 1991, pp 45-60.
19. Tatsunori I, Katsutoshi Y , Satoko O, Toshiko Y. Dilated cardiomyopathy associated with natural killer cell deficiency Am Heart J 1985; 115: 1326-27
20. Anderson JL, Carlquist J F , Hammond EH,. Deficient natural killer cell activity in patients with idiopathic dilated cardiomyopathy. Lancet 1982 ; 2 : 1124-27
21. Hurncgel G, Maisch B: Expression of MHC Class I and II antigens and the IL-2 receptor in rejection myocarditis and dilated cardiomyopathy Eur heart J;1991:12:137-40
22. Klappacher G,Mehrabi M, Franzen P, Plesch K. Endomyocardial HLA expression is increased to the same extent in idiopathic and secondary dilated cardiomyopathy Immunology 1994 ;41: 59-66
23. Van Der Wal, Das PK. Atherosclerotic lesion in humans. In situ immunophenotypic analysis suggesting an immune mediated response. Lab. Invest. 1989; 61: 166-70
24. Robert EF, Charles PB, Edward BS. Defective in vitro supressor cell function in idiopathic congestive cardiomyopathy. Circulation 1979; 59: 483-9
25. Wilson FM, Miranda QR, Chason JL, Lerner AM. Residual pathologic changes following murine coxsackie A and B myocarditis. Am. J Pathol. 1969; 55: 253-65
26. Das SK, Callen JP, Dodson VN. Immunoglobulin binding in cardiomyopathic hearts. Circulation 1971; 44: 612
27. Gown AM, Tsukada T, Ross R. Human atherosclerosis II. immunocytochemical analysis of the cellular composition of human atherosclerotic lesions. Am. J. Pathol. 1986; 125: 191-207
28. Ross R. The pathogenesis of atherosclerosis. N. Engl. J. Med. 1986; 314: 488-500
29. Limas CJ. HLA-DRW6 linkage in chronic congestive heart failure secondary to coronary artery disease (ischemic cardiomyopathy) . Am. J. Cardiol 1988; 62: 483-7. 30. caforio AL, Martinelli M, Schwarz G, Bonifacio E : Idiopathic dilated cardiomyopathy : lack of association between sirculating organ- spesific cardiac antibodies and HLA DR antigens. Tissue Antigens 1992; 39: 236-40
31. Limas CJ, Limas C; Beta adrenoreceptor antibodies and genetics in dilated cardiomyopathy - an overview and review. Eur heart J 1991 ; 12: 175-7
32. Carlquist JF, Ward RH, Huseybe D: major histocompatibility complex class II gene frequencies by serologic and deoxyribonucleic acid genomic typing in idiopathic dilated cardiomyopathy Am j cardiol 1994; 74: 918-20
33. Coughlin SS, Woosley RL: Familial dilated cardiomyopathy (letter) N Eng J Med 1992; 326: 1635-6
34. Zerbe TR, Kauffmann C, Colson Y. Associations of HLA - A,B,DR antigens with primary disease in cardiac allograft recipients . Am J Cardiol 1988; 61: 1359-61
35. Grant SC, Sheldons, Oyer PA: Do spesific HLA antigens prdispose to ischeamic heart disease or idiopathic dilated cardiomyopathy. Br. Heart J 1994; 71:76-8

JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 109-111

ANTIBIOTIC RESISTANCE IN METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

Hande Arslan* • Ayşe Willke**

SUMMARY

Methicillin resistant S. aureus (MRSA) is the term used to refer strains of S. aureus which have intrinsic resistance to all β-lactam antibiotics. These strains are also resistant to many other antibiotics and effective treatment of serious infections has often proved to be difficult. In this study antibiotic resistance patterns were determined for 42 MRSA strains isolated from nosocomial infections by using disc diffusion method. Resistance to antibiotics ranged from 26-92%. All strains were sensitive to vancomycin. Thirty-one (73%) of the strains were found to be multidrug resistant and the severity of multiresistance ranged from five to nine antibiotics.

Key Words: *Methicillin resistant S. aureus, multidrug resistance, nosocomial infection*

Staphylococcus aureus (S. aureus) continues to be a major pathogen affecting patients of all ages. Methicillin resistant S. aureus (MRSA) was first recognized at almost the same time that methicillin was marketed for human clinical use in 1960 (1). Then it is widely recognized that resistance to methicillin is usually accompanied by concomitant resistance to a number of unrelated classes of antimicrobials (2-6). Thus, strains of MRSA are almost invariably multiply drug resistance. Therefore, choosing appropriate agents to treat infections caused by MRSA has become a considerable problem.

This study was undertaken to document drug resistance seen among methicillin resistant S. aureus isolates of nosocomial infections at a teaching hospital.

MATERIALS AND METHODS

A total of 42 strains of MRSA isolated from specimens submitted to Clinical Bacteriology and Infectious Disease Laboratory and Central Laboratory of Ibn-i Sina Hospital were studied. Only one strain per patient was considered. Methicillin resistance was proven by using oxacillin agar screening method (7).

The following antimicrobial agents were evaluated by using Kirby-Bauer disk diffusion method (7); Erythromycin, tetracycline, gentamicin, amikacin, ciprofloxacin, ofloxacin, trimethoprim-sulphamethoxazole (TMP/SMX), chloramphenicol, rifampin, vancomycin, netilmicin.

RESULTS

Antibiotic resistance patterns were determined for these 42 strains. Resistance to antimicrobial agents tested ranged 26% to 92%. On the other hand all strains were sensitive to vancomycin. Table 1 summarises the prevalance of resistant strains to these 10 antibiotics.

Table 1: The prevalance of resistance in MRSA strains to various antibiotics (total number of strains tested: 42)

	No.of.Strains	Ratio %
Erythromycin	39	92
Tetracyclin	37	86
Gentamicilin	31	61
Amikacin	25	59
Netilmicin	21	50
Ofloxacin	19	45
Ciprofloxacin	19	45
TMP/SMX	14	33
Chloramphenicol	11	26
Rifampicin	11	26
Vancomycin	0	0

* Specialist, Başkent University School of Medicine, Department of Clinical Microbiology and Infectious Disease
** Professor, Ankara University School of Medicine, Department of Clinical Bacteriology and Infectious Disease

Received: Jan 13, 1997 Accepted: May 27, 1997

Thirty-one (73%) of these strains were found to be multidrug resistant and the severity of multiresistance ranged from 5 to 9 antibiotics. Figure 1 shows the degree of multiresistance.

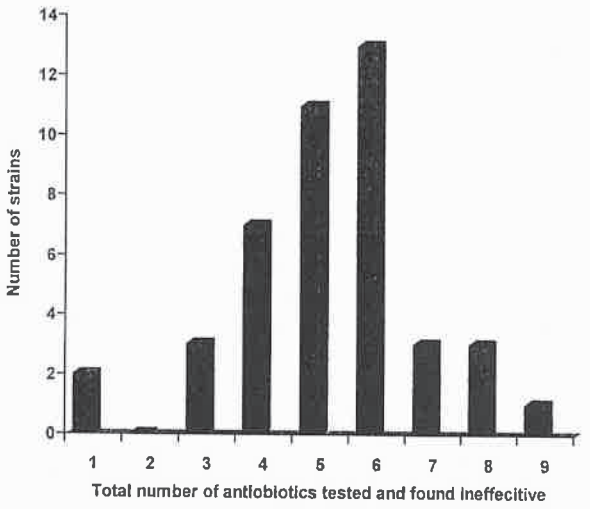

Fig. 1. Degree of multiresistance in MRSA strains.

DISCUSSION

S. aureus is responsible for many of the suppurative infections encountered in clinical practice and is the most important staphylococcal pathogen. Rapid development of antibiotic resistance soon after a drug becomes available is a well-known feature of these species (8,9).

MRSA is the term used to refer to strains of S.aureus that possesses intrinsic resistance to methicillin, oxacillin, nafcillin, cephalosporins, imipenem and other β lactams. These strains are also resistant to many other antibiotics and effective treatment of serious infections has often proved to be difficult.

In this study majority of the isolates have shown high resistance for multiple antibiotics. Although the severity of multiresistance ranged from 5 to 9 antibiotics, the choice of effectively recognized chemotherapy in many instances was limited to TMP/SMX, rifampin and vancomycin. For the highly multiresistant strains vancomycin was the only antibiotic for clinical use. S.aureus has remained sensitive to vancomycin, although resistant coagulase negative staphylococci strains were reported (10,11). MRSA has emerged as a significant cause of nosocomial infections (12-14). During the past decade, the incidence of serious infections caused by MRSA has been increasing while the options for effective antimicrobial chemotherapy have become limited(8,9).In studies reported in Turkey MRSA ratioes ranged from 15-59 % (15-19). And also 54 % of these strains were found to be multidrug resistant (20).

Today the antimicrobial agents available against MRSA are often potentially toxic, limited in number, difficult to administer and expensive. These agents also carries the risk of developing resistance. Therefore it is felt that there is a need for agreed guidelines to help the control of these organisms and to prevent the spread of infection.

REFERENCES

1. Jevons MP. "Celbenin" resistant staphylococci. Br Med J 1961; 1:124-125
2. Brumfitt W, Hamilton Miller J. Methicillin-resistant Staphylococus aureus. N Engl J Med 1989; 320: 1188-96.
3. Blumberg HM, Rimland D, Carrol DJ, Terry P, Wachsmuth IK. Rapid development of ciprofloxacin resistance in methicillin susceptible and resistant S. aureus. J Infect Dis 1991;163:1279-1285.
4. Trucksis M, Hooper DC, Wolfson JS. Emerging resistance to fluoroquinolones in staphylococci; an alert. Ann Int Med 1991;114:424-426.
5. Locksley RM, Cohen ML, Quinn TC, Tompkins LS, Coyle MB, Karihara JM, Counts GV. Multiply antibiotic resistant S.aureus; Introduction, transmission and evaluation of nosocomial infections. Ann Int Med 1982;97:317-324.
6. Peacock JE, Mooreman DR, Wenzel RP, Mandel GP. MRSA; Microbiologic characterisitcs, antimicrobial susceptibilities and assessment of virulance of an epidemic strain. J Infect Dis 1981;144:575-582.
7. Thornsberry C. Methicillin-resistant (heteroresistant) staphyloccocci. ANNLDO 1984; 116: 43-50.
8. Maple PAC, Hamilton-Miller J, Brumfitt W. Antibiotic resistance in methicillin resistant Staphylococcus aureus. Lancet 1989; 11: 537-539
9. Wodsworth S, Kim KH, Satishchandron V, Alexrod P, Truant AL. Development of new antibiotic resistance in methicillin resistant but not methicillin susceptible S.aureus. J Antimicrob Chemother 1992; 30: 821-26.
10. Schwalbe RS, Ritz WJ, Verma PR, Barranco EA, Gilligan PH,.Selection for vancomycin resistance in clinical isolates of Staphylococcus haemolyticus. J Infect Dis 1990;161:45-51.
11. Leclergy R, Derlot E, Weber M, Duval D, Courvalin P. Transferable vancomycin and teicoplanin resistance in Enterococcus faecium Antimicrob Agent Chemother 1989; 33: 10-15.
12. Peacock J, Marsik FJ, Wenzel RP. Methicilin-resistant S. aureus; introduction and spread within a hospital. Ann of Int Med 1980; 93: 526-32.

13. Saravolatz L, Markowitz N, Arking L, Pohlad D, Fisher E. Methicillin-resistant S. aureus, epidemiolgic observations during a community acquired outbreak. Ann Int Med 1982; 96:11-6.
14. Muder R, Brennen C, Wagener M, Vickers RM, Rish JD, Hancock GA. Methicillin-resistant staphylococcal colonisation and infection in a long term care facility. Ann Int Med 1991; 114: 107-12.
15. Akalın HE, Çelik E, Baykal M, Kardeş T. Metisiline dirençli stafilokokların bazı antibiyotiklere karşı invitro duyarlılıkları.Ankem 1987; 1:22
16. Özsan M, Tan G, Özenci H. Çeşitli klinik örneklerden izole edilen Staphylococcus aureus suşlarının antibakteriyallere duyarlılıkları. Mikrobiyoloji Bülteni 1989;23:246
17. Gürler N, Sarpel C, Töreci K, Çetin ET. Muayene maddelerinden izole edilen S. aureus suşlarının kemoteröpetiklere duyarlılıkları. Ankem 1984;3:189
18. Razlıghi R ,Derbentli Ş. Staphylococcus aureus suşlarındaki metisilin direncinin belirlenmesinde mikrodilusyon, disk diffüzyon ve agar tarama yöntemlerinin karşılaştırılması. Ankem 1994;8:62-68
19. Ertuğrul N, Başkaya I, Tural D, Altay G. Stafilokok suşlarının penisilin,oksasilin vankomisin ve ampisilin-sulbaktama duyarlılıkları. Ankem 1988;2:108
20. Metin T, Dündar V, Akgül A, Selçuk S. Haydarpaşa Numune hastanesinde burun taşıyıcılarından izole edilen oksasiline dirençli Staphylococcus aureus suşlarının epidemiyolojisinin antibiyotik tiplendirme yöntemiyle tiplendirilmesi. Ankem 1991; 5: 160.

JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 113-116

SERUM SOLUBLE INTERCELLULAR ADHESION MOLECULE-1 (sICAM-1) LEVELS IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Murat Turgay* • Göksal Keskin* • Murat Duman* • Mansur Kayataş* • Gülay Kınıklı* • Hüseyin Tutkak*
Abdülkadir Dökmeci* • Güner Tokgöz*

SUMMARY

Several cytokines and cell adhesion molecules have been associated with inflammatory bowel disease and may contribute to the characteristic inflammatory state. We measured levels of soluble ICAM-1 in sera of 38 patients with ulcerative colitis (18 men, 20 women: age range; 18-56, 18 active and 20 inactive patients), 11 patients with Crohn's disease (5 men and 6 women: age range; 18-45 years, 6 active and 5 inactive patients), and 16 healthy controls (8 men and 8 women; age range: 24-43 years). All inactive patients with Crohn's disease and 17 of 20 inactive patients with ulcerative colitis were on prednisone and sulphasalazine and 3 of 20 patients were on azathioprine and prednisone. All active patients took no medication or sulphasalazine only.

The mean serum sICAM-1 levels, in active patients with Crohn's disease was 267.7 ± 54.5 ng/ml, in inactive patients with Crohn's disease 127.9 ± 7.8 ng/ml, in active patients with ulcerative colitis 244.7 ± 64.2 ng/ml, in inactive patients with ulcerative colitis 143.2 ± 21.1 ng/ml and in healthy subjects was 194.1 ± 70.3 ng/ml. We found a significantly increased concentration of sICAM-1 in the serum samples of patients with active Crohn's disease and 18 patients with active ulcerative colitis compared to 16 normal healthy subjects ($P<0.05$ and $P<0.05$ respectively).

In conclusion, serum sICAM-1 levels may be a useful parameter to establish exacerbation and remission period.

Key Words: *Soluble ICAM-1, inflammatory bowel disease*

The complex immune mechanisms that mediate local inflammatory reactions in inflammatory bowel disease (IBD) are poorly understood (1). Immunological abnormalities have long been considered as one of the multiple factors contributing to the aetiology of disease (2).

Several cytokines and cell adhesion molecules have been associated with IBD (3) and may contribute to the characteristic inflammatory state (3, 4). The development of monoclonal antibodies that react with adhesion molecules and the use of these antibodies for immunohistochemical studies has provided new insights into the pathogenesis of many inflammatory diseases (5). The adhesion molecules, such as immunoglobulin super family may be useful monitors of disease activity (6, 7). The cell surface expression of many of these, such as intercellular adhesion molecule-1 (ICAM-1), is upregulated following activation during inflammatory responses, mediating both cell migration and activation (3, 4, 8, 9). Intercellular adhesion molecule-1 represents a cell surface bound glycoprotein of 70 to 110 kilodaltons. Intercellular adhesion molecule-1 is an important early marker of immune activation and response (10). It mediates adhesion dependent cell to cell interactions and is expressed on haematopoietic cells such as tissue macrophages, monocytes, B-cells, activated T cells, germinal centre dendritic cells in tonsils, lymph nodes, and Peyer's patches (11).

Recently, a soluble form of ICAM-1 (sICAM-1) has been detected in human serum samples (12). The cellular source of sICAM-1 in samples of healthy blood donors seemed to be from mononuclear cells, because sICAM-1 was only detectable in cell culture supernatants of lymphoid cell lines and peripheral blood mononuclear cell cultures (3). It has been suggested that sICAM-1 may prove useful in the investigation, diagnosis and therapeutic monitoring of various inflammatory, neoplastic, allergic and immune disorders (13-17).

In inflammatory bowel disease, an increased expression of ICAM-1 on mucosal mononuclear phagocytes on colonic biopsy specimens was shown to be associated with the maintenance of chronic inflammati-

* Departments of Immunology and Gastroenterology, Medical School of Ankara University, İbn-i Sina Hospital, Ankara

Received: Mar 7, 1997 Accepted: July 14, 1997

on (1). In this study, we measured sICAM-1 levels in the serum samples and searched for its correlation with disease activity in patients with IBD.

MATERIALS AND METHODS

We studied 38 patients with ulcerative colitis (UC) and 11 patients with Crohn's disease (CD) who were attending İbn-i Sina Hospital. The serum samples were obtained from all the patients with inflammatory bowel disease (IBD).

The patients with UC consisted of 18 men and 20 women (age range; 18-56, median 36.0 years) and the average disease duration was 29.9 (range; 2-96) months. Eighteen of 38 patients with UC were active and 20 patients were inactive. The diagnosis of UC or CD was based on accepted clinical and endoscopic criteria supported by radiological and pathological findings (18). The activity, extent, and severity of UC were scored active or inactive on basis of history, physical examination, laboratory results, endoscopy, radiology, and the clinician's overall opinion (19). Active disease was subsequently graded by endoscopic findings in which severity of the inflammation was graded 0-2, 2 being spontaneously bleeding, 1 being bleeding only after contact, 0 being nonbleeding (20). Active disease was diagnosed in 18 patients, median score at endoscopy was 1.9. Three patients had proctitis, 9 had left colitis and 6 had pancolitis (Table 1).

Table 1: Association of anatomical extent and severity of disease in 38 patients with ulcerative colitis

	Anatomical extent		
Severity of disease	**Proctitis (n=7)**	**Left colitis (n=23)**	**Pancolitis (n=8)**
Mild (0)	4	14	2
Moderate (1)	0	1	1
Severe (2)	3	8	5

The patients with CD consisted of 5 men and 6 women (age range; 18-45 years, median 29.6 years) and the average disease duration was 22.0 (range; 1-96) months. Six of 11 patients with CD were active and 5 patients were inactive. Crohn's disease activity was scored as active or inactive based on The Bristol simple index (21). Active disease was defined as a disease index > 4 (21). Crohn's disease was localized in the ileum only in 2 patients, in the colon only in 4 patients, and in both ileum and colon in 5 patients.

All inactive patients with CD and 17 of 20 inactive patients with UC were already on prednisone and sulphasalazine and other 3 patients were already on azathioprine and prednisone, 12 active patients with UC were not receiving any medication and 6 were on sulphasalazine only (Table 2).

Table 2: Patients with ulcerative colitis and Crohn's disease in relation to localization of disease, disease activity and medication used.

	Crohn's disease (n=11)	**Ulcerative colitis (n=38)**
Colonic	2	-
Ileal	4	-
Ileocolonic	5	-
AZT + P	-	3
S + P	5	17
Active	6	18
Inactive	5	20

AZT: Azathioprine
P: Prednisone
S: Sulphasalazine

Sixteen healthy, age and sex-matched subjects (8 men and 8 women; age range: 24-43 years, median 32.0 years) were studied as controls. Peripheral venous blood was drown from each patient and control, and stored at -20∞ C until tested.

The sICAM-1 levels in serum samples of the control group and the patients with UC and CD were determinated by enzyme linked immunosorbent assay (ELISA) using kits purchased from British Bio-technology products Ltd., Abington, U.K.

Statistical analysis: Multiple comparisons between groups were made by one-way analysis of variance and Kruskal-Wallis variance analysis.

RESULTS

Serum sICAM-1 levels in the active patients with CD ranged from 221.9 to 353.6 ng/ml (median; 267.7 ± 54.5 ng/ml), in the inactive patients with CD ranged from 119.9 to 137.6 (median; 127.9 ± 7.8 ng/ml), in the inactive patients with UC ranged from 115.5 to 181.6 ng/ml (median; 143.2 ± 21.1 ng/ml), in the active patients with UC ranged from 186.1 to 393.5 ng/ml (median; 244.7 ± 64.2 ng/ml) and in normal healthy subjects ranged from 110.2 to 300.7 ng/ml (median; 194.1 ± 70.3 ng/ml). We found significantly increased concentration of sICAM-1 in the serum samples of 6 patients with active CD and 18 patients with active UC compared to 16 normal healthy subjects ($P<0.05$, $P<0.05$ respectively). In inactive patients with CD and UC, serum sICAM-1 levels were significantly lower when compared to the active patients with CD and UC, and control group ($P<0.05$, $P<0.05$, $P<0.05$ respectively). Results are shown in Figure 1.

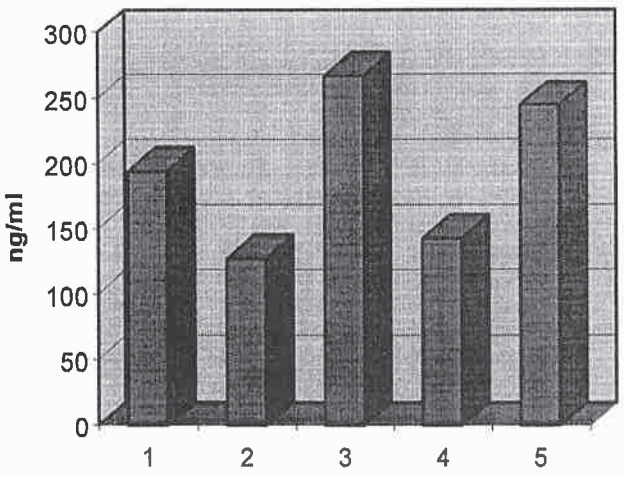

Fig. 1. Soluble ICAM-1 levels in serum samples of patients with Crohn's disease (CD), ulcerative colitis (UC) and control group: 1. Control group, 2. Patients with inactive CD, 3. Patients with active CD, 4. Patients with inactive UC, 5. Patients with active UC. Serum sICAM-1 levels were high in groups 3 and 5 compared to others ($p<0.05$).

Serum sICAM-1 levels were higher in patients with severe UC when compared with patients who had mild and moderate disease activity but this did not reach to statistical significance.

DISCUSSION

A number of studies have investigated the distribution of E selectin, ICAM-1, and VCAM-1 on healthy and diseased tissues. ICAM-1 shows a very broad distribution. It is basally expressed on vascular endothelium and shows marked upregulation on most tissues in acute and chronic inflammatory disease (22-26). Most studies reported have used monoclonal antibodies which bind either to ICAM-1 or its beta-2 integrin ligand (5, 27, 28). It has been reported that ELISA can be used for detecting soluble forms of ICAM-1 (29). The ELISA has been used to demonstrate the existence of soluble adhesins in culture supernatants of cytokine-activated endothelial cells, and in normal and pathological human sera. Assays for soluble forms of the ICAM-1 may be useful diagnostic or prognostic tools. There are a lot of reports that sICAM-1 may be a useful marker for the diagnosis and management of patients with rheumatic disease (14-16), idiopathic pulmonary fibrosis (30), allergic rhinitis (17) and asthma (29) and graft rejection (23, 24), chronic liver disease (31) or autoimmune liver disease (32, 33).

In our study, we evaluated serum sICAM-1 levels with ELISA, in patients with UC and CD. In inflammatory bowel disease, a pattern of remissions and exacerbations is usual. In a exacerbation period, we studied whether serum sICAM-1 level was a useful parameter or not. We found high serum sICAM-1 levels in active patients with CD and UC compared to in inactive patients with CD and UC, and control group. In inactive patients with CD and UC, serum sICAM-1 levels were low compared to control group, because most of inactive patients were receiving 2gr/day sulphasalazin and 10 mg/day prednisone (both therapy at least 3 months).

Recently, it has been reported that corticosteroids down-regulate the expression of E selectin and ICAM-1 (34). Furthermore, a recent study has shown that 5 -ASA (the active moiety of sulphasalazine) can prevent an increase in markers of cellular activation, namely interleukin-2 (IL-2) and transferrin receptors on peripheral blood mononuclear cells in response to pokeweed mitogen (35).

Dippold et al (3) found high sICAM-1 values in serum samples of patients with colonic carcinoma and active IBD. In a previous report on a de novo expression of ICAM-1 by mucosal mononuclear phagocytes in IBD, the percentage of mononuclear phagocytes was increased 7 % in controls, 70 % in UC and 45 % in CD (1). According to the analysis of Malizia (1) and Dippold data (3), however, the source of soluble ICAM-1 seems to be mononuclear phagocytes in IBD and the tumour cells in colonic cancer. Malizia et al (1) suggest that in active IBD the augmented expression of ICAM-1 on tissue macrophages may reflect increased cell adhesiveness, facilitating interaction with T cells and therefore local immune response.

In conclusion, intercellular adhesion molecule-1 levels in the serum of patients with IBD is consistent with a condition of immunological activation possibly induced by the local release of proinflammatory cytokines. The induction of ICAM-1 on colonic mononuclear phagocytes might be an important factor in the maintenance of chronic inflammation. Therefore, serum sICAM-1 levels may be a useful parameter to establish exacerbation and remission period.

REFERENCES

1. Malizia G, Calabrese A, Cottone M et al. Expression of leukocyte adhesion molecules by mucosal mononuclear phagocytes in inflammatory bowel disease. Gastroenterology 1991;100:150-159.
2. Lagercrantz R, Perlman P, Hammerstrom S. Immunological studies in ulcerative colitis. V. family studies. Gastroenterology 1971;60:381-389.
3. Dippold W, Wittig B, Schwaeble W, Mayet W, Meyer zum Büschenfelde K-H. Expression of intercellular adhesion molecule 1 (ICAM-1, CD54) in colonic epithelial cells. Gut 1993;34:1593-1597.
4. Larry Borish, Bobby Z. Joseph. Inflammation and the allergic response. Med Clin North Am 1992; 76: 765-789.
5. Editorial- Adhesion Molecules in Diagnosis and treatment of Inflammatory Disease. Lancet 1990; 336: 1351-1352.
6. Andrew J. H. Gearing, Walter Newman. Circulating Adhesion Molecules in Disease. Immunol Today 1993;14:506-512.
7. Michael P. Bevilacqua. Endothelial-leukocyte adhesion molecules. Ann Rev Immunol 1993; 11:767-804.
8. Greenfield SM, Hamblin AS, Shakoor ZS, Teare JP, Punchard NA, Thompson RPH. Inhibition of leukocyte adhesion molecule upregulation by tumour necrosis factor alfa: a novel mechanism of action of sulphasalazine. Gut 1993;34:252-256.
9. Beat A. Imhof, Dominique Dunon. Leukocyte migration and adhesion. Adv Immunol 1995;58:345-405.
10. Charles R. Mackay, Beat A. Imhof. Cell adhesion in the immune system. Immunol Today 1993;14:99-102.
11. Springer TA, Dustin ML, Kishimoto TK, Marlin SD. The lymphocyte function associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system. Annu Rev Immunol 1987;5:223-252.
12. Rothlein R, Mainolfi EA, Czajkowski M, Marlin SD. A form of circulating ICAM-1 in human serum. J Immunol 1991;147:3788-3793.
13. Seth R., Raymond F. D., Makgoba M. W. Circulating ICAM-1 Isoforms: Diagnostic Prospects for Inflammatory and Immune Disease. Lancet 1991; 338:83-84.
14. Szekanecz Z, Haines GK, Lin TR et al. Differential distribution of intercellular adhesion molecules (ICAM-1, ICAM-2 and ICAM-3) and the MS-1 antigen in normal and diseased human synovia. Arthritis Rheum 1994;37:221-231.
15. Michael Belmont H, Buyon J, Giorno R, Abramson S. Up-regulation of Endothelial cell adhesion molecules characterizes disease activity in systemic lupus erythematosus. Arthritis Rheum 1994; 37:376-383.
16. Justin C. Mason, Pankaj Kapahi, Dorian O. Haskard. Detection of Increased Levels of Circulating Intercellular Adhesion Molecule-1 in Some Patients with Rheumatoid Arthritis but not in Patients with Systemic Lupus Erythematosus. Arthritis Rheum 1993;36:519-527.
17. Turgay M, Keskin G, Kınıklı G et al. Soluble intercellular adhesion molecule-1 (sICAM-1) in patients with allergic rhinoconjunctivitis. Allergol et Immunopathol 1996;24:129-131.
18. O'Morain C, Tobin A, Leen E, Suzuki Y, O'Riordan T. Criteria of case definition in Crohn's disease and ulcerative colitis. Scand J Gastroenterol 1989;24:7-11.
19. Talstad I, Gjone E. The disease activity of ulcerative colitis and Crohn's disease. Scand J Gastroenterol 1976;11:403-408.
20. Powell-Truck J, Day DW, Buckel NA, Wadsworth J, Lennard-Jones JE. Correlations between defined sigmoidoscopic appearences and other measures of disease activity in ulcerative colitis. Dig Dis Sci 1982;27:533-537.
21. Harvey RF, Bradshaw JM. A simple index of Crohn's disease activity. Lancet 1980;1:514.
22. Dustin ML, Rothlein R, Bhan AK et al. Induction by IL-1 and interferon gamma: Tissue distribution, biochemistry and function of a natural adherence molecule (ICAM-1). J Immunol 1986; 137:245-254.
23. Adams DH, Hubscher SG, Shaw J, Rothlein R, Neuberger JM. ICAM-1 on liver allografts during rejection. Lancet 1989;2:1122-1125.
24. Faull RJ, Russ GR. Tubuler expression of ICAM-1 during renal allograft rejection. Transplantation 1989;48:226-230.
25. Sobel RA, Mitchell ME, Fondren G. ICAM-1 in central immune reactions in the human CNS. Am J Pathol 1990;136:1309-1316.
26. Norris P, Poston RN, Thomas DS et al. The expression of ELAM-1, ICAM-1 and VCAM-1 in experimental cutaneous inflammation: A comparison of Uvb erythema and delayed hypersensitivity. J Invest Dermatol 1991;96:763-770.
27. Andrew J.H.Gearing, Ian Hemingway, Rod Pigott et al. Soluble form of Vascular Adhesion Molecules, E-Selectin, ICAM-1, and VCAM-1:Pathological Significance. Annals NY Acad Sci 1992;667:324-331.
28. Ciprandi G., Buscaglia S., Pesce G. et al. Allergic Subjects Express Intercellular Adhesion Molecule-1 (ICAM-1 or CD54) on Epithelial Cells of Conjunctiva After Allergen Challenge. J Allergy Clin Immunol 1993;91:783-792.
29. Wegner CD., Gundel RH., Reilly P., Haynes N., Letts LG et al. Intercellular Adhesion Molecule-1(ICAM-1) in the Pathogenesis of Asthma. Science 1990;247:456-9.
30. Shijubo N., Imai K., Aoki S., Hirasawa M. et al. Circulating Intercellular Adhesion Molecule-1 (ICAM-1) Antigen in Sera of Patients with Idiopathic Pulmonary Fibrosis. Clin Exp Immunol 1992;89:58-62.
31. Adams DH, Mainolfi E, Burra P et al. Detection of circulating intercellular adhesion molecule-1 in chronic liver disease. Hepatology 1992;16:810-814.
32. Satoh S, Nüssler AK, Liu ZZ, Thomson AW. Proinflammatory cytokines and endotoxin stimulate ICAM-1 gene expression and secretion by normal human hepatocytes. Immunology 1994;82:571-576.
33. Thomson AW, Satoh S, Nüssler AK, Tamura K, Woo J et al. Circulating intercellular adhesion molecule-1 (ICAM-1) in autoimmune liver disease and evidence for the production of ICAM-1 by cytokine-stimulated human hepatocytes. Clin Exp Immunol 1994;95:83-90.
34. Cronstein BN, Kimmel SC, Levin RI, Martiniuk F, Weissman G. A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of ELAM-1 and ICAM-1. Proc Natl Acad Sci USA 1992;89:9991-9995.
35. Schreiber S, Macdermott RP, Raedler A, Pinnau R. et al. Increased activation of isolated intestinal lamina propria mononuclear cells in inflammatory bowel disease. Gastroenterology 1991;101:1020-1030.

JOURNAL OF ANKARA MEDICAL SCHOOL Vol 19, No 2, 1997 117–120

HEMATURIA AS GUIDELINE FOR RADIODIAGNOSTIC ASSESSMENT IN PEDIATRIC UROGENITAL TRAUMA

Murat Çakmak* • Ali Naycı* • Hüseyin Dindar* • Meral Barlas* • Haluk Gökçora* Selçuk Yücesan*

SUMMARY

Pediatric trauma patients were retrospectively evaluated to investigate the correlation between urogenital tract injuries and hematuria degree in 43 consecutive patients who presented with a history of trauma and hematuria. Urogenital trauma were detected in 21 of 43 patients. The mechanisms of urogenital trauma were blunt in 17 (81 %) and penetrating in four (19 %) of the 21 patients.

An intravenous urogram and/or an ultrasonography were performed as an emergency procedure to the patients with hematuria. Contrast computed tomography and retrograde urethrogram were also performed by clinical suspicion.

None of eighteen patients with 0-4 RBCs/HPF, one (9.1%) of eleven patients with 4-20 RBCs/HPF, seven (87.5%) of eight patients with 20-35 and more RBCs/HPF on their urinalysis and all patients with macroscopic hematuria had urinary tract injury. The distribution of urogenital trauma of 21 patients were as follows, seven renal, one bladder, five urethral, four vaginal, two vulvo-perineal and two scrotal injuries. The occurrence of a urinary tract injury was higher with the increasing severity degree of hematuria.

Key Words: *Children, Urogenital tract injury, Hematuria, Guideline*

Trauma is the major cause of morbidity and mortality in children (1). Anatomic differences between children and adults reportedly render the pediatric patient more prone to renal and bladder injuries (1).

Optimal management of urinary tract injuries in trauma patients requires accurate diagnosis of the injuries. Usually, decisions on radiodiagnostic procedures are based on the results of the analysis of the first urine obtained after the trauma and on physical examination. At which degree of hematuria diagnostic evaluation of the urinary tract is required, is controversial in the literature. Most authors advocate the performance of diagnostic procedures when either macroscopic hematuria combined with shock or associated injuries is present in patients with abdominal trauma(2). Others maintain that a complete diagnostic evaluation of the urinary tract should take place in all patients with abdominal trauma presenting with microscopic hematuria and sometimes even in the absence of hematuria (3,4).

We have investigated the incidence of urinary tract injuries in multiple injured patients and discussed a guidelines for the initial assessment of these injuries. An additional objective was to set up the efficacy of diagnostic procedures in demonstrating these injuries.

PATIENTS AND METHODS

A retrospective review was performed on 43 patients with hematuria of 97 patients admitted for trauma between January 1984 and June 1995. Information was obtained by reviewing patient records, radiology reports and x-ray films.

Urinalysis were obtained by spontaneous voiding within 2 hours of admission. Based on the red cell counts in the urine sediment, four groups were distinguished: A finding of less than 4 RBCs/HPF was considered to be physiological. In group A, eighteen patients had 0-4 RBCs/HPF on their urinalysis. One IVU and five USG were performed. In group B, eleven patients had 4-20 RBCs/HPF on their urinalysis. Five IVU, three USG and one contrast CT were performed. In group C and D, fourteen patients had 20-35 and more RBCs/HPF, macroscopic hematuria on their urinalysis. Seven IVU, seven USG, four contrast CT and five retrograde sistourethrographies were performed.

An emergency intravenous urogram (IVU) was performed when a urinary tract injury was suspected on the basis of physical examination, urinalysis or radiodiagnostic evaluation of the trunk. Extravasation of contrast in any part of the urinary tract or decreased

* Department of Pediatric Surgery, Medical Faculty of Ankara University, Ankara

Received: Dec 6, 1996 Accepted: May 21, 1997

Table 1: Evaluation of the urinary tract injury and the eritrocyte number of the groups.

Urine Sediment	Patients (n)	Evaluation
0-4 Eritrocyte HPF	**18**	
-	**0**	
>5 Eritrocyte HPF	**11**	
Renal contussion	1	(USG)
20-30 Eritrocyte HPF	**8**	
Renal contussion	1	(USG)
Intrarenal hematoma	2	(USG)
Renal laseration	1	(IVU, CT)
Retroperitoneal hematoma	1	(USG)
Perivesical hematoma	1	(USG)
Macroscopic Hematuria	**6**	
Renal contussion	1	(USG)
Renal laseration	1	(IVU, CT)
Intravesical hematoma	2	(IVU, CT)
Urethra ruptures	5	(Sistourethrogram)

visualization of the kidneys was classified as abnormal. The kidneys were regarded as abnormal when masses or irregularities of tissue, perirenal fluid or retroperitoneal hematomas were found. In patients with a pelvic fracture and macroscopic hematuria or blood at the meatus were suggestive of a lower urinary tract injury and a retrograde urethrogram was performed (5,6). Urinary tract injuries were defined as traumatic anatomical lesions demonstrated by the diagnostic procedures mentioned above or at laparotomy. Vascular lesions, large and moderate ruptures of the kidney and ruptures of ureter, bladder and urethra were regarded as serious injuries. Contusions and small ruptures of the kidney were defined as minor injures (1).

RESULTS

The mean age was 8.2 (3.50 (1-15 years). Twenty-eight (65%) were male and fifteen (35%) were female.

The mechanisms of trauma were blunt in 38 (88.4 %) and penetrating in five (11.6 %) of the 43 patients. One patient died as a result of head and chest trauma.

Hematuria was presented of 43 (44 %) trauma patients. None of the 18 patients in group A with 0-4 RBC/HPF on their urinalysis had urinary tract injury. Only one (9.1%) of eleven patients in group B with 4-20 RBCs/HPF on their urinalysis had perirenal contussion. Seven (87.5%) of eight in group C with 20-35 and more RBCs/HPF on their urinalysis had urinary tract injuries. All of the six patients in group D with macroscopic hematuria had urinary tract injury (Table 1). The distribution and management of urogenital trauma are illustrated in figure 1. In the entire group 13 (13.4 %) urinary, eight (8.3 %) genital, total 21 (21.7 %) genito-urinary injuries were detected. There were seven renal, one bladder, five urethral, four vaginal, two vulvo-perineal and two scrotal injuries detected during this period. No ureteral injury was sustained.

Five of eight patients with pelvic fracture had associated lower urinary tract injury. Pelvic fracture was seen in five of six patients with lower urinary tract injury.

DISCUSSION

Initial assessment of multiple injured patients should aim for complete diagnosis of injuries in all systems. In urinary tract,the primary concern is the criterion for diagnostic evaluation. Secondly the sequence of the diagnostic procedures to demonstrate urinary tract injuries should be established.

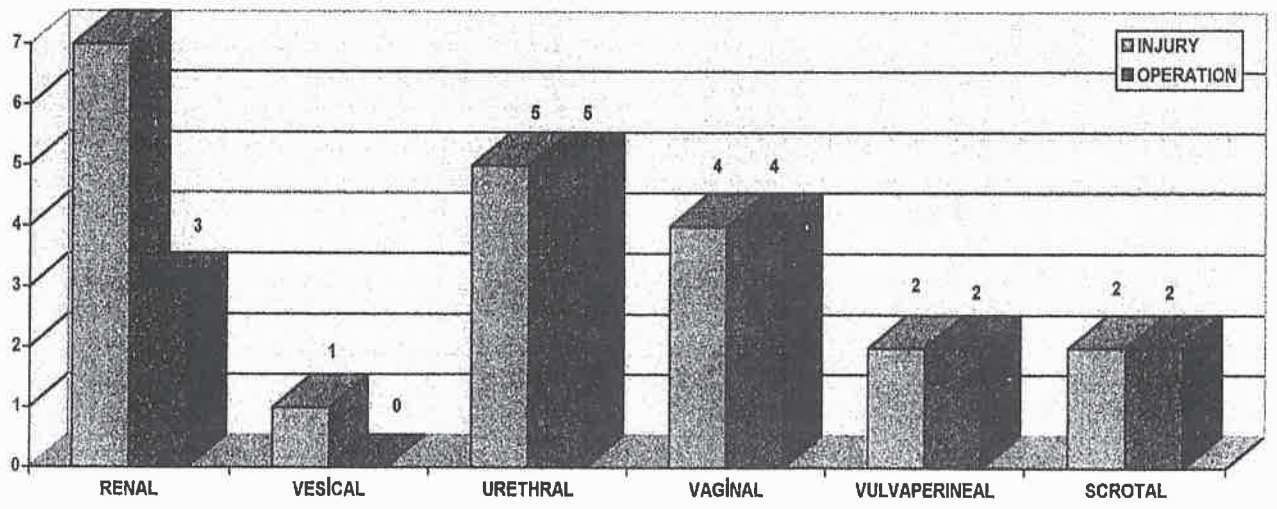

Fig. 1. Site of the urogenital tract injuries and the number of the operations.

The indications for an imaging workup of the urinary system following blunt trauma are controversial. Significant renal injuries may be present with any degree of hematuria but usually major renal injuries are accompanied by either gross hematuria or hypotension.

Werkeman et al. (4) reported that diagnostic imaging procedures performed based on the criterion of more than 35 RBCs/HPF in the sediment missed no major urinary tract injury. Hardeman et al.(7) found just one significant renal injury among 1080 patients presenting with only microscopic hematuria and 21 of 25 patients with documented renal injuries had gross hematuria. In a recent study by Thomason et al.(8) only one (1.3%) of 76 patients with microscopic hematuria after blunt abdominal injury had an abnormal IVU, but required no surgery, while seven (25%) of 26 patients with gross hematuria had abnormal IVU, and two (7.7%) required urologic surgery. Two other studies documented major renal injury 1.2% and 0.8% in blunt trauma patients also presented with neither gross hematuria or hypotension on admission(3). Mee et al.(9) reported significant renal injuries in 44 patients (4.4%) with blunt trauma and gross hematuria or microscopic hematuria associated with shock, and in 88 patients (63%) with penetrating trauma. They conclude that complete radiographic staging was mandatory in patients with penetrating trauma and in patients with blunt trauma associated with either gross hematuria or microscopic hematuria and shock. Bass et al.(2) reported that IVU has negligible influence on the management of children with minor degrees of asymptomatic hematuria after blunt trauma but they advocate preoperative IVU in all patients. Griffen et al.(11) have advocated IVU in all patients with history of blunt abdominal trauma even in the absence of hematuria. Wong et al.(12) recommended that IVU performed on all patients with hematuria and identified IVU abnormalities in 34 (25%) of 139 patients with blunt abdominal trauma, but could nor predict the likehood of an abnormal IVU or the need for surgery based on degree of hematuria..

In the present series only one of 29 patients with microscopic hematuria after blunt abdominal trauma had perirenal contussion which required no surgery. Eight of 12 patients with gross hematuria required urologic surgery. We agree with complete radiographic staging in patients with penetrating trauma and in patients with blunt trauma associated with either gross hematuria or microscopic hematuria and shock but we also prefer it in blunt trauma with more than 4 eritrocytes in their urinalysis. Trauma patients always are being evaluated with minimum a plain abdominal film. So it doesn't take time to evaluate such patients with IV. contrast for IVU. On the other hand urinary tract system can be evaluated with USG or contrast CT at the same time with the evaluation for the other solid organs.

The role of IVU, USG and CT scanning for suspected renal injury is also controversial. Patients who are hemodynamically unstable upon admission and who require urgent surgical investigation may undergo a "one shot" IVU. It allows the surgical team to verify bilateral renal function and indicates the need for exploration of the retroperitoneum when grossly abnormal. IVU has 12% false negative rate for renal laceration and 30% false negative rate for vascular pedicle injuries (3.13). Ultrasonogram can detect renal injuries such as contusions and ruptures but a small percentage of the injuries to the bladder. The remaining urinary tract injuries will be missed. In the present series, three intrarenal and a subcapsular hematoma visualized by ultrasonography or by contrast computed tomography,which were not demonstrated by the intravenous urogram. A major renal laseration and a pedicule fracture visualized by computed tomography, were demostrated as intrarenal and retroperitoneal hematoma by the ultrasonography. Contrast enhanced CT (CECT) provides a much more accuracy in urinary tract injuries. CECT scan is the best study to determine extent of solid organ injury (14), however CT evaluates completely neither the presence of intrarenal vascular injury nor the presence of active arterial bleeding (13,15) and is inferior to cystourethrography to diagnose bladder or urethral injuries (10.15).

Lower urinary tract injury coincide with pelvic fractures (16,17) Bladder injury occurs in 10 to 15 % of all patients with major pelvic fractures (3). Blunt injury to the posterior urethra occurs in approximately 10 per cent of patients with pelvic fracture and is rare in the absence of disruption of the bony pelvis (18,19). In many studies the type of pelvic fractures has been related to the urinary tract injuries. Several authors have emphasized the importance of the location as well as the severity of the pelvic fractures as a cause of urinary tract injuries. In the present series, eight patients had pelvic fracture, five had associated lower urinary tract injuries. Pelvic fractures were seen in five of the six patients with lower urinary tract injuries.

Urine should be obtained in all multiple injured patients. If it is not possible to obtain urine by spontaneous voiding, the presence of a pelvic fracture and its location will indicate whether the performance of a retrograde urethrogram should take place before catheterisation. This is obligatory when blood is found at the meatus (18).

After obtaining urine, the results of urinalysis should indicate the procedure to be followed. We advise diagnostic evaluation of the urinary tract if more than 5 RBCs/HPF are found in the sediment. An IVU with USG, or a contrast CT is safe and diagnostic in upper urinary tract injury, but by suspicion of bladder or urethra injury, a retrograde urethrosistography is more useful. There were no delayed urinary diagnosis in our series when followed this initial assessment. With the proposed guidelines, it will be possible to diagnose all urinary tract injuries in multiple injured patients with a minimum of interference with the management of other serious injuries and other diagnostic procedures.

REFERENCES

1. Mc.Aleer IM, Kaplan GW, Scherz MG et al. Genitourinary Trauma in The Pediatric Patient. Urol 1993; 42:563-8.
2. Bass DH, Semple PL, Cywes S. Investigation and Management of Blunt Renal Injuries in Children: A review of 11 Yearsí Experience .J Ped Surg 1991; 26:196-200.
3. Myrvis SE. Diagnostic Imaging of The Urinary System Following Blunt Trauma, Clinical Imaging. 1989; 13:269-80.
4. Werkeman HA, Jansen C, Klein JP et al. Urinary Tract Injuries In Multiple-Injured Patients: A Rational Guideline For The Initial Assessment. Injury 1991; 22:471-4.
5. Carroll PR, Mcaninch JW. Major Bladder Trauma: Mechanisms of Injury and a Unified Method of Diagnosis and Repair. J Urol 1984; 32:254-7.
6. Corriere JN, Gillenwater JY, Grayhack JT et al. Trauma to the Lower Urinary Tract in Adult and Pediatric Urology.pp 499-523 Mosby Year Book,Inc.1991 2.Edition St.Louis.
7. Hardeman SW, Husmann DA, Chinn HKW et al. Blunt Urinary Tract Trauma:Identifying Those Patients Who Require Radiological Diagnostic Studies. J Urol 1987; 138:99-101.
8. Thomason RB, Julian JS, Mostellar HC et al. Microscopic Hematuria After Blunt Trauma: Is Pyelography Necessary? Am Surg 1989, 55:145-50.
9. Mee SL, McAninch JW, Robinson AL et al. Radiographic Assessment of Renal Trauma: A 10-Year Prospective Study of Patient Selection. J Urol 1989; 141:1095-8.
10. Corriere JN, Gillenwater JY, Grayhack JT, Howards SS, Duckett JW. Ureteral Injuries in Adult and Pediatric Urology.pp 491-499 Mosby Year Book,Inc.1991 2.Edition St.Louis.
11. Griffen WO, Behn RP, Ernot CB. Intravenous Pyelography in Abdominal Trauma. J Trauma1978, 18:387-392
12. Wong L, Waxman K, Smolin M et al. The Role of IVU in Blunt Trauma. J Trauma 1988, 28:502-4.
13. Kantor A, Sclafani SJA, Scalea T et al. The Role of Interventional Radiology in the Management of Genitourinary Trauma. Urol Clin North Am 1989; 16:255-65.
14. Gay SB, Sistrom CL, Computed Tomographic Evaluation of Blunt Abdominal Trauma. Rad Clin North Am 1992; 30:367-88.
15. Presti JC, Carol PR, Mcaninch JW. Ureteral and Renal Pelvic Injuries from External Trauma: Diagnosis and Management. J Trauma 1989; 29:375-385.
16. Corriere JN., Sandler CM. Managment of the Ruptured Bladder:Seven Years of Experience with 111 Cases. J Trauma 1986; 26:830-3.
17. Hayes EE, Sandler CM, Corriere JN. Management of The Ruptured Bladder Secondary to Blunt Abdominal Trauma. J Urol 1983; 129:946-8.
18. Malangoni MA, Miller FB, Cryer HM et al. The Management of Penetrating Pelvic Trauma. Am Surg 1990; 56:61-5.
19. Malek RS, O'Dea MJ, Kelalis PP. Management of Ruptured Posterior Urethra in Childhood. J Urol 1977; 117:105-9.