Egyptian Journal of Surgery Vol. (22), No. (1), Jan., 2003

CAN OXIDANT, ANTIOXIDANT, AND ADHESION MOLECULES LEVELS PREDICT PROGNOSIS IN PATIENTS WITH VARICOSE VEINS?

By

Hesham A. Sharaf El-Din*, Mohamed A. Atwa**, Mohamed Abdel Latif, Iman El-Shabrawy***, and Ekbal Abo-Hashem**.***

Vascular Surgery*, Clinical Pathology**, and Biochemistry Departments***, Faculty of Medicine, Mansoura University.

Background: ***Varicose veins represent a worldwide problem. Physiologic changes and clinical signs of chronic venous insufficiency (CVI) are not static but progress from simple varicose veins to skin ulceration. Failure of local antioxidant scavenging systems in the face of overwhelming production of oxidants is central to the injury associated with oxidative process.***

Aim of the work: ***The aim of the present work was to study the effects of varicose veins on levels of some antioxidant (GPx), oxidant (MDA) and adhesion molecules (VCAM-1).***

Patients and methods: ***This study was conducted on 40 patients with varicose veins aged 35±10 years in addition to 20 healthy controls. The patients were divided into two groups: group I:20 patients with varicose veins without skin changes, and group II:20 patients with varicose veins with skin changes (pigmentation & ulceration). Blood samples were obtained from peripheral veins for patients and controls and calf varix of lower limb veins of patients. One month after surgery, blood samples were obtained from patients in group I (n=20) and group II (n=20). Serum malondialdehyde (MDA) was estimated by a procedure depending on the acid catalyzed thermal decomposition of lipid peroxide to MDA which reacts with thiobarbituric acid to form a colored adduct. Glutathione peroxidase (GPx) was assayed by spectrophotometric determination of NAD (P) H oxidation of blood hemolysate. Vascular cell adhesion molecule (VCAM-1) was measured by Elisa technique.***

Results: ***MDA and VCAM-1 were significantly increased while GPx was significantly decreased in peripheral blood in groups I and II in comparison to control group. Moreover, results of blood from calf varix of lower limb showed significant increase of MDA and VCAM-1 and significant decrease of GPx in group II in comparison to results of blood from peripheral veins. One month after surgery, MDA, VCAM-I and GPx showed no significant change in group I in comparison to control while in patients in group II, MDA and VCAM-1 were still significantly increased and GPx was significantly decreased in comparison to control. On the other hand, MDA and VCAM-1 were significantly decreased and GPx was significantly increased after surgery in comparison to before surgery.***

Conclusion: ***In patients with varicose veins, MDA and VACM-1 were significantly increased while GPx was significantly decreased especially in the presence of pigmentation and ulceration. So, early treatment is recommended since unopposed free radical production may be important in the pathogenesis of venous ulceration and cutaneous complications.***

Key words: Varicose veins – oxidants -antioxidants - adhesion molecules.

INTRODUCTION

Varicose veins are a worldwide problem [(1)]. High number of procedures consumes large amounts of health service resources. The benefit of surgery for the treatment of varicose veins is understandably being questioned, because it is not clear how effective the various forms of treatment are for this condition, the lack of a consistent outcome measure being a major problem [(2 &3)].

The commonly accepted hypothesis is that physiologic changes and clinical signs of CVI are not static but rather progress from simple varicose veins to skin ulceration. This

acceptance is based mainly on clinical observations. Only a few studies have focused on changes in both the clinical picture and venous function in patients with primary disease [4].

It is supposed that an inflammatory reaction is one of the major factors responsible for the chronic venous insufficiency (CVI) of lower limbs which cause leg ulcers [5]. Several theories have been suggested to explain the skin changes and ulcerations. However, none of these can be fully account for all the alterations [6]. Leukocyte binding to endothelial cells is thought to contribute to the pathogenesis of leg ulcers caused by chonic venous insufficiency. Such binding may be mediated by adhesion molecules [7]. The activated leukocytes may release active oxygen intermediates that cause tissue damage [8]. Failure of local antioxidant scavenging systems in the face of the overwhelming production of oxidants is central to the injury associated with oxidative process [9].

This study was designed to explore the level of some oxidant (MDA), antioxidant (GPx) and adhesion molecule (VCAM-1) in patients with varicose veins before and after surgical treatment. Also, the relation between these levels and the occurance of venous and cutaneous complications of varicose veins before and after surgery.

PATIENTS AND METHODS

This study was conducted on 40 patients with varicose veins, aged 35± 10 years in addition to age and sex matched 20 healthy controls. The patients were divided into 2 groups:

Group I: 20 patients (5 males & 15 females) with varicose veins without skin changes.

Group II: 20 patients (5 males & 15 females) with skin changes (pigmentation and ulceration).

One month after surgery , blood samples were obtained from patients in group I (no=20) and group II (no=20).

None of the patients were receiving antioxidant therapy or suffered from associating diabetes mellitus, congestive heart failure or advanced liver disease.

Surgical procedures included phlebectomy (venous stripping) with direct attacks to ligate communicators and perforators of the affected limb either surgical or endoscopic subfacial ligation after accurate mapping the varicosity on patients's skin. Clinical results of the surgical treatment were depending on perfect clinical examination confirmed by duplex scanning.

One month after surgery, clinical evaluation of the patient's limb and duplex scanning were performed and blood samples were obtained from patients in group I and group II.

Fasting blood samples were obtained from peripheral vein of patients and controls. Blood samples were obtained from calf varix of lower limb veins of patients. Also blood samples were obtained one month after surgery in group I & II. The samples were divided into:-

5 ml blood in plain tube & unhemolyzed sera were used for serum MDA & VCAM-1.

3 ml blood onto heparinized tube and erythrocyte hemolysate was used for glutathions peroxidase determination (GPx).

Serum malondialdehyde (MDA): the degradation product of lipid peroxide was estimated depending on the acid catalyzed thermal decomposition of lipid peroxide to MDA which reacts with thiobarbituric acid to form a colored adduct [10].

Glutathione peroxidase (GPx, EC 1.11.19) was assayed according to the method of Rotruck et al., [11] which based on the spectrophotometric determination of NAD (P) H oxidation of blood hemolysate (RANSEL, Randox Laboratories Ltd, Antrim, UK).

sVCAM 1 was measured by sandwich enzyme immunoassay kit (BioSource International, Inc., California, USA).

Statistical analysis was carried out with SPSS 7.5 for windows statistical software package. MDA, GPx and sVCAM-1 were tested by paired t test. The criterion of significance was of value when $P<0.05$. Correlation was calculated with Pearson's method.

RESULTS

MDA and VCAM-1 were significantly increased while GPx was significantly decreased in peripheral blood in group I and II in comparison to control group (Table 1). Also, MDA and VCAM-1 were significantly increased while GPx was significantly decreased in group II in comparison to group I (Table1)&(Fig.1). Moreover, results of blood from calf varix of lower limb showed significant increase of MDA and VCAM-1 and significant decrease of FGPx in group II in comparison to results of blood from peripheral veins (Table 2)&(Fig.4).

One month after surgery, MDA, VCAM-1 and GPX showed no significant change in group I in comparison to control while in group II , MDA and VCAM-1 were still significantly increased and GPX was significantly

decreased in comparison to control (Table 3). On the other hand, values of MDA and VCAM-1 were significantly decreased and GPX was significantly incresed after surgery in comparison to those before surgery (Table 4)&(Fig.2, 3).

Table (1) : ***Comparison of MDA, GPx and VCAM-1 among group I,II and control (before surgery)***

Group	*MDA (µmol/l)*	*GPx (U/ml)*	*VCAM-1 (ng/ml)*
Control (n=20)	2.7 ± 0.4	14.4 ± 1.7	404 ± 60
Group I (n=20)	4.6 ± 0.8	10.3 ± 2.1	470 ± 70
Group II (n=20)	6.4 ±1.1	7.50 ±1.8	540 ± 60
p1	<0.001	<0.001	0.01
p2	<0.001	<0.001	<0.001
p3	<0.001	<0.001	0.01

p1 comparison between group I and control
p2 comparison between group II and control
p3 comparison between group I and group II

Table (2): ***Comparison of MDA, GPx and VCAM-1 between blood from peripheral and calf varix of group II.***

Sample	*MDA (µmol/l)*	*GPx (U/ml)*	*VCAM-1 (ng/ml)*
Peripheral blood (n=20)	6.4 ±1.1	7.50 ±1.8	540 ± 60
Calf varix blood (n=20)	10.5 ±2.4	4.5 ±1.4	650 ±110
p	<0.001	<0.001	<0.001

Table (3): ***Comparison of MDA, GPx and VCAM-1 among group I, II and control one month after surgery***

Group	*MDA (µmol/l)*	*GPx (U/ml)*	*VCAM-1 (ng/ml)*
Control (n=20)	2.7 ± 0.4	14.4 ± 1.7	404 ± 60
Group I (n=20)	2.9 ± 0.5	13.9 ± 2.1	430 ± 50
Group II (n=15)	44 ± 0.7	10.8 ±1.9	480 ± 40
p1	>0.05	>0.05	>0.05
p2	<0.001	<0.001	0.01

p1 comparison between group I and control
p2 comparison between group II and control

Table (4): ***Comparison of MDA, GPx and VCAM-1 in group I and II before and one month after surgery***

Group	*MDA (µmol/l)*	*GPx (U/ml)*	*VCAM-1 (ng/ml)*
Group I (n=20)			
Before	4.6 ± 0.8	10.3 ± 2.1	470 ± 70
After	2.9 ± 0.5	13.9 ± 2.1	430 ± 50
Group II (n=20)			
Before	6.4 ± 1.1	7.50 ±1.8	540 ± 60
After	44 ± 0.7	10.8 ±1.9	480 ± 40
p1	<0.001	<0.001	0.01
p2	<0.001	<0.001	<0.001

p1 before and after surgery in group I.
p2 before and after surgery in group II.

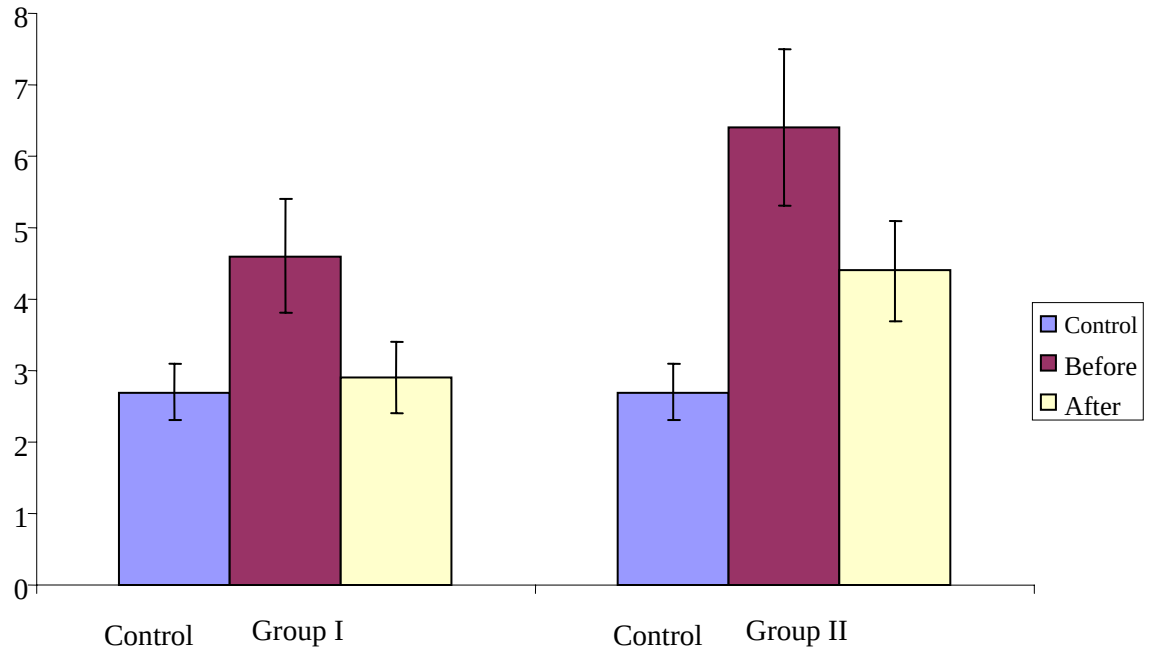

Fig (1): MDA before and after surgery in studied groups

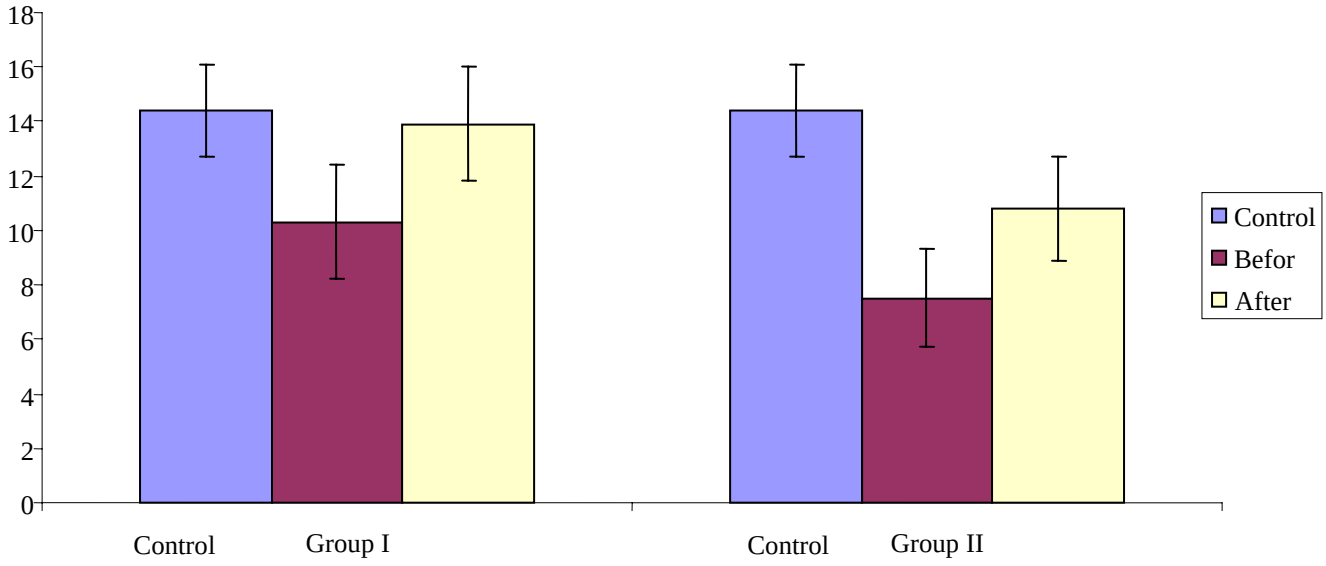

Fig (2): GPXx before and after surgery in studied groups

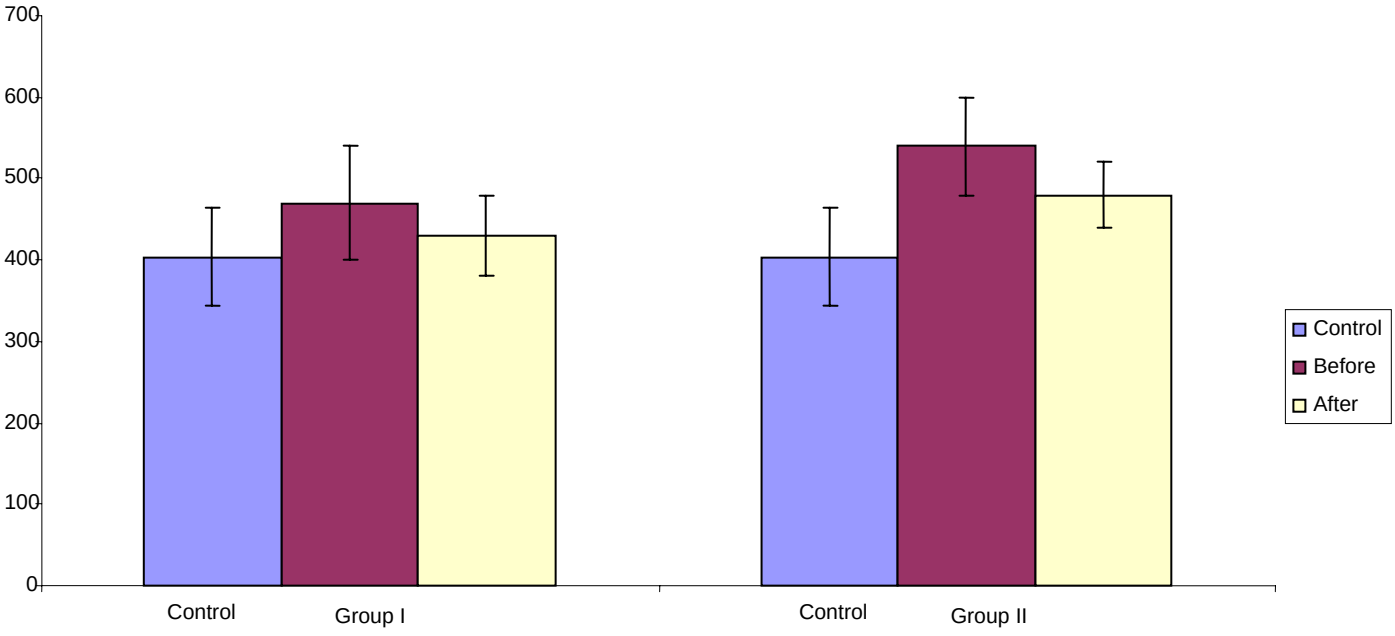

Fig (3): VCAM-1 before and after surgery in studied groups

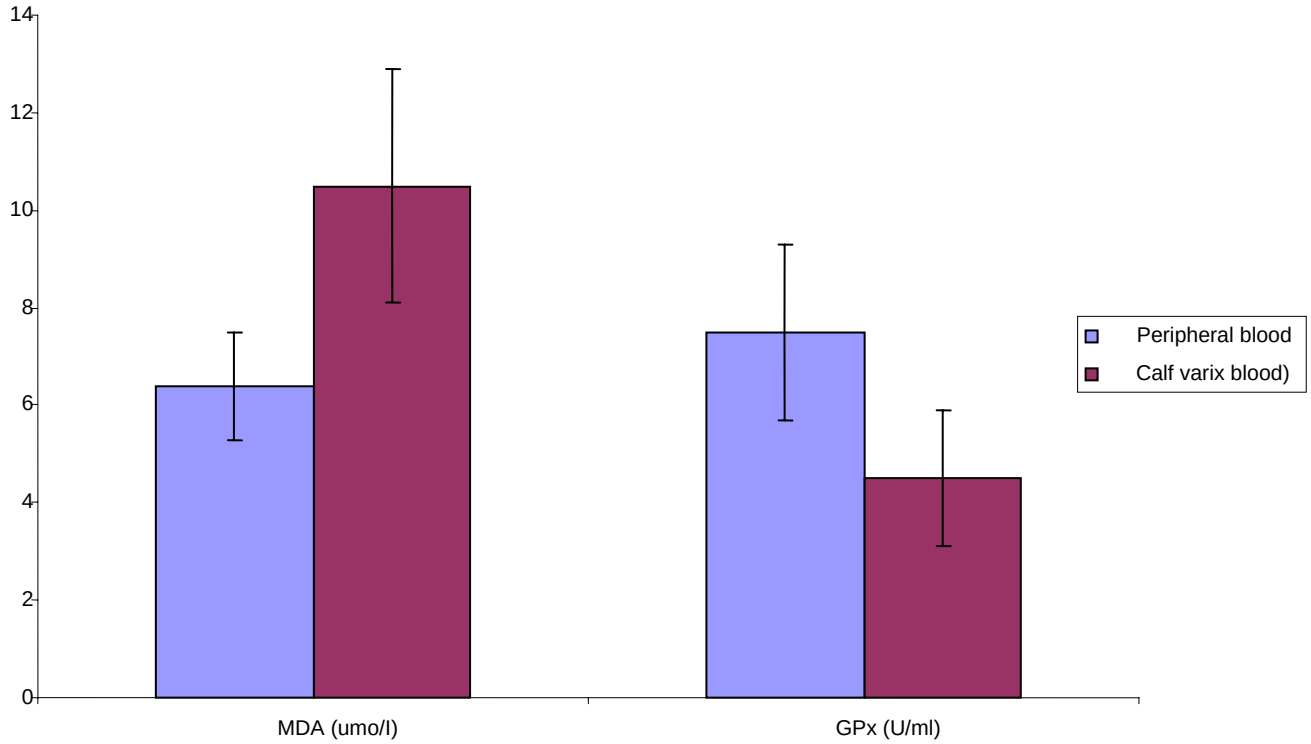

Fig (4): MDA and GPx in peripheral blood and calf varix

DISCUSSION

In the present study, MDA was significantly increased while GPx was significantly decreased in peripheral blood in groups I and II in comparison to control group (Table 1). Also, MDA was significantly increased while GPx was significantly decreased in group II in comparison to group I (Table1). These results come in agreement with Takase et. al. [8] who stated that the patient plasma produced significantly higher hydrogen peroxide values than did the control.

The enzme glutathione peroxidase (GPx) catalyses the reduction of organic hydroperoxides, and thus is an important intracellular antioxidant. Its measurement has been described as a reliable measure of antioxidant status [12]. Selenium and methionine supplementation has been shown to enhance the production of GPx. Levels of GPx can be enhanced by preloading with combination antioxidant supplements and levels of this important endogenous antioxidant remain significantly raised after temporary combined arterial and venous occlusion with delayed venous reflow. This may be protective against oxidative injury, as less lipid peroxidation, tissue oedema were observed in the group treated with antioxidant in comparison to the group that did not receive such pretreatment [13].

Although it is clear that venous hypertension from chronic venous obstruction and valvular incompetence predispose individuals toward development of the chronic skin pigmentation, subcutaneous fibrosis, and skin ulceration that are the hallmark of the disease, the pathophysiology at a cellular level is a matter of ongoing research [14].

Also, the impairments in leukocyte rheology and granulocyte production of oxygen free radicals cause capillary plugging and possibly damage to microcirculatory vessel walls in venous disease [15].

In the present study, results of blood from calf varix of lower limb showed significant increased of MDA and significant decreased of GPx in group II in comparison to results of blood from peripheral veins (Table 2)&(Fig.4). This is cope with the results of Wali et al., who suggested that peroxide radicals play an important role in the pathogenesis of varicose veins.

Because activated cells may release reactive oxygen intermediates that cause such tissue damage, the hypothesis was proposed that soluble markers of leukocyte adhesion and activation could be detected in the plasma of individuals with venous insufficiency [8].

Leuckocytes have been further implicated in the genesis of venous stasis and ulceration noticed by Thomas et al. [17] who found that 24% fewer white blood cells left the dependent foot in patients with venous hypertension than in either healthy volunteers or patients with uncomplicated varicose veins. Monocytes, as a percentage of total white blood cells, decreased by 28.3% with dependency in the patients with venous hypertension.

The neutrophils of patients with CVI are primed and /or activated because they are able to release higher amount of superoxide, lysosomal enzymes and express elevated number of adhesion molecules. It may serve as one of the important evidences of an inflammatory mechanisms involved in the pathogenesis of chronic venous insufficiency [4].

Activated leukocytes may be more likely to adhere to the endothelium in the pockets of venous valves and may exhibit more enhanced levels of cytotoxicity. This hypothesis is in line with the increased infiltration of leukocytes observed in the venous wall and valve leaflet of patients with venous dysfunction [18]. Also, this may be associated with the enhanced expression of endothelial adhesion molecules [8].

In the present study, VCAM-1 was significantly increased in blood in groups I and II in comparison to control group (Table1)&(Fig.3). Also, VCAM-1 was significantly increased in group II in comparison to group I (Table1). Moreover, results of blood from calf varix of lower limb of group II showed significant increase in VCAM-1 in comparison to results of blood from peripheral veins (Table 2) as in Glowinski [19] study who concluded that : patients with varicose veins especially those with complications like skin ulceration and supeficial thrombophlebitis showed incrased level of free radical generation.

Quarmby et al. [20] reported small but significantly higher levels of vascular cell adhesion molecule-1 in blood taken from veins in which experimental thrombi had formed, compared with controls.

Basal levels of plasma VCAM-1 of blood from foot vein were higher in patients with varicose veins with skin changes in comparison to controls. Moreover, the magnitude fires of VCAM-1 was greater in patients with varicose veins with skin changes in comparison to patients with varicose veins without skin changes. Increased VCAM-1 which is a counterligand for receptors expressed by monocytes and lymphocyses signify that these cells may be more important in the development of skin changes [21].

Thrombosis is thought to involve interactions among endopthelial cells, platelets, and leukocytes mediated by cell adhesion molecules produced by each cell-type [22]. Leukocyte adhesion and migration through the vessel wall,

resulting in endothelial damage and desquamation, has been postulated to be an initiating mechanism in venous thrombosis. It is possible, therefore, that there may be changes in the release of molecules that facilitate these processes when thrombosis occurs [20].

Ciuffetti et al., [23] reported that persistent high levels of circulating adhesion molecules may contribute to worsen microvascular perfusion, which leads to the onset of trophic damage in chronic venous insufficiency (CVI).

The vascular cell adhesion molecule is not stored, but is normally expressed by endothelial cells and monocytes activated by substances such as thrombin. The increased levels of soluble VCAM-1 in blood samples can be explained by shedding of VCAM-1 already present on the cell surface, because the process of synthesis would normally take several hours. Increased VCAM-1 release may, therefore, be the product of acute endothelial cell activation. Binding of VCAM-1 to its counter receptor very late, activation of antigen-4 is thought to be a mechanism by which leukocytes firmly adhere to the endothelium before migration in inflammatory situations [24 & 25].

In the present study, one month after surgery , MDA, VCAM-1 and GPx showed no significant change in group I in comparison to control while in group II, MDA and VCAM-1 were still significantly increased and GPx was significantly decreased in comparison to control (Table 3). On the other hand, MDA and VCAM-1 were significantly decreased and GPx was significantly increased after surgery in comparison to before surgery (Table 4).

Ramelet [26] reported that phlebotropic drug (flavonoids) decreases capillary permeability and increases capillary resistance and explained these changes by inhibition of leukocyte activation, migration and adhesion. This inhibition is linked to a significant decrease in plasma levels of endotnelial adhesion molecules (VCAM-1 and ICAM-1) after treatment. So, the chronic venous insufficiency induced damage to the microcirculation is counteracted by treatment. Antioxidant drug therapy may be of great value to guard against the complications of varicose veins till the definitive treatment [26] . Early surgical treatment of patients with varicose veins protect the affected limb from cutaneous complications like (eczyma, pigmentation and ulceration) may be essentially due to protection of the patient's skin from the injurious effect of the increased plasma levels of some oxidants like MDA and adhesion molecules like VCAM-1 and enhances the effect of endogenous antioxidants like glutathione peroxidase (GPx) [4]. The higher the plasma and varicael levels of some oxidants like MDA and adhesion molecules like VCAM-1 and the lower the the plasma and varicael levels of some antioxidants like glutathione peroxidase (GPx) indicates the importance of rapid surgical management of varicose veins before the occurance of venous or cutaneous complications.

REFERENCES

1. Callum MJ. Epidemiology of varicose veins. Br J Surg 1994; 81:167-73.

2. Scott HJ, McMullin GM, Coleridge-Smith PD, et. al., : Venous disease: Investigation and treatment, fact or fictions Ann R Coll Surg Engl 1990; 72:188-92.

3. Smith JJ, Garratt AM,. Guest M,et.al.,: Evaluating and improving health-related quality of life in patients with varicose veins. J Vasc. Surg. 1999; 30:710-719.

4. Makarova NP, Lurie F and Hmeluiker SM: Does surgical correction of superficial femoral vein valve change the course of varicose disease ? J. Vasc. Surg. 2001;33:361-368.

5. Stvrtinova V, Jahnova E, Weissova S, et al., : Inflammatory mechanisms involving neutrophils in chronic venous insufficiency of lower limbs. Bratisl Lek Listy 2001; 102 (5) : 235-239.

6. Veraart JC, verhaegh ME, Neumann HA,et.al.,: Adhesion molecule expression in venous leg ulcers. Vasa 1993; 22 (3):213-8.

7. Weyl A, Vanscheidt W, Weiss JM,et.al., : Expression of the adhesion molecules ICAM-1, VCAM-1, and E-selectin and their ligands VLA-4 and LFA-1 in chronic venous leg ulcers. J Am Acad Dermatol 1996 mar; 34 (3):418-23.

8. Takase S, Schmid-Schonbein G and bergan JJ: leukocyte activation in patients with venous insufficiency. J. Vasc. Surg. 1999; 30:138-156.

9. Walker PM, Lindsay TF, Labbe R,et.al., : Salvage of skeletal muscle with free radical scavengers. J Vasc Surg 1987; 5:68-75.

10. Satoh K : Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chem Acta 1978; 90 : 37.

11. Rotruck JT, Pope AC, Ganther HE, et. al., : Selenium : biochemical role as a component of glutathione peroxidase. Science 1973; 179 : 588-900.

12. McMaster D, Bell N, Anderson P,et.al., : Automated measurement of two indicators of human selenium status, and applicability to population studies. Clin Chem 1990; 36:211-16.

13. Harkin DW, Barros AAB, Yassin MMI,et.al.,: Reperfusion injury is greater with delayed restoration of venous outflow in concurrent arterial and venous limb injury. Br. J. Surg 2000;87:734-741.

14. Peyton BD, Rohrer MJ, Furman MI,et.al.,: Patients with venous stasis ulceration have increased monocyte-platelet aggregation. J Vasc. Surg. 1998;27:1109-1116.

15. Ciuffetti G, Mannarino E, Paltricca R, et. al., : Leucocyte activity in chronic venous insufficiency. Int Angiol 1994; Dec;13(4):312-6.

16. Wali MA, Suleiman SA, Kadoumi OF, et. al., : Superoxide radical concentration and superoxide dismutase (SOD) enzyme activity in varicose veins. Ann Thorac cardiovasc Surg 2002Oct; 8(5):286-90.

17. Thomas PRS, Nash GB, Dormandy JA: White cell accumulation in the dependent legs of patients with venous hypertension: a possible mechanism for trophic changes in the skin. BMJ 1988;296:1693-5.

18. Ono T, Bergen JJ, Schmid-Schonbein GW, et.al.,: Monocyte infiltration into venous valves. J Vasc Surg 1998; 27:158-66.

19. Glowinski J, and Glowinski S : Generation of reactive oxygen metabolites by the varicose vein wall. Eur J Vasc Endovasc Surg 2002 June; 23(6) : 550-5.

20. Quarmby J, Smith A, Collins M,et.al., : A model of in vivo human venous thrombosis that confirms changes in the release of specific soluble cell adhesion molecules in experimental venous thrombogenesis. J. Vasc. Surg. 1999;30:139-147.

21. Saharay M, Shields DA, Georgiannos SN, et.al., : Endothelial activation in patients with chronic venous disease. Eur J Vasc Endovasc Surg 1998 Apr; 15(4):342-9.

22. Downing LJ, Wakefield TW, Stricter RM, et. al., : Anti-P-selectin antibody decreases inflammation and thrombus formation in venous thrombosis. J Vasc Surg 1997; 25:816-28.

23. Ciuffetti G, Lombardini R, Pasqualini L, et.al., : Circulating leucocyte adhesion molecules in chronic venous insufficiency, Vasa 1999 Aug; 28 (3):156-9.

24. Adams DH, and Shaw S. : Leukocyte-endothelial interactions and regulation of leukocyte migration. Lancet 1994; 343:831-6.

25. Matic M, Duran V, Ivkov-Simic M, et.al., : Microcirculatory changes in chronic venous insufficiency. Med pregl 2000 Nov Dec; 53 (11-12):579-83.

26. Ramelet AA: Pharmacologic aspects of a phlebotropic drug in CVI-associated edema. Angiology 2000 Jan; 51 (1):19-23.