

Research Article

A comparative study on the lipid peroxidation and total antioxidant status in patients with and without varicose veins

Gaurav B. Shetty¹, Rajesh Ballal¹, Suchetha Kumari N.^{2*}, Harshini Ullal³

¹Department of General Surgery, ²Department of Biochemistry, ³Central Research Laboratory, K.S. Hegde Medical Academy, Nitte University, Mangalore-575018, Karnataka, India

Received: 09 July 2016

Revised: 25 July 2016

Accepted: 05 August 2016

***Correspondence:**

Dr. Suchetha Kumari N.,

E-mail: drsuchetha@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: The prevalence of varicose veins was increasing with increasing age but a deviance from the normal prevalence of the female patients. The patho- physiology has yet not been clearly delineated but the role of certain enzymes leading to oxidative stress has been implicated in the causation of this disease. Therefore, the present study was conducted to compare the lipid peroxidation and total antioxidant status in the patients with and without varicose veins.

Methods: This study was performed after the institutional ethical clearance and informed consent from all the participants. Patients who were admitted for the surgery of inguinal Hernia were taken as controls. The blood was obtained and its serum was used for the estimation of MDA, GPx, and total antioxidant status using commercially available kit, according to the manufacturer's guidelines in the central research laboratory of the institute. The data was represented as Mean±S.D. and compared using students't' test and a p value of <0.05 was considered the level of significance.

Results: The Serum estimations of MDA, GPX and TAC on comparison to Normal Individuals did not show any statistical significance (p>0.05).

Conclusions: The levels of MDA, GPX and TAC were at the upper limit of the normal ranges in patients of Varicose veins indicating that more on antioxidants as a measure of global stress index in varicose veins are needed to be performed in the future with different Antioxidants and Advent of investigative modalities for the same.

Keywords: Varicose veins, Melondialdehyde, Glutathione peroxidase, Total antioxidant capacity

INTRODUCTION

Varicose veins (VV) are tortuous, elongated and dilated veins in the lower limbs attributed to changes in the vessel wall and valve incompetence. The patho-physiology has yet not been clearly delineated but the role of certain enzymes leading to oxidative stress has been implicated in the causation of this disease.

In an attempt to unravel the causative factors this study has is focused on three elements which are acquired from

the serum and vein of an individual with varicose veins with an end point of them being as markers of oxidative stress for the disease based on the previous studies conducted on the three parameters by various researchers.

In a study conducted by Glowinski J et al to evaluate the content of lipid peroxidation products in VV with Superficial thrombophlebitis and VV without sequelae revealed an increased free radical generation in patients of Superficial thrombophlebitis.¹ Later, two studies were performed by research groups with preliminary aim of

establishing the role of oxidative stress in VV. The first study was conducted by Condezo-Hoyos et al where blood samples were obtained from patients of CVI of CEAP C2 stage patients versus healthy controls revealing a significant increase in MDA in the CVI patients.²

The importance of this study was the interjection of biochemical analysis on VV which was further seconded by a study in the same year by Hesham A et al, a study on 60 patients with 20 healthy controls and 40 patients with varicose veins were divided into two groups of 20 patients each with skin changes of pigmentation and ulceration and without skin changes.³ After a month of surgery their blood samples were estimated for Glutathione Peroxidase revealing a significant decrement and Malondialdehyde revealing a significant increment in patients with varicose veins.³

This index, with the appropriate validation in a larger population, could be used for early detection or progression of cardiovascular injuries. A study conducted by Kozka et al on obese patients with VV estimated the MDA levels and was found to be an increment in patients with VV on contrasting with normal individuals with an accentuated level in obese patients as it increases the risk of lipid peroxidation.⁴

Karatepe O et al on 39 consecutive patients of VV with Saphenectomy were divided into two groups of persistent ulcer and healed venous ulcers. The analysis of the valves revealed increased levels of MDA.⁵ Krzysciak W et al denounced that local iron overload lead due to RBC extravasation in CVI resulted in oxidative stress causing VV, the study analyzed TBARS and GPX levels in competent and incompetent regions of the Vein stripped during surgery showed statistically significant levels of TBARS and GPX in the latter group.⁶

Therefore, the present study is designed to evaluate the total antioxidant status and other antioxidant studies to evaluate the same as biomarkers of oxidative stress in varicose veins. Hence, the review of literature for our study has helped in establishing a yardstick in terms of the qualitative analysis, sample size, feasibility of the analysis with the future prospective of use of these Biochemical parameters as a global stress index for varicose veins.

METHODS

This hospital based, case control study was performed after the institutional ethical clearance and informed consent from all the participants. This study included 119 patients admitted in a surgery department of a tertiary care hospital. The primary, symptomatic, varicose veins undergoing surgery and who are ready to give informed written consent were included in the study. Those who have undergone interventions such as sclerotherapy, recurrent varicose veins and inability to give informed written consent were excluded from the study. Patients

who were admitted for the surgery of inguinal Hernia and ready to give informed written consent were taken as controls. The blood was obtained in the preoperative period is centrifuged and preserved in cold storage. The varicose vein (VV) is resected during surgery and divided into two with each fragment placed in Saline and Formalin followed by preservation in a cold storage facility. The serum was used for the estimation of Malondialdehyde (MDA), Glutathione Peroxidase, and total antioxidant status using commercially available kit, according to the manufacturer's guidelines in the central research laboratory of the institute.

Statistical analysis

The data obtained in the present study was represented as Mean±S.D. The means of case and controls were compared using students't' test in SPSS (Version-18) software. The p value of <0.05 was considered the level of significance.

RESULTS

The sample size collected in the permitted duration was 230 out of which 30 samples were rejected as they did not consent for Preoperative acquisition of blood. On commencing analysis it was realized that 47 samples out of 200 were denatured, termed as Loss of samples. 119 samples met the Inclusion criteria inclusive of Both Case and Control Veins.

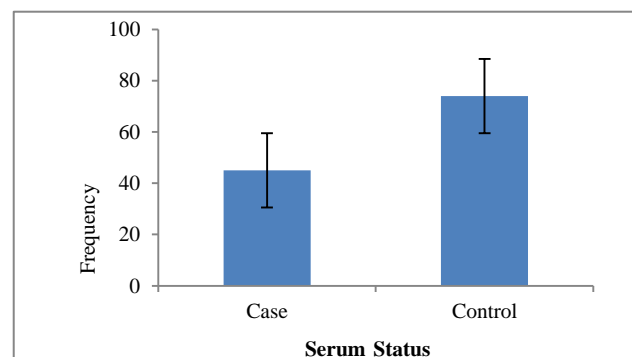


Figure 1: Case and control distribution of study population.

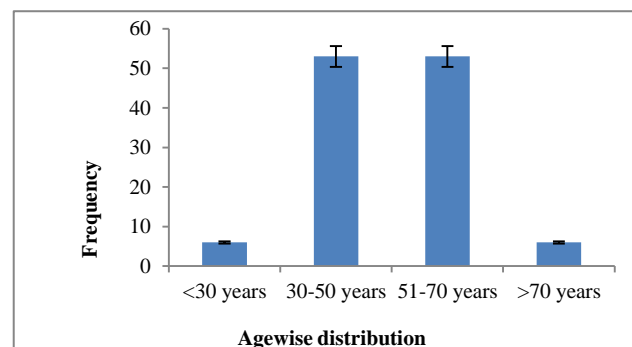


Figure 2: Age wise distribution of study population.

34 samples designated as No comorbidity samples are of those patients who are free from any co- morbidity such as T2DM, HTN, COPD, these samples are preserved in a cold storage facility for further studies on sensitive enzymes indicative of Global Oxidative Stress. The Sample Size of the Case group was 45 and the Sample size of the Control group was 74 (Figure 1-3 and Table 1 and 2).

The Pooled data was tabulated in Microsoft Excel and Incorporated into SPSS Version 19, the Case was designated as 1 and Controls as 0. Gender designations for Male was 1 and Female was 0.

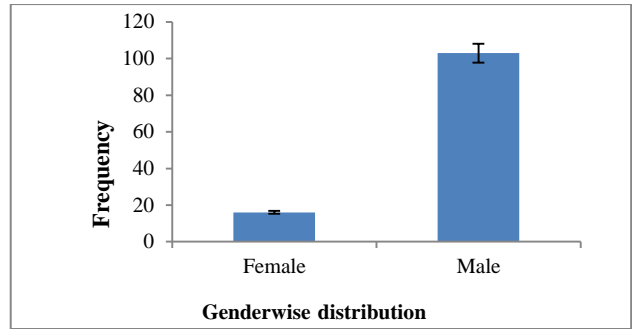


Figure 3: Gender wise distribution of study population.

Table 1: Descriptive statistics of age wise distribution.

Age in Years	Frequency	Percent	Valid Percent	Cumulative percent	
Valid	<30 years	6	5.0	5.1	5.1
	30-50 years	53	44.5	44.9	50.0
	51-70 years	53	44.5	44.9	94.9
	>70 years	6	5.0	5.1	100.0
	Total	118	99.2	100.0	
Missing	System	1	0.8		
Total		119	100.0		

Table 2: Descriptive statistics of age and gender wise distribution.

Case		N	Min.	Max.	Mean	SD
		Gender	45	0.00	1.00	0.84
	Age	45	23.00	72.00	47.20	10.73
Control		N	Min.	Max.	Mean	SD
		Gender	74	0.00	1.00	0.84
	Age	74	18.00	72.00	52.58	13.63

Table 3: Levine’s test for equality of variances and t-test for equality of means.

		Levine’s test for equality of variances		Test for equality of means						
		F	Sig	t	df	Sig (2 tailed)	Mean diff	S.E of diff	95% CI	
									Upper	Lower
GPx	Equal variances assumed			0.343	117	0.732	-2.1717	6.334	-14.716	10.372
	Equal variances not assumed	1.313	0.254	-0.330	81.774	0.742	-2.1717	6.584	-15.276	10.932
MDA	Equal variances assumed			-1.524	117	0.130	-0.251	0.166	-0.578	0.075
	Equal variances not assumed	3.769	0.055	-1.470	82.701	0.145	-0.251	0.171	-0.592	0.088
TAC	Equal variances assumed			-1.447	117	0.151	-0.177	0.122	-0.420	0.065
	Equal variances not assumed	3.001	0.086	-1.409	85.240	0.163	-0.177	0.126	-0.428	0.073

Table 4: Statistical significance values with degrees of freedom and 'p'-value.

	t- value	Degree of freedom	Std. Error Difference	Mean Difference	Sig. (2-tailed)
MDA	-1.524	117	0.16512	-0.25158	0.130
GPX	-0.343	117	6.33404	-2.17177	0.732
TAC	-1.446	117	0.12280	-0.17767	0.151

Levine's test for Equality of Variances and t-Test for Equality of Means was calculated the tabulations of the same are made and shown in Table-3 and 4. The Serum estimations of MDA, GPX and TAC on comparison to Normal Individuals were not statistically significant ($p > 0.05$).

DISCUSSION

The present study included 119 patients meeting the inclusion criteria were included in the study. The patients were both male and female in the VV group whereas only Males in the Control Group. The mean age of patients in the Case group is 47.2 ± 10.7 years and the mean age in the Control group is 52.58 ± 13.63 years. Majority of patients were in the group of 30-50 and 51-70 years equally distributed, 6 (5.0%) patients were in the age group of Less than 30 years, 53 (44.5%) patients equally distributed in the age group of 30-50 and 51-70 respectively and 6 (5.0%) patients in the age group of more than 70 years of age.

In most of the previous studies, the prevalence of VV increases with increasing age with a theory that the onset of CVI is at an early age and progresses overtime.⁷ In the RELIEF study the mean age of patients recorded with CEAP C0 were 40.3 years and in C4 it was 54.2 years.⁸ The RELIEF study was able to connect the relationship between reflux and severity of the disease. As the prevalence of VV increases with increasing age, this must be taken into account in investigating possible risk factors.

From the previous epidemiologic studies it is evident that the prevalence of VV is higher in females when compared to males, in the same context its worthy of mention that the sex ratio decreases with increasing age.⁹ The study conducted by us had only 16 female participants. The western studies report of women taking part extensively in the studies for the fact that they are more concerned about cosmetic blemishes caused by VV. This may also introduce selection bias that overestimates the female predominance in the prevalence of VV.

According to Glowinski J et al, who evaluated the lipid peroxidation content in VV with and without Superficial Thrombophlebitis revealed increment in the Serum of the ones with Superficial Thrombophlebitis. In the present study on VV and not its sequelae, complications or adverse manifestations, there has been a rise of these markers in numerous cases of VV.¹

According to Condezo-Hoyos et al, who compared the levels of MDA in patients with CVI versus Normal Individuals, and found marked levels of MDA in patients with CVI.² But these patients of CVI were graded as CEAP C2 stage whereas most of our patients do not complement with the equivalent staging of the CEAP Classification. Hence this study is similar and not the same on the lines of the CEAP Classification. The prominent point in our study is that every patient was defined by the CEAP Classification which in the previous epidemiologic studies performed fell short of defining the samples with CEAP Classification.

Another study by Hesham et al encompassed of estimation of blood samples after a month of the surgery revealing significant increment of MDA and significant decrement of GPX after a month of the surgery.³ As the study was a case control study without any intention of follow up there was no scope in our study for postoperative levels of these biochemical markers.

Controversy exists about the role of overweight in CVI and particularly regarding the importance of obesity as a risk factor between the sexes. The previous studies conducted reveal that obesity is most probably a risk factor in its own right. This thought was seconded by a study conducted which denounced that there was a relationship between the clinical presentation and BMI, independently of reflux measurements, indicating that the effect of overweight may involve a mechanism separate from local effects on venous flow, and that overweight might be a cause of disease progression.¹⁰

In a study conducted by Kozka et al on Obese patients with VV estimated the MDA levels and was found to be an increment in patients with VV on contrasting with normal individuals with an accentuated levels in obese patients as it increases the risk of lipid peroxidation.⁴ As per the study conducted by us there was no significant correlation of VV with obesity and out of the 45 patients with VV, most of the patients were well under the WHO ranges of BMI between 25-29 which designates overweight and not obesity.

A study by Karatepe O et al reported an increased level of MDA.⁵ This is an insight for a future prospective of the study as the there are long vein segments with valves in the cold storage facility on which further MDA testing can be performed. These vein segments are from patients who do not suffer from any co morbidities such as T2DM and HTN hence enlisted as pure samples in the study.

CONCLUSION

The levels of MDA, GPX and TAC were at the upper limit of the normal ranges in patients of Varicose veins indicating that more on antioxidants as a measure of global stress index in varicose veins are needed to be performed in the future with different Antioxidants and Advent of investigative modalities for the same.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Glowinski J, Glowinski S; Generation of reactive oxygen metabolites by the varicose vein wall: Eur J Vasc Endovasc Surg. 2002;23(6):550-5.
2. Condezo-Hoyos L. A plasma oxidative stress global index in early stages of chronic venous insufficiency: J Vasc Surg. 2003;57:205-13.
3. Hesham A. Can oxidant, antioxidant, and adhesion molecules levels predict prognosis in patients with varicose veins? Egyptian Journal of Surgery. 2003;22:23-30.
4. Kozka M, Krzysciak W, Pietrzycka A, Stepniewski M. Obesity and its influence on Reactive Oxygen Species (ROS) in the blood of patients with Varicose Veins of the lower limbs. Rzepl Lek. 2009;66(5):213-7.
5. Karatepe O, Unal O, Ugurlucan M, Kemik A, Karahan S, Aksoy M, et al. The Impact of Valvular Oxidative stress on the development of venous stasis ulcer valvular oxidative stress and venous ulcers. Angiology. 2010;61(3):283-8.
6. Krzysciak W, Kowalska J, Kozka M, Papiez MA, Kwiatek WM. Iron content (PIXE) in competent and incompetent veins is related to the vein wall morphology and tissue antioxidant enzymes. Bioelectrochemistry. 2012;87:114-23.
7. Schultz-Ehrenburg U, Weindorf N, Von Uslar D. Prospektive epidemiologische Studie uber die Entstehungsweise der Krampfaden bei Kindern und Jugendlichen (Bochumer Studie I und II). Phlebol Proktol. 1989;18:3-11.
8. Jantet G. The RELIEF Study group: Chronic Venous Insufficiency: Worldwide results of the RELIEF Study. Angiology. 2001;53:245-56.
9. Fowkes FGR, Evans CJ, Lee AJ. Prevalence and risk factors of Chronic Venous Insufficiency. Angiology. 2001;52(suppl 1):s5-s15.
10. Danielsson G, Eklof B, Grandinetti A. The influence of obesity on Chronic Venous Disease. J Vasc Endovasc Surg. 2002;36:271-6.

Cite this article as: Shetty GB, Ballal R, Kumari SN, Ullal H. A comparative study on the lipid peroxidation and total antioxidant status in patients with and without varicose veins. Int J Res Med Sci 2016;4:4037-41.