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Research Article

STUDY OF MATRIX METALLOPROTEINASE LEVELS IN VARICOSE VEINS

Gaurav Shetty[1]., Rajesh Ballal[1]., Suchetha Kumari N[2]* and Ullal Harshini Devi[3]

[1]Department of General Surgery, KSHEMA, Mangalore, Karnataka, India
[2]Department of Biochemistry, KSHEMA, Mangalore, Karnataka, India
[3]Central Research Laboratory, KSHEMA, Mangalore, Karnataka, India

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ABSTRACT

Varicose veins are a common cause of morbidity, etiologic factors predisposing to dilatation, elongation, and tortuosity of the saphenous vein and its tributaries are poorly understood. MMPs have a crucial role in the pathogenesis of VV, but limited work has been done in Indian population. The aim of this study is to study the levels of MMP9 in Varicose veins. A total of 74 control and 45 cases were included in the present study. The estimations of MMP-9 in Serum and Vein samples on comparison to Normal Individuals were not statistically significant. The levels of MMP-9 were at the upper limit of the normal ranges in patients of Varicose veins indicating that more studies on MMP's as a measure of blood stasis in Varicose veins are needed to be performed in the future with different MMP's and Advent of investigative modalities for the same.

INTRODUCTION

Varicose veins are a common venous disease of the lower extremity is an important cause of morbidity. Varicose veins are elongated, dilated, and tortuous veins. Risk factors for varicose veins include increasing age, female gender, dietary factors, obesity, physical activity, standing occupations, connective tissue abnormalities, and genetic predisposition.[1,2] Matrix metalloproteinases (MMPs) are proteolytic enzymes that have been identified in many tissues and organs including the venous system. MMPs play a major role in tissue remodeling and the continuous turnover of collagen, elastin and other proteins of the extracellular matrix (ECM), and have been implicated in cardiovascular remodeling and vascular diseases. In addition to their proteolytic properties on ECM, MMPs may have early effects on other cellular components of the vein wall including endothelial cells and vascular smooth muscle.[3]

Loss of tone due to structural weakness of the venous wall resulting from imbalance in synthesis and degradation of matrix proteins has received increasing atten tion.[4-6] Because matrix metalloproteinases (MMP) and their inhibitors, tissue inhibitors of metalloproteinases (TIMP), are important in synthesis and degradation of extracellular matrix.[7]

The study on varicose veins and its association with MMP's have been conducted between 2002 – 2011 with significant results but the specificity and the feasibility of the detection have not been established, also the studies conducted were only on serum samples. The study conducted by us is inclusive of serum and tissue which is subjected to biochemical evaluation of MMP-9 with a large sample size of case and controls designating this study of ours as an authority on the association of MMP in Varicose veins. In this study we estimated the levels MMP-9 in Serum and Vein samples of patients and controls with Varicose Veins.

MATERIALS AND METHOD

The study was conducted after the approval from the Institutional ethics committee (INST.EC/E.C/29/2013-14). It is a Hospital Based Study conducted on 119 patients admitted in the In-Patient block of J.S.K.S.H.C.H. from the period of August 2013 to April 2015.

Sample Selection

Inclusion Criteria

Inclusion criteria for cases

1. Primary, symptomatic, varicose veins undergoing

*Corresponding author: **Suchetha Kumari N***
Department of General Surgery, KSHEMA, Mangalore, Karnataka, India

surgery.
2. Ability to give informed written consent.

Inclusion Criteria for Controls

1. Inguinal Hernia, symptomatic, undergoing surgery.
2. Ability to give informed written consent.

Exclusion Criteria

Exclusion criteria for cases

1. Those who have undergone interventions such as sclerotherapy.
2. Recurrent Varicose Veins
3. Inability to give informed written consent

Exclusion criteria for controls

1. Those who have undergone previous Inguinal Surgeries.
2. Recurrent Inguinal Hernias
3. Those who have undergone interventions like sclerotherapy or any surgery pertaining to Varicose Veins
4. Inguinal Hernias posted on emergency basis

Matrix Metalloproteinase-9 (MMP-9) was estimated on use of a modified prourokinase.

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 realised 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. 34 samples designated as No comorbidity samples are of those patients who are free from any comorbidity such as T2DM, HTN, BA, 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.

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. Levine's test for Equality of Variances and t-Test for Equality of Means was calculated the tabulations of the same are as mentioned below.

DISCUSSION

Totally 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 previously conducted 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.[8] 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.[9] 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. The Assessment of the Levels of MMP-9 in Serum and Vein Samples were performed in the Lab of K.S.H.E.M.A. under the guidance of personnel who are well versed with the estimations of these elements and a standard protocol was followed. The Levels of these parameters were increased with respect to the Normal ranges mentioned but fall short on comparing and contrasting with the Serum and Vein of Normal Individuals. The previous studies conducted have shown fluctuations in these parameters. A study conducted by Paul-Jacob *et al* signified MMP-9 as a marker of blood stasis in varicose veins. The study we have conducted have shown increments of MMP-9 but on comparison with that of normal healthy individuals have failed to establish a statistical significance. The study conducted by D. L Gillespie *et al* on varicose veins with estimations of MMP-1 and MMP-13 revealed significant increments of MMP-1. The study we have conducted is on MMP-9 hence our paper cannot be extrapolatory to the one mentioned here. Also the study conducted by D.L. Gillespie *et al* involved RT-PCR, Western Blot, mRNA analysis. Another study relevant to the one being conducted is on MMP-2,-13 and TIMP-4 in varicose veins. The study showed statistical significance for MMP-2 but not for MMP-13. The understanding is that MMP-13 has shown declining results in studies hence a shift of focus from this MMP is to be considered, highlighting that MMP-9 the study being conducted by us is an estimation which is feasible and definitive.

CONCLUSION

The estimations of MMP-9 in Serum and Vein samples on comparison to Normal Individuals were not statistically significant. The levels of MMP-9 were at the upper limit of the normal ranges in patients of Varicose veins indicating that more studies on MMP's as a measure of blood stasis in Varicose veins are needed to be performed in the future with different MMP's and Advent of investigative modalities for the same. The study has given in an insight with a future prospective to conduct studies on the Histology of VV with Normal Veins via the vein segments stored in the cold storage facility and also further studies on MDA in the Valves and Iron Overload in veins leading to a oxidative stress in Varicose Veins via the vein segments stored in Saline in our cold storage facility. These studies are preempted as there has been an association of Antioxidants and MMP's in varicose veins.

Table 1 Descriptive statistics of Age wise distribution

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Less than 30	6	5.0	5.1	5.1
	30-50	53	44.5	44.9	50.0
	51-70	53	44.5	44.9	94.9
	More than 70	6	5.0	5.1	100.0
	Total	118	99.2	100.0	
Missing	System	1	.8		
Total		119	100.0		

Table 2 Descriptive Statistics of Age & Gender wise distribution

CASE	N	Minimum	Maximum	Mean	Std. Deviation
Gender	45	.00	1.00	.8444	.36653
Age	45	23.00	72.00	47.2000	10.73355

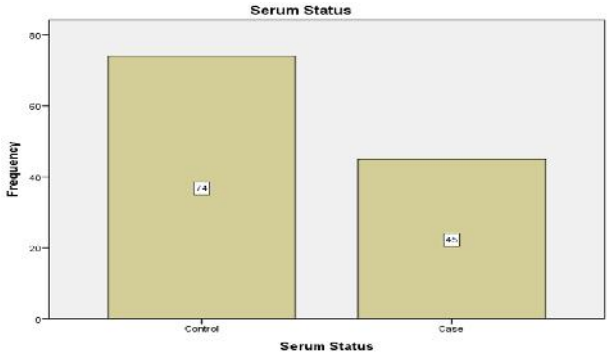
CONTROL	N	Minimum	Maximum	Mean	Std. Deviation
Gender	74	.00	1.00	.8784	.32908
Age	74	18.00	77.00	52.5811	13.63116

Table 3 Group Statistics of MMP-9 levels of Serum and Vein Samples in Cases and Controls.

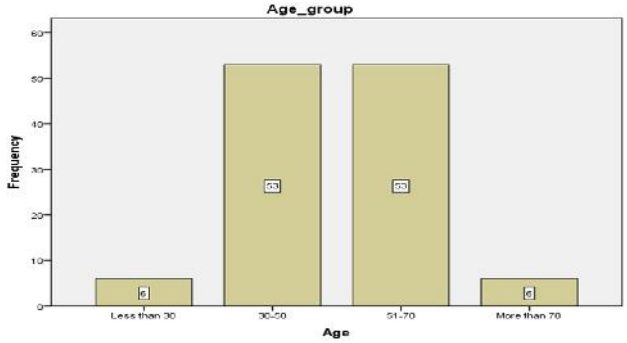
	STATUS	N	Mean	Std. Deviation	Std. Error Mean
Serum level	Control	54	8.6953	4.11067	.55939
	Case	34	9.8277	3.28596	.56354
Vein Level	Control	54	5.0085	2.36990	.32250
	Case	34	5.9966	2.32141	.39812

Table 4 Statistical Significance values with't'-Test for Equality of Means and p-value.

		Mean	Std Devn	't' – value	'p' - value
SERUM	Case	9.8277	3.28596	1.457	.149
	Control	8.6953	4.11067		
VEIN	Case	5.9966	2.32141	1.884	.063
	Control	5.0085	2.36990		



Graph 1 Case and Control distribution of study population



Graph 2 Age wise distribution of study population

Graph 3 Gender wise distribution of study population

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