



## To study enzyme imbalance for the development of varicosity in young adult male population of South India

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### Abstract

Varicose veins are tortuous, widened veins in the subcutaneous tissues of the legs and are often easily visible. Their valves are usually incompetent so that reflux of blood occurs, and the resulting venous hypertension. Varicose veins are widely seen as medically unimportant and deserving low priority for treatment. For the great majority of people varicose veins cause no symptoms and never cause harm. The primary cause of varicose vein formation is not clear. Epidemiological studies indicate involvement of hereditary factors. Both vein valve dysfunction and hydrostatic venous pressure appear to play a critical role in the initiation and progression of the disease. The role of imbalance of certain metalloproteinases are thought to play some significant role in the development of varicose veins and hence the study aimed to investigate the role of matrix metalloproteinases (MMPs) imbalance in development of varicosity.

**Key words:** Etiology; Hernia; MMPs; Pathogenesis; Varicose veins

### Introduction

Varicose veins are veins that have become enlarged and tortuous. The term commonly refers to the veins on the leg, although varicose veins can occur elsewhere. Veins have valves to prevent blood from flowing backwards. Leg muscles pump the veins to return blood to the heart, against the effects of gravity [1]. When veins become varicose, the valves no longer meet properly, and the valves do not work. This allows blood to flow backwards and they enlarge even more. Histologically, varicose veins are characterized by significant disruption of the regular architectural pattern observed in normal veins. These findings appear as skip or alternate lesions and may not involve the entire circumference of the venous wall.

The intima may be intact, but asymmetric areas of intimal thickening or fibrosis interspersed with areas of normal-appearing intima are not uncommon. These intimal changes are associated with increased collagen deposition and plaques below the endothelial lining. In the media, remodeling of the components of the extracellular matrix scaffold results in disruption of the longitudinal and circular muscle fiber bundle arrangement. Smooth muscle cells appear enlarged and have lost their elongated morphology, suggesting a phenotypic change from their contractile to a synthetic

state. The adventitia is characterized by an increase in smooth muscle cells, fibroblasts, and collagen.

Thrombus in various stages of organization is not an infrequent finding on the luminal surface. Because these morphologic changes may not be uniformly distributed along the entire circumference of the vein, it is not uncommon to find normal venous segments interspersed with abnormal varicose changes within the venous wall [2,3]. Expression of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) has been documented to different degrees in varicose veins MMP-1 is increased in varicose veins and localizes to the endothelium and more diffusely throughout the media in smooth muscle cells, fibroblasts, and, rarely, adventitial microvessels.

Levels of pro-MMP-2 are increased with or without a significant increase or decrease in MMP-2 expression or activity. MMP-3 can also be significantly increased in the wall of varicose veins. MMP-9 protein expression is variable and localizes to the endothelial cell monolayer, adventitial microvessels, and, especially, the smooth muscle cells in the media. MMP-12 expression is also similar to normal veins and localizes to smooth muscle cells and fibroblasts [4]. The precise role of metalloproteinases (MMPs) in the development and progression of varicose veins awaits further study.

## Materials and Methods

**Source of data:** This study will include 50 male patients between the age 18-35years admitted to K.S Hegde Charitable hospital recently for the last 2years. The control will have 50 male patients of the same age group undergoing groin hernia repair. **Study design:** Case control study. **Study sample:** 47 in each arm.

### Methods of Collection of data :

The present study to be carried out at K.S Hegde Charitable hospital. Relevant data to be collected from the patients admitted with varicose veins., fit for surgery which will include name ,age, sex, hospital number and diagnosis. All patients to undergo routine preoperative investigations to obtain fitness for surgery. Sample to be collected from the patient who is undergoing surgery for varicose vein. Segment of involved vein to be isolated and sent for the study. In the control group who are undergoing groin hernia repair -a segment of the tributaries of the saphenous vein to be isolated and sent for examination. In both the cases utmost care will be taken, .not to use diathermy to avoid structural damage to the vein.

The study got clearance by the Institutional Ethical Committee.

**Inclusion criteria for cases:** Primary, symptomatic, varicose veins. 18-35year old males undergoing surgery.

Ability to give informed written consent. **Exclusion**

**criteria for cases:** Those who have undergone interventions such as sclerotherapy or major surgery.

Age above 35 years and below 18 years. Inability to give informed written consent. **Inclusion criteria for**

**controls:** Male patients undergoing groin hernia repair between the ages of 18-35 years. Those male patients

undergoing elective surgery for groin hernia. Ability to give written consent. **Exclusion criteria for controls:**

Those who have undergone interventions like sclerotherapy or major surgery. Age above 35 years and below 18 years. Inability to give informed written consent.

### Statistical analysis:

Proportions of the variables will be taken in to consideration, correlation co efficient to find the correlation and chi square test to find the correlation between dependant and independent variables. Multivariate analysis will be done for independent correlation of variables.

### Method:

Assay based on use of modified prourokinase in which the activation sequence, normally recognized by plasmin. was replaced by MMPs. A chromogenic peptide substrate for urokinase is then used to measure

the active urokinase generated through mmp activation of modified urokinase. .MMP-2 is captured from biological fluids or standards (proMMP2). Immobilised latent MMP-2 is then activated using p-amino phenyl mercuric acetate (APMA) to measure total MMP-2. Diiferential activation enables the analysis of the activity of both the already active and latent forms of MMP-2.

Samples were homogenized in triton buffer, Centrifuged at 3000 rpm and the supernatant was taken. 100microlitre of the supernatant and 100 micro litre of the standard are taken ,50micro litre APMA added and 50 micro litre detection reagent (prourokinase and substrate) added .incubation carried out for one and half hours at room temperature and then the optical density is read at 405 nm [5,6].

So far we have received 17 samples which includes.8 varicose vein samples and 9 hernia samples. Assay was done for 8 samples each. Assay for the control sample, which we received recently is yet to be done. The project is ongoing, the results are given only to the analysis of the samples that are received.

## Results

It appears that there is increased expression of matrix metalloproteinases in varicose veins. But since the sample size is small [the samples are very rare to get] no conclusions can be reached. We propose to reach the target figure such that this study assumes statistical significance. The available data shows the significant increase in levels of MMPs compared to controls.

**Table 1: Readings from cases and controls of MMP**

CONTROLS-HERNIA MMP-2 activity(ng/ml)	CASES- VARICOSEVEINS MMP-2 activity (ng/ml)
120	208
165	256
115	85
134	397
13	234
198	57
206	129
109	213

## Discussion

The present study aimed to explain the possible role of MMPT in the aetiopathogenesis of the development of varicose veins. Nomura, says matrix metalloproteinases (MMPs) have been implicated in tissue degradation in varicose veins. The aim of their study was to investigate the effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) on the activity of MMPs in varicose veins. MMP-9 was present at significantly higher levels in varicose veins than in controls and was localized mainly in smooth muscle cells at the tunica media, where marked degradation of the extracellular matrix was observed [4]. Woodside et al say for the remodeling process, MMP-9 may be produced in the Varicose Vein wall and degrade elastic lamellae and other extracellular components of the venous wall [7]. Marie Paule Jacob says the marked increase in plasma pro-MMP-9 activity provides evidence of polymorphonuclear activation and granule release in the varicose vein in response to postural blood stasis. Similarly, detection in the plasma of membrane proteins shed from the endothelium or leukocytes provides evidence of pericellular proteolysis [8]. There are distinct differences in the structural architecture and localization of MMP expression in normal and varicose veins. Although the changes observed are not sufficiently definitive to enable a causal relationship, they do suggest a possible mechanism for the alterations in matrix composition observed between normal and varicose veins [5]. Raffetto et al observed that MMP2 causes significant relaxation of inferior venacava via hyperpolarisation and activation of K<sup>+</sup> channel [9]. Gillespie et al observed increased MMP1 in varicose veins [10].

## Conclusion

There is definitely the probable role of MMPT in the aetiopathogenesis of varicose veins but it should be studied in detail with the analysis of proportion of enzyme assay and the severity of varicose veins with or without complications of varicose veins. Further in depth study is needed to conclude the conclusive role of MMPT and development of varicose veins.

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