



MOLECULAR GENETICS OF VARICOSE VEINS OCCURRENCE IN HUMAN

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Abstract

This is a prospective study done in inpatients of Pt. BD Sharma University of Health Sciences, Haryana. A total of 61 patients were analysed for finding their prevalence, risk factors, family history, clinical features and surgical data outcomes were studied over a period of 1 years. In our study of 61 patients we found the mean age of the study population to be 45.6% with a range of 21 to 70 years. Male patients (38%) outnumbered the females (62%). In our study, we encountered 47 cases of complication out of 61 patients, the commonest being haematoma in 18 cases which cleared conservatively followed by 15 cases of DVT but literature shows the incidence to be very low at 0.01%. Varicose veins are a frequent occurrence. The commonest age group of patients suffering from varicose veins is 20-30 years. Most of the patient presented to the hospital for one or the other complications not for the cosmetic purpose. The great saphenous and the communicating systems are most commonly involved, followed by great saphenous system alone. Pain is the most common presenting symptom and pigmentation the most common complication of the disease. The treatment depends on the site of incompetence and should hence be tailor-made for each case.

Key words: Complications, Varicose veins, Haematoma, Surgery and Varicose surgery.

Introduction

Varicose veins are a common condition affecting the lower limbs. Apart from being a cosmetic problem, it can have some serious complications if not treated in time. It is common vascular abnormalities in the world. It occurs, when the one way valves in the vein stop working properly. Veins are blood vessels that returns deoxygenated blood from the outer part of body back to the heart and lungs. When vein becomes abnormal, full of the twists and turns, or enlarged, they are called Varicose Veins. The vein becomes weakened, enlarged and dilated. The veins have permanently lost their ability to carry blood from the legs back up to the heart against the force of gravity. As the blood falls down the legs and pools due to gravity, the veins overfill giving them their typical unsightly bulging appearance. Varicose veins are abnormally swollen (dilated) and tortuous (twisted) veins and usually situated quite near the surface and visible beneath the skin. Vein size can vary from quite small (2-3mm across) to vary large (2-3 cm across). Varicose veins may occur anywhere in the body, but they are most often located in the legs. These veins in the leg may be superficial or deep. The prevalence of varicose veins in general population increases in those over 35 years of age, ranging from 30% with minor varicosities to 6% with severe symptoms (Evans *et al.*, 1999). The most striking about the epidemiology of varicose veins is the geographical

variation in prevalence rate. Western countries have high rate of prevalence as compared to traditional living countries (Burkitt *et al.*, 1972). According to available statistics, approximately 10% of the U.S. and European populations suffers from varicose syndrome by the age of 30 year. Varicose veins more common in women than in men and are linked to heredity. However, in Edinburgh study, prevalence of varicosity was 40% in men and 32% in women (Evans *et al.*, 1999).

Multiple factors have been ascribed to the development of varicose veins; there is little scientific evidence to suggest any thing, other than genetics, in the development of varicose veins. The overwhelming majority of people with vein problem have relative (mother, grandmother and aunt etc.) with similar problems. Although very obese people and vary tall people have particularly troublesome varicose veins. A number of studies have found that varicose veins are common in people who stand up at work- particularly those who stand still for long period. Reflux in deep vein may be a cause of recurrent veins but this is often not treatable (Ali and Callam, 2007). The incidence of varicose veins is much higher and onset earlier, in individual with Lymphedema- Distichasis (LD) syndrome than in general population. LD syndrome is commonly referred to as swelling of leg with double row of eye lashes. About 50% of individuals with Lymphedema-

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distichasis syndrome have varicose veins (Brice *et al.*, 2002). Environmental factors such as food, occupation, life style are known to influence the development of varicose veins and possible familial clustering and estimation of heritability based on twin studies have been reported (Khalid *et al.*, 2008). It has been found that lack of fiber in diet and sitting straining on the lavatory (rather than squatting briefly to pass a bulky stool) might predispose to varicose veins. A genetics predisposing to varicose veins has been proposed for many years, and venous functions in twins, indicate a strong genetic influence (Brinsuk *et al.*, 2004). Individuals are more likely to be affected by varicose veins when parents and sibling have varicose veins (Lee *et al.*, 2003). FOXC2 is the 1st gene in which mutation has been strongly associated with primary venous valve failure in the both superficial and deep veins in the lower limb and is a cause of varicose veins. This gene is a regulatory transcription factor and is located on chromosome 16q24.3 (Bell *et al.*, 2001 and Fang *et al.*, 2000). Cytogenetic investigation of varicose veins patients has revealed the presence of metaphase with structural abnormalities, clonal trisomies of chromosomes 7, 12 and 18 and monosomy of chromosome 14 (Scappaticci *et al.*, 1994). There are many treatment modalities available of varicose veins. Different vein respond differentially to each of the treatment options. So, appropriate therapy for each individual may involve different therapeutic option applied at different points in legs. Color duplex is the best technique available for the examination of venous function (Baker *et al.*, 1993). Mutation in ten FOXC2 gene is strongly associated with venous reflux varicose patients. Venous disease is a common condition, and further studies are required to determine whether FOXC2 abnormalities play a role in the development of varicose veins in the general population. Cytogenetic result in varicose veins will suggest karyotypic variation and can be associated with the genotypic constitution responsible for longer duration of disease or with sporadic varicosities. Such studies will facilitate in the prognosis or in the treatment of varicose veins. In Indian scenario the disease is one of the common surgical problems in low socio-economic class people, which at times compel the patient to change his occupation which is very disturbing (Pramod *et al.*, 2011). Researcher personally has observed that most of the Indians were unaware of varicose veins, and also their preventive measures. So there is a need to educate the people regarding this condition in order to prevent it. The present study will provide self-instructional module regarding prevention of varicose veins.

Research Methodology

This prospective study on outcome of stab avulsion in conventional varicose vein surgery was conducted in department of surgery at Pt. B. D. Sharma University of Health Sciences, belonging to population groups known to have Varicose Veins disorder was chosen for this study. During this period 61 patients having primary varicose veins

were selected randomly. All cases of varicose veins presenting to the OPD were subjected to duplex scan to rule out secondary causes. Patients admitted with varicose vein who satisfied the inclusion and exclusion criteria were included in the study. All the required data was collected from patients during their stay in the hospital, during follow up at regular intervals and from medical records.

Inclusion criteria

All patients clinically diagnosed of symptomatic or complicated primary lower limb varicose veins with saphenofemoral and/or saphenopopliteal incompetence with or without perforator incompetence.

Exclusion criteria

1. Patients presenting with recurrent varicose veins.
2. Patients with concurrent deep venous thrombosis.
3. Patients having secondary varicosities.
4. Patients less than 18 years of age.
5. Patients have Diabetes.
6. Patients not fit for surgery

Mutation analysis

For mutation analysis DNA of varicose vein patients was extracted from peripheral venous blood by a standard procedure after the informed consent of the patients. To amplify the DNA, oligonucleotide primers was designed. Using these primers, PCR was performed. PCR product will be then electrophoresed horizontally. Agarose gel electrophoresis was used to detect PCR amplification.

All 61 patients were inpatients in the surgical ward, their history was taken, symptoms and signs recorded followed by general and local examination. Secondary causes were ruled out using the duplex scan. Cases with complications were initially treated conservatively in order to improve the associated complications like ulcers eczema and dermatitis and later subjected to operative treatment. Patients who presented with bilateral varicose veins with symptoms in one leg got their symptomatic limb operated on first. No bilateral surgeries were performed. Surgeries were performed based on the site of incompetence. Post-operative compressive dressing was applied for 6 months (only during daytime). Follow-up was done on regular basis for up to 6 months (Figure 1).

Results

In our study of 61 patients we found the mean age of the study population to be 45.6% with a range of 18 to 70 years. Age of diagnosis of varicose cases was studied and analysed. The age range of this series was of 18-70 years. It was found that there were most diagnosed cases between age of 20-30 (37.70%) (Figure 1). Out of 61 patients studied, 23 were male

and 38 were female (Table 1). Women are more frequent because of the sex hormone estrogen and progesterone cause blood vessels to relax, thus separating the valves, so that they do not meet to back the flow of blood.

Symptomatology

Patients studied were presented with varied symptoms, out of which dilated vein was most common 58 (95.08%) followed by aching pain 52 (85.24%) (Table 2, Figure 2).

Rural Urban Ratio

Out of 61 patients studied, 62% of patients belongs to rural region whereas 38% were from urban region (Figure 3).

Risk factors

Patients studied were presented with varied risk factors, out of which standing for long time was most common 55.17%, followed by obesity which was 41.37% (Figure 4). Right limb was more affected 39 cases than left limb 08 cases. In 14 cases both the limb were involved (Figure 5).

Groups based on the types of work

The patients were divided into three groups based on the types of work. Out of which heavy weight loading workers were more affected than other groups of working (Table 3). Results shown only 03% of patients were from official background.

Occupation

Out of 61 patients studied 57 patients in their occupation involved, either prolonged periods of standing or heavy load working or both (Figure 6). Farmers were most affected due to violent muscular efforts during their working.

Associated Complications

Total number of patients which have complications was 47, out of which hematoma and DVT was commonest with 18 and 15 cases respectively. There was no recurrence of varicosity in any case because of less number of patients studied in three month of time (Figure 7).

Family history

Out of 61 patients with varicose veins studied 15(25%) patients had family history of varicose veins occurring in several members of same family. Family member which have varicose veins were not diagnosed but shown the symptoms of varicose in past.

Comparison of Age and Sex Ratio

According to age and sex ratio, prevalence of female patients was higher than male patients (Figure 8). Age group 41-50 shown the high frequency of male patients.

Comparison of the profession and Sex

Prevalence of females was higher in group II whereas male were higher in group III (Figure 9). Men were frequent in group III because of prolonged standing and violent muscular efforts. varicose veins was significantly higher in men in all three professional groups.

Mutation Detection in Varicose Patients

Patients of varicose veins were subjected to molecular analysis to identify the FOXC2 mutation in these patients. Out of 61 patients of varicose veins 54 patients have shown the mutations. However all the 61 patients were subjected to further DNA analysis for mutation detection.

DNA Extraction and Purification

DNA preparation from whole blood using a rapid improvised isolation of mammalian DNA technique was used (Sambrook, 2002). Optimization of the protocol resulted in 80ng to 100ng of DNA. Purity of DNA sample was checked at the OD_{260}/OD_{280} . All the samples were found to be in desirable reference ratio of 1.65 to 1.85. The samples which were found to fluctuate from the reference range were purified again by RNase and Proteinase K treatment. The purified DNA was stored in the TE buffer (pH 7.6) at -20°C. DNA product was determined by using agarose gel electrophoresis (Figure 10 A, B).

Amplification of DNA by Polymerised Chain Reaction

Purified DNA was amplified using polymerised chain reaction. All the 61 selected patients of varicose veins were screened for FOXC2 mutation. Detection of FOXC2 mutation was done with Allele Specific PCR.

Mutation detection

A) FOXC2 (-91 C→G) Mutation: Amplification product range for FOXC2 varied from -413 to + 194 bp. Out of 61 patients of varicose veins 19 were detected with (-91 C→G) mutation. However among the Male and female groups, there was 15 female and 4 male patients were shown this mutation (Figure 11).

B) FOXC2 (-41 G→A) Mutation: Amplification product range for FOXC2 varied from 50 to 318 bp. Out of 61 patients of varicose veins 52 were detected with (-41 G→A) mutation. However among the Male and female groups, there was 33 female and 19 male patients were shown this mutation (Figure 12).

C) FOXC2 (-41 G→T) Mutation: Amplification product range for FOXC2 varied from 206 to 655 bp. Out of 61 patients of varicose veins 56 were detected with (-41 G→T) mutation. However among the Male and female groups, there was 33 female and 20 male patients were shown this mutation (Figure 13).

Comparison of all three mutations with Sex Ratio

Total 82% of varicose patients were affected by FOXC2 mutations which were studied. Female patients were more affected by all mutation than male. (-41 G→T) transversion mutation was more frequent (59.01% in females and 32.78% in males), followed by (-41 G→A) transversion (54.01% in female and 31.14% in male) (Table 4, Figure 14).

Varicose veins and their treatment have been commented upon since antiquity. Although the surgical treatment of ligation and stripping of the greater saphenous veins has been fairly standard for nearly the last 100 years, more recent studies have questioned this approach. It is the purpose of this study is to review the pathophysiology, diagnosis, surgical treatment of varicose veins, and their outcomes. In 1978 Widmer reported data from a defined population of factory workers'. He found a higher incidence of varicose veins in men (5.2%) than in women (3.2%), with the overall incidence of varicose veins being 4.2 %. The prevalence of venous disease increases with age. Varicose veins are a known occupational disease, found in people required to stand for prolonged periods. We found that our study had 47.14% farmers, who admitted that their occupation required standing for long intervals. Also worth noticing was the fact that the other people affected were policemen and teachers, jobs associated with prolonged standing. Analysing the data regarding systems involved, we derive that the great saphenous system is the most commonly involved (75.71%), the communicating system is the next commonest (60%), the small saphenous is the least involved system. Two or more systems were seen to be involved frequently than isolated system insufficiency. In accordance with other studies we too noted that pain was the commonest presenting symptom and pigmentation was the commonest presenting complication. Surgeries were based on the site of incompetence, junctional reflux noted on duplex scanning was treated by either by Trendelenberg's procedure (flush ligation of the SFJ) or by ligation of the SPJ, whichever was involved. The GSV was stripped, interrupted or preserved as per surgeons' preference. The perforator incompetence was dealt by sub-fascial ligation. The blowouts and tributaries were stab avulsed using a small incision overlying the area with a stab knife. Multiple such avulsions were carried out depending on the size and extension of the varicosities. When each component is considered alone, Trendelenberg's procedure was done in 62 (88.57%) limbs, stripping was done for 46 (65.71%), SPJ ligation was done in 13 (18.57%) limbs and perforators were ligated in 40 (57.14%) limbs. So, Trendelenberg's surgery amounts for the maximum number of cases, followed by stripping, perforator ligation and SPJ ligation, in that order of frequency. It should be borne in mind that procedures are combined based on the patient's requirements. Hence multiple

combinations of the above mentioned procedures are done to alleviate the patient's symptoms. Among complications of surgery a total of 6 (8.57%) recurrences were noted, most of them were secondary to new perforator incompetences occurring atleast an year after primary surgery. Other complications such as seroma, hematoma, wound infection and neuropathies were noted and were treated accordingly.

Conclusion

This study successfully identified single and combined clinical characteristics that were predictive of the risk of ulceration in patients with varicose veins. Varicose veins are a frequent occurrence. Commonly affect middle aged males, required to work standing for prolonged hours. The great saphenous and the communicating systems are most commonly involved, followed by great saphenous system alone. Pain is the most common presenting symptom and pigmentation the most common complication of the disease. The treatment depends on the site of incompetence and should hence be tailor-made for each case. These surgical procedures are associated with complications, seroma being the commonest.

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Table 1: Sex distributions among varicose patients

Gender	No. of cases	Percentage
Male	23	38
Female	38	62
Total	61	100

Table 2: Symptoms present in varicose patients

Symptoms	No. of cases	Percentage
Pain	52	85.24
Dilated vein	58	95.08
Limb edema (Swelling)	23	37.70
Ulcer	07	11.47
Others (Skin Changes etc.)	22	36.06

Table 3: Patients effected in different groups of working

Groups of patients	No. of cases	Percentage
Official work	02	03.27
Light physical laborers	25	40.98
heavier physical laborers	32	52.45

Table 4: Comparison of all three mutations in male and female

Mutation	Male Patients		Female patients	
	No. of patients	Percentage	No. of patients	Percentage
(-91 C→G) Mutation	04	06.55	15	24.59
(-41 G→A) Mutation	19	31.14	33	54.09
(-41 G→T) Mutation	20	32.78	36	59.01

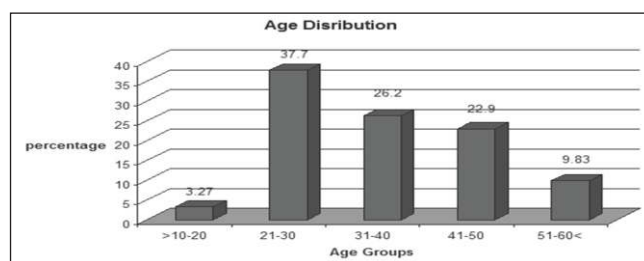


Figure 1: Age Diagnosis of Patients in different age groups

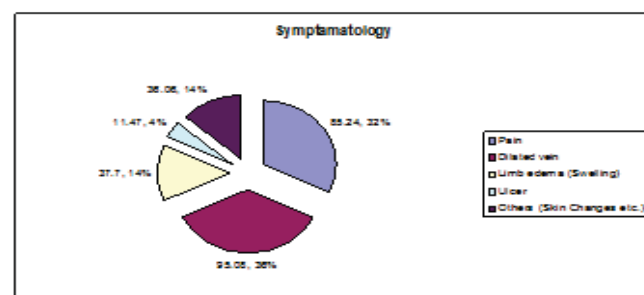


Figure 2: Percentage frequency of symptoms present in patients

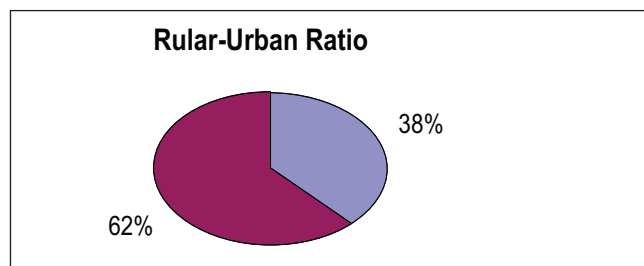


Figure 3: Percentage frequency of rural -urban ratio

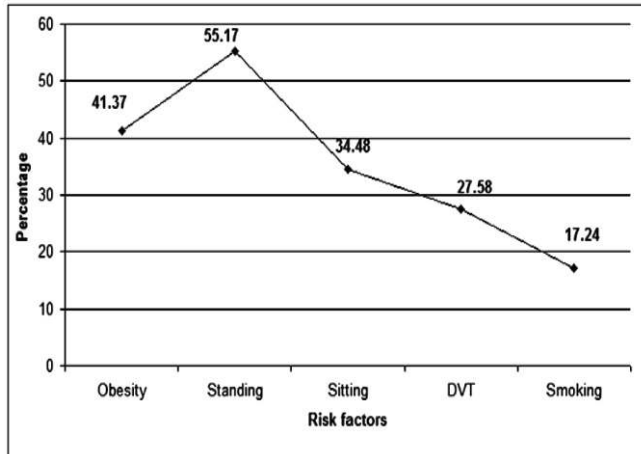


Figure 4: Percentage frequency of risk factors of Varicose Veins patients

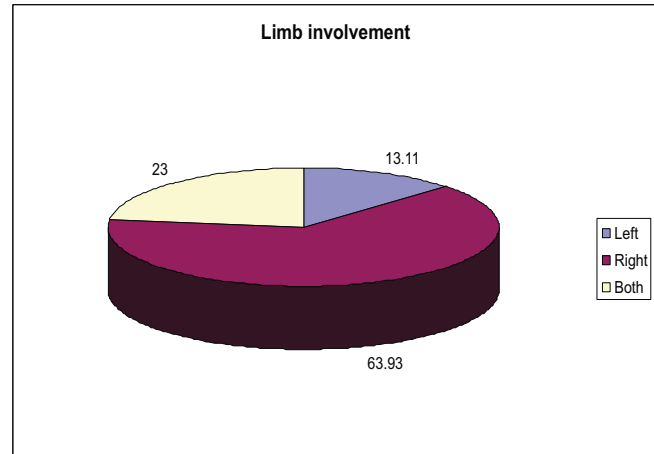


Figure 5: Side effected of limb in varicose patients

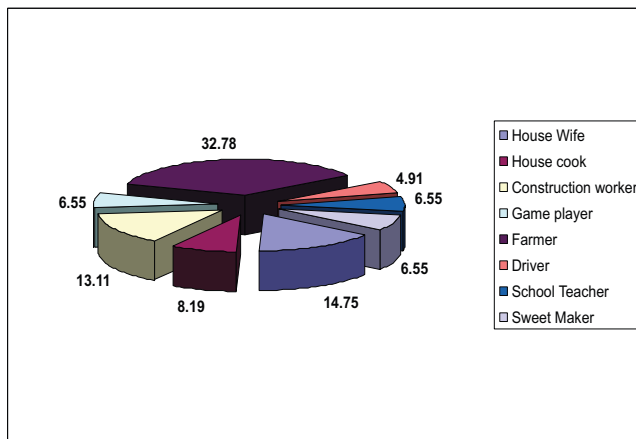


Figure 6: Percentage frequency of patients involved in their occupation

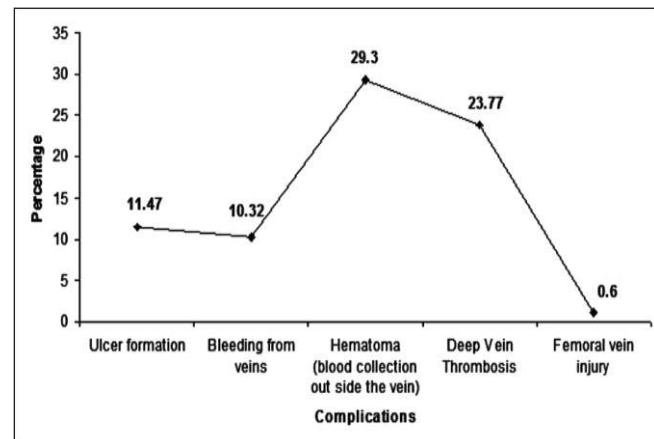


Figure 7: percentage frequency of complications in varicose patients

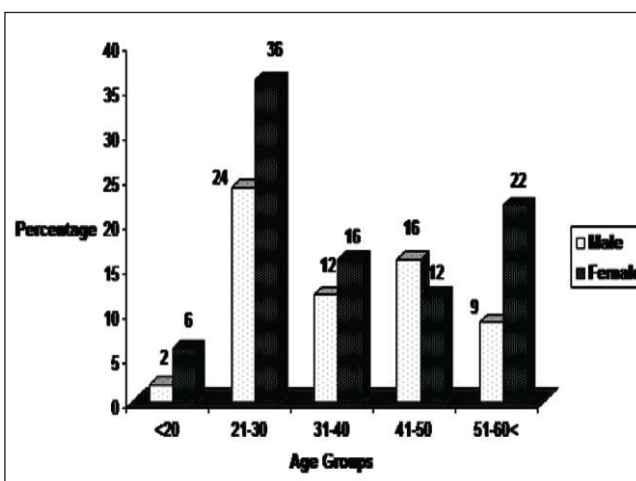


Figure 8: The frequency of varicose veins in comparison to the age groups and sex

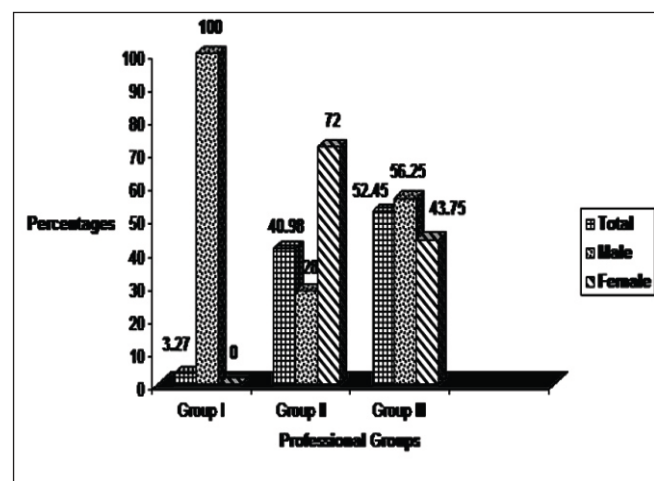


Figure 9: The prevalence of varicose veins in comparison to the profession and sex

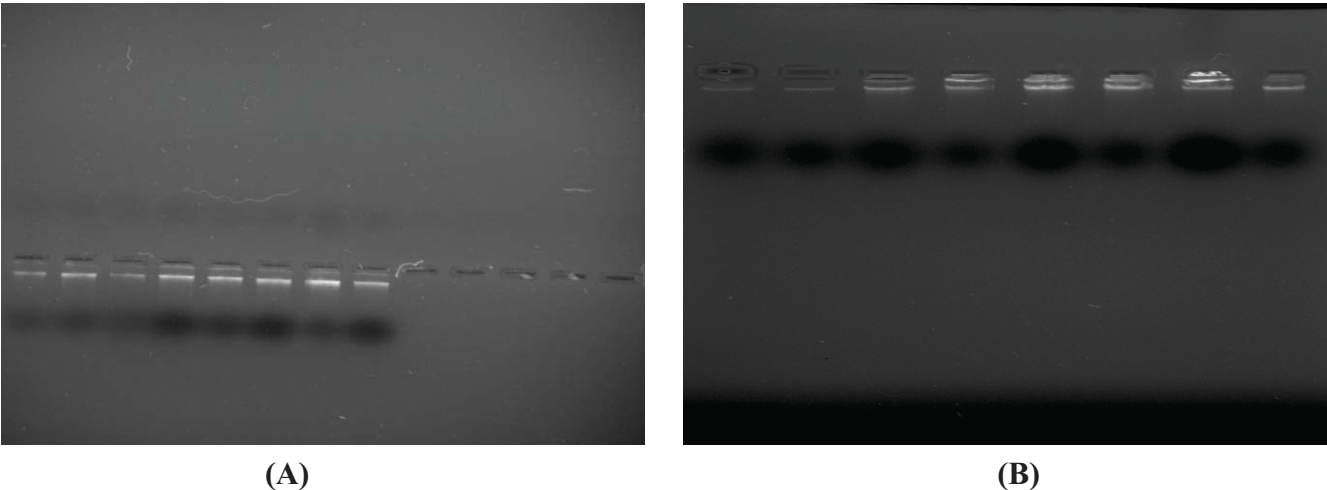


Figure 10 A & B: DNA amplification by mammalian DNA technique was used (Sambrook 2002)

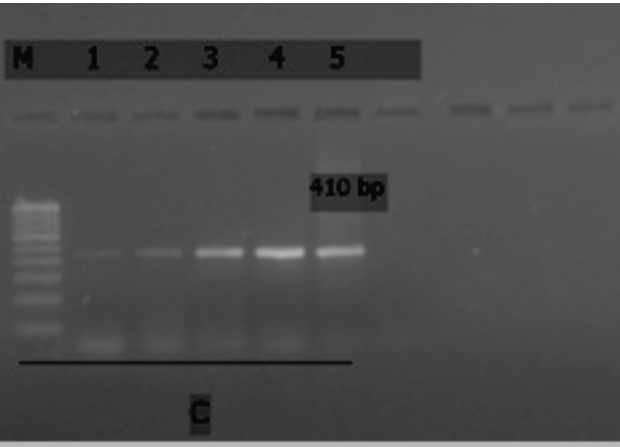


Figure 11: FOXC2 (-91 C→G) Mutation

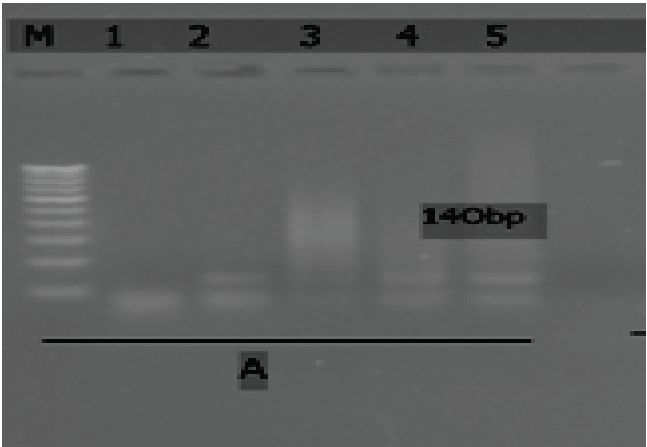


Figure 12: FOXC2 (-41 G→A) Mutation

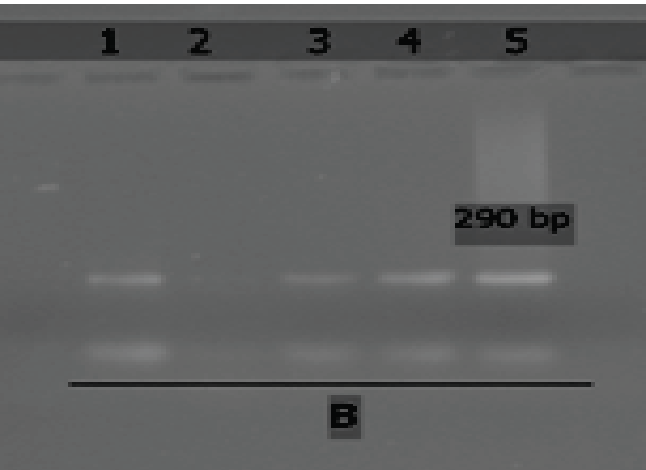


Figure 13: FOXC2 (-41 G→T) Mutation

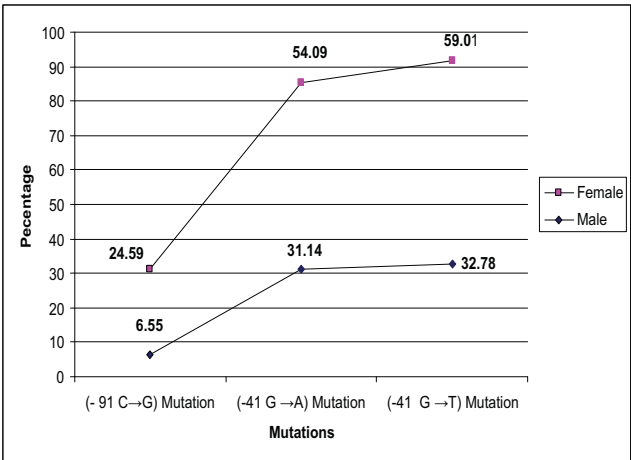


Figure 14: All three Foxc2 mutation in varicose patients