CHAPTER- 1
INTRODUCTION

Varicose veins are visible surface manifestations of an underlying venous insufficiency syndrome. Venous blood escapes from a normal path and circulates in a retrograde manner in an already congested leg. These 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 (2-3 mm across) to vary large (2-3 cm across) (Borden et al. 1997, Badier et al. 2000 and Eklof et al. 2004). 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. They are usually bilateral affecting both legs (75-76%) (Abramson et al. 1981, Maffei et al. 1986, Hirai et al. 1990 and Komsuoglu et al. 1994) and when unilateral they are detected with the same frequency on each leg (Maffei et al. 1986, Hirai et al. 1990 and Komsuoglu et al. 1994).

Understanding varicose veins means understanding the complex system of veins that make up our legs. Our legs are comprised of a network of veins that are similar to branches on a tree. They contain large or major veins and increasingly smaller veins. The veins of the leg are divided into superficial and deep vein system. From the venules to the pelvic veins, the veins contain valves that can resist gravitational and muscles pressure and direct the blood flow towards the heart. Venous disease (also called vein insufficiency or venous reflux), occurs if these valves become damaged, allowing the backward flow of blood in the legs. Because gravity works on the legs more than on other parts of the body, these vein walls are under tremendous pressure. When blood cannot be properly returned through the vein, it can pool, leading to a feeling of heaviness and fatigue, causing varicose veins and other skin changes. Over time, this increased pressure can cause additional valves to fail. If left untreated, it can lead to leg pain, swelling, ulcers and other health problems.

Varicose veins patients have swelling of the feet and ankles due to fluid from stagnant blood leaking through the walls of the veins into surrounding tissues. A feelings of heaviness, tiredness and aching, especially at the end of day or after periods of prolonged standing may occur. They may be change in skin color, the affected skin
may turn into brownish grey color, especially around the ankles. Minor injuries to the area may bleed more than normal and/or take a long time to heal. In some people abnormally high pressure in the leg veins can damage the skin, and eventually lead to ulcer. Development of carcinoma in varicose patients can occur due to long standing venous ulcers. There have been over 100 reported case of malignant transformation and the rate is reported as 0.4% to 1% (Goldman 1995). Restless legs syndrome appears to be a common overlapping clinical syndrome in patients with varicose veins and other chronic venous insufficiency. The "more severe" are the ones that result in serious problems, as bleeding, ulcers (wounds), eczema, infections, redness, stains, thickness of the skin, pain, phlebitis and even the lung clot, that although rare in primary varicose veins, may put in risk the patient's life.

Varicose veins have been recognized as chronic disorder since ancient times. Hippocrates discussed them 2500 years ago (Johnson 1994). With increasing population, increased lifespan and changes in lifestyle and occupation, prevalence has been variously reported from as little as 2% to over 20% in population studies (Russell et al. 2007). This enormous variation results from the different populations studied, different definitions applied and the different assessment or examination techniques used. Western studies have shown that 20% population suffers from varicose vein and 1% has skin changes proceeding to venous ulceration (Russell et al. 2007). 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 1972). The Edinburgh Venous study (Carpentier 2004) examined over 1000 adults in UK, showed that 50.3% of men and 32.2% of women had a dilated tortuous trunk of the long and/or short saphenous vein. In India it is estimated that 15 to 20% of the general population is suffering from varicose veins disease these days (Vardhan 2010).

Women have greater risk of varicose veins, because the female sex hormone estrogen and progesterone cause blood vessels to relax, thus separating the valves. So that they don't meet to block the back flow of blood and varicose occurs in women.

The prevalence of small reticular varicosities was even higher at over 80% for both males and females. Earlier studies showed a higher prevalence of varicose veins in women than men (Weddell 1969 and Coon et al. 1973). In special survey for
varicose veins in England, reported prevalence have varied from 7 to 38% in men and from 14 to 51% in women (Abramson et al. 1981). In Finland the incidence rate was significantly higher in women in all cohorts studied (from 40 to 60-year-olds) (Mäkivaara et al. 2004). Although it was reported in the literature that varicose veins are common in women. There are also some studies which suggest that the prevalence of varicose veins is higher in men (Colin 1972, Stanhope 1975, Evans et al. 1999, Chiesa et al. 2005a and Chiesa et al. 2007). Recently an Indian study found that 75% of men and 25% of women had varicose veins (Mirji et al. 2011).

Pregnancy appears to be a major predisposing factor for the development of varicose veins and is likely a major reason why the prevalence of varicose veins is twice as high in women as in men (Bassi 1967, Nahoum et al. 1974, Widmar 1978, Basellini et al. 1985 and Guerrine et al. 1987). The development of new varicose veins occurs in up to 28% of pregnancies and the incidence rises with increasing parity (Stansby 2000). The prevalence of varicose veins in women with zero, one, two, three, and four or more pregnancies was reported as 32%, 38%, 43%, 48%, and 59%, respectively (Laurikka et al. 2002).

In most studies the prevalence of varicose veins increased with age in both genders (Laurikka et al. 1993, Criqui et al. 2003 and Kroeger et al. 2004). The Edinburgh vein study reported the prevalence of varicose veins increases from 11.5% in 18 to 24 year olds age to 55.7% in 55 to 64 year olds (Evans et al. 1999). In India a survey of 1,000 young industrial employees, it was found that 10% had definite varicose veins. In an older group of department store employees, all over 40, the incidence had risen to 40% among the men and 70% among the women (Mirji et al. 2011). The same was reported earlier in the studies of male Indian railroad workers (Malhotra 1972). In an Indian report increased prevalence of varicose veins with age was reported in men (Mirji et al. 2011).

The establishment of the cause of varicose veins is essential for prognosis, management, and understanding the genetic of varicose. The causes of varicose veins are numerous and include genetic and environmental factors.

Environmental factors such as food, occupation, life style are known to influence the development of varicose veins and possible familial clustering (Khalid et al. 2008). Today a lot of occupations and professions have sprung up where a person is
required to either constantly stand up for a long time or made to sit with legs hanging down for a considerable time. Case control study carried out on tobacco smokers, reported lower limb venous insufficiency to be significantly associated with tobacco smoking with dose effect relation in both genders (Ducimetiere et al. 1981). A longitudinal follow-up survey on men reported smokers to be more likely to develop varicose veins than non-smokers (Scott et al. 2004). Work involving heavy lifting was also related to the higher prevalence of varicose veins in one study (Weddell 1969). Working in a standing position was even associated with subsequent hospitalization due to varicose veins for both men and women in the Danish study (Tuchsen et al. 2000). Computer professionals, Nurses, House made, Constructors, Receptionists, Security guards, Traffic policemen, Salesmen, Teachers and persons doing Desk jobs, Work involving heavy lifting etc. are the worst sufferers of Varicose veins. Significant associations were found with a standing posture at work and varicose veins in both sexes (Abramson et al. 1981, Tuchsen et al. 2000 and Kroeger et al. 2004) or in women alone (Mekky et al. 1969, Sisto et al. 1995, Jawien et al. 2003 and Lee et al. 2003). In India a growing number of traffic policemen are suffering from varicose veins, a painful ailment which often develops due to high pressure while standing and walking. Over 80% of people studied in a population based evaluation were found to have visible evidence of venous disease (Marston 2010). Thus, the identification of cause is important in planning for the medical, educational, and treatment needs of a particular individual.

Although these risk factors may contribute to varicose vein formation, many individuals exposed to these risk factors do not develop the disease. Furthermore, the so-called environmental risk factors may also contain substantial genetic components. For example, obesity has an estimated heritability of up to 80% (Bell et al. 2005). Varicose vein show an autosomal dominant pattern of inheritance with reduced penetrance (Rosbothan et al. 2000). Individuals are more likely to be affected by varicose veins when parents and sibling have varicose veins (Lee et al. 2003). The majority of earlier studies showed self reported positive family history to be a risk factor for varicose veins (Schultz-Ehrenburg et al. 1992, Komsuoglu et al 1994, Scott et al. 1995, Jawien 2003, Carpentier et al. 2004 and Criqui et al. 2007).
A genetic predisposing to varicose veins has been proposed for many years, and venous functions in twins, indicate a strong genetic influence (Brinsuk et al. 2004). A study in a twin cohort indicated linkage of varicose veins to candidate marker D16S520 on chromosome 16 (Ng et al. 2005). This region of chromosome 16 contains genes coding for, among others, FOXC2, FOXL1, FOXF1, and IRF8. 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 (Russell and Glen 2007). The official name of this gene is “Forkhead box C2 (MFH-1, mesenchyme forkhead 1). FOXC2 gene encodes a 2.2Kb transcript with a 1.5Kb single exon coding region that is highly GC rich (>70%) and is expressed in the developing cardio-vascular system (Miura et al. 1997).

FOXC2 gene is a regulatory transcription factor and is located on chromosome 16q24.3 (Fang et al. 2000 and Bell et al. 2001). The forkhead family of transcription factors includes 180 members in various species, these transcription factor bind DNA through a highly conserved 100-aminoacid forkhead motif (Kaestner et al. 1993). FOXC2 gene is implicated in both lymphatic and vascular development (Kume et al. 2001 and Kriedermam et al. 2003). In animal models FOXC2 gene is expressed in developing mesenchyme cells that later develop into connective tissue, blood vessels, and lymphatic vessels (Miura et al. 1993 and Wilm et al. 2004). At a later stage of development, FOXC2 gene is expressed on both the endothelial and smooth muscle cells of developing blood vessels (Kume et al. 2001) and on the venous and lymphatic valve leaflets (Petrova et al. 2004). The FOXC2 gene provides instructions for making a protein that plays a critical role in the formation of many organs and tissues before birth. This protein is a transcription factor, which means that it attaches (binds) to specific regions of DNA and helps control the activity of many other genes. Researchers believe that the FOXC2 protein has a role in a variety of developmental processes, such as the formation of veins and the development of the lungs, eyes, kidneys and urinary tract, cardiovascular system, and the transport system for immune cells (lymphatic vessels). More than 50 mutations in the FOXC2 gene can cause lymphedema-distichiasis syndrome and varicose veins, about 50% of individuals with Lymphedema-distichiasis syndrome have varicose veins (Brice et al. 2002). Most of these mutations are insert or delete a few DNA building blocks (nucleotides), which
results in a premature stop signal in the instructions for making the FOXC2 protein. These mutations lead to the production of a FOXC2 protein that is abnormally small and cannot effectively attach (bind) to DNA. As a result, the altered protein cannot regulate the activity of other genes. Other mutations change one protein building block (amino acid) in the area of the FOXC2 protein that binds to DNA, preventing the protein from regulating gene activity.

In a study for genetic variation among varicose veins patients, 655bp of the single exon of FOXC2 gene as well as an additional 413 bp of upstream UTR was sequenced. Three SNPs were identified in proximal region, 91 C→G, -41 G→A and -41 G→T (Khalid et al. 2008). Seven other novel mutations were found in the FOXC2 gene in varicose veins patients, of which five were missense and two were frameshift mutations. These mutations alter the transcriptional activity of FOXC2 gene (Steensel et al. 2009).

Cytogenetic investigation of primary cell cultures of varicose veins of with familial varicosity and patients with the sporadic type revealed the presence of metaphases with structural abnormalities, clonal trisomies of chromosomes 7, 12, and 18, and monosomy of chromosome 14 only in cases with the familial type, while the sporadic cases had no similar chromosome aberrations. The immunophenotypical results are consistent with fibroblast lineage of the cultured cells. These results suggest that karyotypic variations in familial varicose vein tissue cultures could in some way be associated either with the genotypic constitution responsible for the familial type or a longer duration of disease on average than those with sporadic varicosities (Susi et al. 1994).

Recognition of the dilated veins is usually made by physical examination. The lack of accuracy of diagnosis based only on visualization and palpation is obvious and many small lesions may escape detection. Color Duplex ultrasound is the best technique available for the noninvasive examination of venous function (Baker et al. 1993). The wearing of graduated compression stocking with a pressure of 30-40 mmHg has been shown to correct the swelling, nutritional exchange, and improve the microcirculation in legs affected by varicose veins (Curri 1989). The symptoms of varicose veins can be controlled to an extent with the treatment and exercise. Advices
about regular exercise sound sensible but are neither supported by any evidence (Campbell 2006). Active medical intervention in varicose veins can be divided into surgical and non-surgical treatments.

For a family the diagnosis marks the end of uncertainty about the cause of patient’s difficulties. The family studies may be initiated which bring forward the diagnosis of some affected relative.

The current consensus is that both environmental and genetic factors are associated with the development of varicose veins (Ng et al. 2005, White and Ryjewski 2005, Raffetto and Kahlil 2008). Knowledge of the diagnosis can direct the patient to appropriate information and to aware the people.

**NEED OF THE STUDY:**

India consists of various populations with different types of occupations. The list of occupational hazards just seems to be increasing. People are expected to respond to a variety of situations that may arise while they are on duty. Varicose veins have become a serious threat to the lives of millions of people across the globe and is said to be ignored by people living across India. There is an urgent need to spread awareness about varicose veins in India. Many people suffer from it, but most tend to ignore it and that is not good as it can lead to complications in the advanced stage. Though there is high prevalence of varicose veins in India, but very few studies have been conducted in India. Researcher personally has observed that most of the people were unaware of varicose veins, and also their preventive measures. Hence, there is an urgent need to conduct a study to assess the effectiveness of a self instructional module on knowledge regarding varicose vein among all the people. Over time emerging technologies are likely to change the evaluation of patients in future. It is likely that in the next several years, whole genome sequencing will become a cost effective method of identifying genetic causes and conditions associated with unexplained reason of varicose veins. Mutation in FOXC2 gene is strongly associated with venous reflux varicose patients and studies are required to determine whether FOXC2 abnormalities play a role in the development of varicose veins in the general population. Thus identification of cause would be important in planning for the
medical, educational and treatment need of a particular individual. Hence present study has been conducted with the following objectives:

1. To find the prevalence of Varicose Veins in Haryana population.
2. Dermatoglyphics analysis of patients having Varicose Veins syndrome.
3. Cytogenetic analysis to find out karyotypic abnormalities of Varicose Veins patients.
4. Molecular cytogenetic analysis using Polymerase Chain Reaction, to find out mutation in Varicose Veins patients.
5. Parental screening to identify predisposing factors.