CHAPTER- 4
RESULTS

In the present study of “Dermatoglyphics and molecular cytogenetic characterization of varicose veins” 216 patients of varicose veins were studied from Pandit Bhagwat Dayal Sharma University of Health Sciences, Rohtak, Haryana. A questionnaire for history, types of varicose veins (Bulging, Rope like, Twisted and change to Ulcer), occupation of patients, life style and nutrition was developed and got filled with the help of patients and information was compiled. These patients were further evaluated for age of diagnosis, sex ratio, clinical features and molecular cytogenetic detection of FOXC2 mutation. Results were statistically analysed to know the role various risk factors and to findout the etiopathogenesis of varicose veins.

4.1. IDENTIFICATION OF PATIENTS OF VARICOSE VEINS:

Two hundred sixteen patients of varicose veins were studied during Nov 2010 to Sept 2013. Patients of varicose veins were identified with the help of clinician and selected for further analysis.

4.2. AGE OF DIAGNOSIS

All the 216 cases of varicose veins were confirmed by clinicians after their Doppler examination. Information as per requirement of questionnaire was collected. Age of diagnosis of varicose veins cases was noted and analysed. Less than 1% cases were diagnosed before the age of 10 years and 03.70 % cases could be diagnosed from eleven to twenty years of age. From twenty one to thirty years of age 04.16% cases of varicose veins patients were detected. There were 24.53% cases who showed varicose veins between the age of thirty one to forty. Seventeen point twelve percent of patients were diagnosed between forty one to fifty years of age. Thirty patients were present between age of fifty one to sixty. There were only 8 cases that showed varicose veins after age of sixty years (Figure- 11). The average age of patient’s diagnosis was the 21 to 30 years, the youngest patient was 09 years old and the oldest was 73 years old.
4.3 SEX RATIO

In the present study male to female ratio in the patients of varicose veins showed variation (Table- 7). There were 127 males and 89 female patients of varicose veins (Figure- 12). Out of 89 females there were 24 female who have diagnosed varicose veins at the time of pregnancy. The percentage frequency of males was approximately double as compared females (Figure- 13). However about equal ratio of males and females was found in age group of 51 to 60 years. Maximum difference was found between male and female patients of varicose veins in the age groups of 21-30 and 31-40 years.

The female patients mainly sought treatment for symptoms and complications rather than for cosmetic reason. The low incidence of varicose veins in female patients in the age group 21 to 40 years was reported in the present study. It may be due to less cosmetic concern in our Indian middle and lower class women. ANOVA on the sample population of varicose veins in different age groups and gender was applied and the interaction of age and gender as factors was studied. Statistical calculation revealed highly significant values for male and female ratio (CI = 95%, df =1 p= 0.0001). Whereas age alone as a factor was found less significant (CI = 95%, df = 4, p= 0.0002). The interaction of gender and age revealed significant values at p<0.0024 (CI = 95%, df = 4) (Table- 8).

Figure 11: % Frequency of varicose veins patients in different age groups.
Table 7: Percentage frequency of Male and Female patients of varicose veins and different age groups.

<table>
<thead>
<tr>
<th>AGE GROUPS</th>
<th>MALES (N)</th>
<th>FEMALES (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>01</td>
<td>00</td>
</tr>
<tr>
<td>11-20</td>
<td>07</td>
<td>02</td>
</tr>
<tr>
<td>21-30</td>
<td>48</td>
<td>30</td>
</tr>
<tr>
<td>31-40</td>
<td>34</td>
<td>19</td>
</tr>
<tr>
<td>41-50</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>51-60</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>&gt;60</td>
<td>05</td>
<td>03</td>
</tr>
<tr>
<td>Mean</td>
<td>18.1</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Table 8: ANOVA between age groups, gender and their interaction in patients of varicose veins.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENDER</td>
<td>6</td>
<td>11.412226</td>
<td>0.0001***</td>
</tr>
<tr>
<td>AGE</td>
<td>1</td>
<td>4.6930</td>
<td>0.0002**</td>
</tr>
<tr>
<td>INTERACTION</td>
<td>6</td>
<td>8.99</td>
<td>0.0024*</td>
</tr>
</tbody>
</table>

(* denotes level of significance at 95% CI)
Figure 12: Sex ratio in patients of varicose veins.

Figure 13: Sex ratio of Male and Female patients of varicose veins and different age groups.
4.4 CLINICAL PRESENTATION OF VARICOSE VEINS

Varicose veins are dilated, tortuous and elongated veins of the lower extremities and classified into four types i.e. saphenous veins, segmented veins, reticular veins and web type veins. There were two hundred sixteen cases of varicose veins. Saphenous type of vein is longest vein in body running along the length of leg and was present in 82% of cases. Thirty eight point two percent patients were having segment type of veins. These veins are the small dilated blue and green veins beneath the skin surface. Reticular veins do not protrude above the skin as varicose veins do. Percentage frequency of reticular type vein was 28.16%. Webbed types of veins were present only in 14% of patients (Figure- 14).

The distribution pattern of varicose veins found of the lower limbs of women and men showed more percentage frequency in the right limb (Table- 9). The prevalence of saphenous varicose veins was 87.9% in men and 76.4% in women. By contrast, a 2-fold higher prevalence of great-saphenous varicose veins was found in left, right and bilateral limb. Comparatively higher frequency of great-saphenous veins was noted in women (Table- 9).

![Pie chart showing percentage frequency of different types of varicose veins.](image)

**Figure 14:** Percentage frequency of different types of varicose veins.
Table 9: Distribution of varicose veins among sex and with respect to limb involvement.

<table>
<thead>
<tr>
<th>Prevalence of varicose veins</th>
<th>Men (n=127)</th>
<th>Women (n=89)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right leg (%)</td>
<td>Left leg (%)</td>
</tr>
<tr>
<td>Great saphenous veins</td>
<td>10.6</td>
<td>4.0</td>
</tr>
<tr>
<td>Small saphenous veins</td>
<td>4.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Non-saphenous vein only</td>
<td>5.2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

4.4.1 CLINICAL SYMPTOMS OF VARICOSE VEINS

Major symptoms in patients of varicose veins were prominent veins, aching, swollen ankles, itching, night cramps and ulcer (Figure- 15-a, b, c, d, e and f). There were 95.08% of the patients with prominent veins. The next most common symptom was aching in leg (85.24%) with prominent veins which occurred alone or in combination with swollen ankle or edemas (37.70%). There were 31.53 % patients with ulcer in one or both limbs. 68.70% of varicose patients complained of night cramps in affected veins (Figure- 16). Frequency distribution of varicose symptoms among male and females showed that, male patients were more affected with all these symptoms. Chi square analysis revealed significant difference between frequency of symptoms among male and female patients ($\chi^2 = 16.221^*$, df= 6, p value= 0.01) (Table-10).
Figure 15: Varicose veins presentation with different type of symptoms:

a) Swollen ankle  

b) Varicose eczema  

c) Dilated Varicose Veins  

d) Prominent varicose veins  

e) Ulcer with varicose veins  

f) Open venous ulcer
Figure 16: Percentage frequency of clinical symptoms in varicose veins patients.

Table 10: Frequency of clinical symptoms in male and female.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>124</td>
<td>126</td>
<td>111</td>
<td>63</td>
<td>108</td>
<td>106</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>60.4</td>
<td>68.4</td>
<td>71.1</td>
<td>72.4</td>
<td>72.9</td>
<td>77.9</td>
<td>70.5</td>
</tr>
<tr>
<td>Female</td>
<td>81</td>
<td>58</td>
<td>45</td>
<td>24</td>
<td>40</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>39.6</td>
<td>31.6</td>
<td>28.9</td>
<td>27.6</td>
<td>27.1</td>
<td>22.1</td>
<td>29.5</td>
</tr>
<tr>
<td>Total</td>
<td>205</td>
<td>184</td>
<td>156</td>
<td>87</td>
<td>148</td>
<td>136</td>
<td>68</td>
</tr>
</tbody>
</table>

\( \chi^2 = 16.221^*, \text{df}= 6, p \text{ value}= 0.01 \)

4.4.2 MANIFESTATION OF CLINICAL SYMPTOMS AND PROFESSION

Male and female patients were divided in three groups on the basis of their profession and working. Group I consisted of office workers or those who do less work or live in same position for long time; group II consisted of light physical laborers mainly including arm and leg movement, no weight lifting and no whole body movements; group III consisted of heavier physical laborers including walking, whole body movement and heavy load handling. Male patients showed significant association between symptoms and professions (\( \chi^2 = 22.494^*, \text{df}= 12, p \text{ value}= 0.03 \))
Male patients in group I were less affected with all clinical symptoms as compared to group II whereas Group III male patients manifested maximum frequency of clinical symptoms. But in case of females no significant association was found between working groups ($\chi^2=16.375$, df= 12, p value= 0.17) (Table- 11). Leg ulcer as complicated manifestation of varicose veins was more frequent in patients of heavy load workers in both sexes (In male 79.3% and in female 65%) (Table- 11).

**Table 11: Frequency of clinical symptoms in the three groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Prominent veins No.</th>
<th>Prominent veins %</th>
<th>Aching No.</th>
<th>Aching %</th>
<th>Itching No.</th>
<th>Itching %</th>
<th>Swollen Ankles No.</th>
<th>Swollen Ankles %</th>
<th>Nights Cramps No.</th>
<th>Nights Cramps %</th>
<th>Varicose Eczema No.</th>
<th>Varicose Eczema %</th>
<th>Ulcer No.</th>
<th>Ulcer %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G I</td>
<td>13</td>
<td>13.7</td>
<td>13</td>
<td>10.3</td>
<td>20</td>
<td>18.0</td>
<td>09</td>
<td>14.2</td>
<td>17</td>
<td>26.9</td>
<td>17</td>
<td>15.7</td>
<td>13</td>
<td>12.2</td>
</tr>
<tr>
<td>G II</td>
<td>36</td>
<td>29.0</td>
<td>29</td>
<td>23.0</td>
<td>36</td>
<td>32.4</td>
<td>17</td>
<td>26.9</td>
<td>37</td>
<td>34.2</td>
<td>33</td>
<td>31.1</td>
<td>19</td>
<td>18.7</td>
</tr>
<tr>
<td>G III</td>
<td>75</td>
<td>60.4</td>
<td>84</td>
<td>66.7</td>
<td>55</td>
<td>49.6</td>
<td>37</td>
<td>49.6</td>
<td>54</td>
<td>50.1</td>
<td>60</td>
<td>50.7</td>
<td>38</td>
<td>79.3</td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>126</td>
<td>111</td>
<td>63</td>
<td>108</td>
<td>106</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G I</td>
<td>07</td>
<td>8.7</td>
<td>10</td>
<td>17.2</td>
<td>3</td>
<td>6.6</td>
<td>3</td>
<td>12.5</td>
<td>10</td>
<td>25.0</td>
<td>4</td>
<td>13.3</td>
<td>1</td>
<td>5.0</td>
</tr>
<tr>
<td>G II</td>
<td>28</td>
<td>34.5</td>
<td>16</td>
<td>27.5</td>
<td>11</td>
<td>24.4</td>
<td>7</td>
<td>29.1</td>
<td>12</td>
<td>30.0</td>
<td>14</td>
<td>46.6</td>
<td>6</td>
<td>30.0</td>
</tr>
<tr>
<td>G III</td>
<td>46</td>
<td>56.8</td>
<td>32</td>
<td>55.3</td>
<td>31</td>
<td>69.0</td>
<td>14</td>
<td>58.4</td>
<td>18</td>
<td>45.0</td>
<td>12</td>
<td>40.1</td>
<td>13</td>
<td>65.0</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>58</td>
<td>45</td>
<td>24</td>
<td>40</td>
<td>30</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $\chi^2= 22.494$, df= 12, p value= 0.03  ** $\chi^2=16.375$, df= 12, p value= 0.17

G I= Office workers
G II= Light physical laborers
G III= Heavier physical laborers

**4.4.3 ASSOCIATED COMPLICATIONS OF VARICOSE VEINS**

Majority of the patients considered in the present study reported to the hospital for some complications of the disease rather than for the treatment of the visible veins itself. Total complication rate observed during the study period was 42%. Leg ulceration, hematoma and deep vein thrombosis (DVT) were commonest associated complications with 31.53%, 29.30% and 27.77% of cases respectively. Hematoma along with DVT were found simultaneously in 24.46% of cases. There were 16% of patients that showed recurrence of varicosity after surgery. Bleeding from superficial
veins was noticed in 10.3% of varicose patients. Only 2.60% of patients were seen with femoral vein injury as associated complication of varicose veins (Figure-17).

![Complications Percentage Frequency](image)

**Figure 17: Associated complication in varicose veins patients**

### 4.4.4 LIMB INVOLVEMENT

The present study showed slightly increased incidence of varicosity on the left limb (63.93%) (Figure-18). The probable reason for increased incidence on left side is that the venous drainage of the left leg follows a more tortuous course through the pelvis, with left common ileac vein traversed by the right common ileac artery. The bilateral varicose veins were seen in 32% of patients. There were 45.3% men with clinical varicose veins who had symptoms in both legs, 33.8% and in the left and 20.9% in the right leg. Women had bilateral symptoms in 14.6%. 51.6% in left leg and 33.8% in the right leg. Frequency of bilateral leg was very low in case of women as compared to men. Fisher’s exact analysis showed the two tailed P value is less than 0.00001 and revealed extremely statistically significant association between both sex and limb involvement (Table-12).
Table 12: Limb involvement of varicose veins patients among both sexes.

<table>
<thead>
<tr>
<th>Limb involved</th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Right</td>
<td>42</td>
<td>(33.8)</td>
<td>46</td>
<td>(51.6)</td>
</tr>
<tr>
<td>Left</td>
<td>20</td>
<td>(20.9)</td>
<td>30</td>
<td>(33.8)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>56</td>
<td>(45.3)</td>
<td>13</td>
<td>(14.6)</td>
</tr>
</tbody>
</table>

(Fisher’s exact, two tailed, *p< 0.00001)

4.5 RURAL AND URBAN POPULATION

Analysis of the patients of varicose veins revealed that more patients belong to rural background. The urban population was 37% whereas rural population was 63% (Figure- 19). Patients of varicose veins of different age groups and gender from urban and rural areas found that in all age groups (Figure- 20) frequency of rural males was comparatively high. Two way analysis of variation between various age groups and urban/rural population of varicose veins was carried out. The interaction of age and urban/rural revealed highly significant values (p<0.001***, CI = 95%) (Table-13).
Figure 19: Percentage frequency of patients of varicose veins in rural and urban areas of Haryana.

Figure 20: Patients of varicose veins of different age groups and gender from Urban and Rural areas.

RF-Rural Female, UF- Urban Female, RM-Rural Male, UM- Urban Male
Table 13: ANOVA between different age groups, Urban –Rural population and their interaction in patients of varicose veins.

<table>
<thead>
<tr>
<th>AGE groups</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>1361.579</td>
<td>16.118</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R/U</td>
<td>1</td>
<td>1950.914</td>
<td>23.095</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>208</td>
<td>84.472</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor 1</td>
<td>6</td>
<td>16.118</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Factor 2</td>
<td>1</td>
<td>23.095</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(* denotes level of significance at 95% CI) (p<0.001)**

4.6 RISK FACTORS

To know the etiology of varicose veins, information about various risk factors was collected and subjected to statistical analysis. Major risk factors like occupation, working groups, smoking, number of pregnancies (in female patients) and obesity were considered and correlated.

4.6.1 OCCUPATION

Out of 216 patients, occupation of 196 patients was either prolonged periods of standing, sitting or violent muscular efforts or both. Sixty one point one percent patients have reported standing position at their work. Farmers working mostly as heavy weight lifting showed 19% frequency of varicose veins. Fruit sellers/Book sellers with varicose veins were 11.60%. Percentage frequency of electricians was 10.18 and followed by laborers and machine turners with 9.72 and 9.25 respectively.
The occupation of these patients with respect to the area of work showed that farmers (70.31%), laborers (70.00%) and fruit sellers (69.23%) with varicose veins were more common among rural patients. Electricians (63.6%) and machine turners (66.6%) with varicose veins belonged to urban area (Table-15).

To estimate the strength of the association between prevalence of various risk factors in varicose veins adjusted odds ratios (ORs) at 95% confidence intervals (CIs) were computed. Each risk factor was compared with all possible combinations to estimate the most strong and least associated factors. Odds ratio within the risk factors revealed the relative association of each factor to varicose veins. An odds ratio greater than 1 indicates that varicose veins is more likely to occur in the numerator risk factor. Odds ratio less than 1 indicates that the condition or event is less likely to occur in the numerator risk factor and more in denominator risk factor.

The odds ratio analysis showed that the standing posture at work was highly significant risk factor as compared to those who primarily sit in their workplace (OR= 3.1358; 95% CI=2.1167 to 4.6456) and also in those who generally work indoor than outdoor workers (OR= 1.8166; 95% CI= 1.2406 to 2.6602). Frequency of patients who work in high temperature for long time was found high as comparison to those who worked in low temperature (OR= 2.115; 95% CI= 1.4413 to 3.1057) (Table-16).

Odds ratio analysis revealed that standing posture at work as a risk factor was more as compared to outdoor workers (OR=2.205, 95% CI=1.4987 to 3.230), indoor workers (OR= 1.212, 95% CI= 0.825 to 1.7819), high temperature at work (OR= 2.3769, 95% CI= 1.615 to 3.497) and low temperature at work (OR= 1.1235, 95% CI= 0.7635 to 1.6531). The analysis of the odds ratio highlighted the role of indoor workers and low temperature at work to be more in comparison to sitting posture during work (OR= 0.3866, 95% CI= 0.2619 to 0.5708 and OR= 0.3583, 95% CI= 0.2424 to 0.5295 respectively) and less in case of outdoor workers and high temperature at work (OR= 0.7024, 95% CI= 0.4758 to 1.0369 and OR= 0.7580, 95% CI= 0.5129 to 1.1203 respectively). Low temperature at work (OR= 0.5101, 95% CI= 0.3479 to 0.7478) were established as a prominent risk factor as compared to outdoor workers as well as compared to indoor workers (OR= 0.9266, 95% CI= 0.632 to 1.358) (Table-17).
Table 14: Percentage frequency of varicose veins patients with different occupation.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Occupation</th>
<th>% Frequency (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Farmer</td>
<td>19.00 (41)</td>
</tr>
<tr>
<td>2</td>
<td>Fruit/ Book Seller</td>
<td>11.60 (25)</td>
</tr>
<tr>
<td>3</td>
<td>Electrician</td>
<td>10.18 (22)</td>
</tr>
<tr>
<td>4</td>
<td>Machine turner</td>
<td>9.72 (21)</td>
</tr>
<tr>
<td>5</td>
<td>Labor</td>
<td>9.25 (20)</td>
</tr>
<tr>
<td>6</td>
<td>Factory worker</td>
<td>7.40 (16)</td>
</tr>
<tr>
<td>7</td>
<td>House constructor</td>
<td>6.48 (14)</td>
</tr>
<tr>
<td>8</td>
<td>House wife</td>
<td>4.62 (10)</td>
</tr>
<tr>
<td>9</td>
<td>House servant (Sweeper, Cook)</td>
<td>4.62 (10)</td>
</tr>
<tr>
<td>10</td>
<td>Tailoring</td>
<td>4.62 (10)</td>
</tr>
<tr>
<td>11</td>
<td>Student</td>
<td>2.77 (06)</td>
</tr>
<tr>
<td>12</td>
<td>Government service (Police man, Bus conductor, School teacher)</td>
<td>2.77 (06)</td>
</tr>
<tr>
<td>13</td>
<td>Sportsman</td>
<td>3.70 (08)</td>
</tr>
<tr>
<td>14</td>
<td>Barber</td>
<td>3.24 (07)</td>
</tr>
</tbody>
</table>
### Table 15: Occupation distribution in urban and rural population of varicose patients.

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Percentage frequency % (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urban</td>
</tr>
<tr>
<td>Farmer</td>
<td>29.69 (12)</td>
</tr>
<tr>
<td>Labor</td>
<td>30.00 (06)</td>
</tr>
<tr>
<td>Electrician</td>
<td>63.63 (14)</td>
</tr>
<tr>
<td>Machine turner</td>
<td>66.66 (07)</td>
</tr>
<tr>
<td>Fruit seller</td>
<td>30.77 (08)</td>
</tr>
</tbody>
</table>

### Table 16: Comparison of varicose veins prevalence in relation to the working posture, location and temperature at work place.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Risk factors</th>
<th>Categories</th>
<th>Present</th>
<th>Absent</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Working Posture</td>
<td>Standing</td>
<td>134</td>
<td>82</td>
<td>3.1358 (2.1167 to 4.6456)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sitting</td>
<td>74</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Working Location</td>
<td>Indoor</td>
<td>124</td>
<td>92</td>
<td>1.8166 (1.2406 to 2.6602)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outdoor</td>
<td>92</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Temperature at workplace</td>
<td>High</td>
<td>128</td>
<td>88</td>
<td>2.115 (1.4413 to 3.1057)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low Temperature</td>
<td>88</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>S.No.</td>
<td>Risk factors</td>
<td>PRESENT</td>
<td>ABSENT</td>
<td>A+C</td>
<td>B+D</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------</td>
<td>---------</td>
<td>--------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>1</td>
<td>Standing Posture***</td>
<td>134</td>
<td>82</td>
<td>226</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>Outdoor workers</td>
<td>92</td>
<td>124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Standing Posture**</td>
<td>134</td>
<td>82</td>
<td>258</td>
<td>174</td>
</tr>
<tr>
<td></td>
<td>Indoor workers</td>
<td>124</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Standing Posture***</td>
<td>134</td>
<td>82</td>
<td>222</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>High temperature workers</td>
<td>88</td>
<td>128</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Standing Posture**</td>
<td>134</td>
<td>82</td>
<td>262</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>Low temperature workers</td>
<td>128</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Sitting posture</td>
<td>74</td>
<td>142</td>
<td>166</td>
<td>266</td>
</tr>
<tr>
<td></td>
<td>Outdoor workers**</td>
<td>92</td>
<td>124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Sitting posture</td>
<td>74</td>
<td>142</td>
<td>198</td>
<td>232</td>
</tr>
<tr>
<td></td>
<td>Indoor workers***</td>
<td>124</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Sitting posture</td>
<td>74</td>
<td>142</td>
<td>162</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>High temperature workers**</td>
<td>88</td>
<td>128</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Sitting posture</td>
<td>74</td>
<td>142</td>
<td>202</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>Low temperature workers***</td>
<td>128</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Outdoor workers</td>
<td>92</td>
<td>124</td>
<td>180</td>
<td>252</td>
</tr>
<tr>
<td></td>
<td>High temperature* workers</td>
<td>88</td>
<td>128</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Outdoor workers</td>
<td>92</td>
<td>124</td>
<td>220</td>
<td>212</td>
</tr>
<tr>
<td></td>
<td>Low temperature** workers</td>
<td>128</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Indoor workers</td>
<td>124</td>
<td>92</td>
<td>212</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td>High temperature workers**</td>
<td>88</td>
<td>128</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Indoor workers</td>
<td>124</td>
<td>92</td>
<td>252</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Low temperature workers*</td>
<td>128</td>
<td>88</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(* Indicate less significant difference in the ratio)
(** Moderately significant difference in the ratio)
(*** Highly significant)

(An odds ratio greater than 1 indicates that the condition or event is more likely to occur in the first group. And an odds ratio less than 1 indicates that the condition or event is less likely to occur in the first group.)
4.6.2 WORKING GROUP

The patients of varicose veins were divided into three groups on the basis of types of work. Group I consisted of office workers or those who do less work or live in same position for long time; group II consisted of light physical laborers mainly including arm and leg movement, no weight lifting and no whole body movements; group III consisted of heavier physical laborers including walking, whole body movement and heavy load handling. Out of total workers, group III workers were most affected 56.48%, followed by group II with 30.98% and Group I worker with 13.27% varicose veins (Table- 18).

Table 18: Percentage frequency of patients of varicose veins in different working groups.

<table>
<thead>
<tr>
<th>Groups of patients (G)</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>G I - Official work</td>
<td>28</td>
<td>13.27</td>
</tr>
<tr>
<td>G II - Light physical laborers</td>
<td>66</td>
<td>30.98</td>
</tr>
<tr>
<td>G III - Heavier physical laborers</td>
<td>122</td>
<td>56.48</td>
</tr>
</tbody>
</table>

4.6.2.1 Sex ratio and working groups

Percentage frequency of varicose veins was significantly higher in men in two professional groups i.e. in group II (60.60%) and group III (63.93%) whereas in group I frequency of females was high i.e. 77.86% (Figure- 21).

Figure 21: The frequency of varicose veins in comparison to the age groups and sex.
Among all three groups of profession heavy physical labors were having maximum number of varicose veins. Odds ratio analysis revealed that group I was less likely as risk factor than group II (OR= 0.3385; 95% CI= 0.2071 to 0.5513). Whereas group III was found to have more risk factors as compared to group II (OR= 2.9497; 95% CI= 1.9868 to 4.3793) as well as Group I (OR= 0.1148; 95% CI= 0.0711 to 0.1854) (Table 19).

Table 19: Odds ratio in different professional groups.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Categories</th>
<th>Present</th>
<th>Absent</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Profession</td>
<td>Group I</td>
<td>28</td>
<td>188</td>
<td>0.3385</td>
<td>0.2071 to 0.5513</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>66</td>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group I</td>
<td>28</td>
<td>188</td>
<td>0.1148</td>
<td>0.0711 to 0.1854</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>122</td>
<td>94</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>122</td>
<td>94</td>
<td>2.9497***</td>
<td>1.9868 to 4.7393</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>66</td>
<td>150</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(* Indicate less significant difference in the ratio)
(** Moderately significant difference in the ratio)
(***) Highly significant

(An odds ratio greater than 1 indicates that the condition or event is more likely to occur in the first group. And an odds ratio less than 1 indicates that the condition or event is less likely to occur in the first group.)

Chi Square analysis revealed significant association between working groups and sex ratio in varicose veins patients ($\chi^2 = 9.629$, df= 2, p value= 0.0081), which conclude that Work involving heavy lifting was significantly related to the presence of varicose veins both for men and women (Table 20).

Table 20: Association between sex ratio and working groups of varicose veins patients.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>9</td>
<td>40</td>
<td>78</td>
<td>127</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>26</td>
<td>44</td>
<td>89</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>66</td>
<td>122</td>
<td>216</td>
</tr>
</tbody>
</table>

* $\chi^2 = 9.629$, df= 2, p value= 0.0081
4.6.3 NUMBER OF PREGNANCIES/ PARITY

Another important risk factor in the development of varicose disease in women is pregnancy. Present study has observed a higher prevalence of varicose disease in female patients who had more than two pregnancy in comparison to nulliparae i.e 50.56% (Figure- 22).

Out of 89 women with varicose veins disease, there were 14 (16.27%) nullipare, 13 (14.60%) primipare, 17 (19.10%) secundipare, and rest 45 (50.56%) had three or more pregnancies. According to number of pregnancies the prevalence of varicose veins in men and women was classified in two different orders i.e between nulliparae and men the proportion was 1:9, whereas between multiparae and men, it was 1:1.7. It was observed that risk of development of varicose veins was six times more in a woman who has given birth twice (primipare) or more as compared to nullipare. Chi square analysis, revealed highly significant values when parity was compared with occurrence of varicose veins (Table- 21).

![Figure 22- Women over the age of 20 years with varicose veins and number of pregnancies.](image-url)
Table 21: Relationship between Parity and working women (different professional groups).

<table>
<thead>
<tr>
<th>Working Groups (Women)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>G I</td>
<td>(14)</td>
<td>15.73</td>
<td>(2)</td>
<td>02.24</td>
<td>(3)</td>
<td>03.37</td>
</tr>
<tr>
<td>G II</td>
<td>(0)</td>
<td>00</td>
<td>(5)</td>
<td>05.61</td>
<td>(5)</td>
<td>05.61</td>
</tr>
<tr>
<td>G III</td>
<td>(0)</td>
<td>00</td>
<td>(6)</td>
<td>10.11</td>
<td>(9)</td>
<td>10.11</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>13</td>
<td>17</td>
<td>20</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

χ²=64.6*, d.f (Degree of Freedom) =8, p<0.0001

G I= Office workers, G II= Light physical laborers and G III= Heavier physical laborers

4.6.4 BODY MASS INDEX

In the present study it was observed that obese people or people with overweight suffered from various manifestations of varicose veins much more frequently. The BMI was calculated from specified weight and height, reached the more than average value of 30.03 ± 4.82 (Table-22 and Figure-23). A standard BMI value up to 24.8 is set for the standard population. In the present study the incidence of varicose veins was higher among both sexes who were obese than those who were of normal weight. In patients the average height was 172.87 ± 18.6 cm (limits, 140 to 192 cm), weight was 87.83 ± 17.98 kg (limits, 35 to 115 kg), body mass index averaged 30.03 ± 4.82 kg/m² (limits, 16.3 to 38.4 kg/cm²). In normal population the average height was 161.45 ± 9.48 cm, weight was 67.68 ± 12.29 kg, body mass index was 19.63 ± 1.63 kg/cm². Increased height as alone was not statistically significant in patients versus the normal persons (P-0.176, Student unpaired t test). However weight and total BMI showed significant values when analysed statistically (P-0.0001 Student unpaired t test) (Table-23). This indicated that the presence of obesity and total body mass index had a definite impact on occurrence of varicose veins.
Table 22: Weight, height, and BMI of patients of varicose veins.

<table>
<thead>
<tr>
<th>Characteristics of the patient</th>
<th>N</th>
<th>Average ± S.d.</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>216</td>
<td>87.83 ± 17.98</td>
<td>35</td>
<td>115</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>205</td>
<td>172.87 ± 18.6</td>
<td>140</td>
<td>192</td>
</tr>
<tr>
<td>BMI</td>
<td>199</td>
<td>30.03 ± 4.82</td>
<td>16.3</td>
<td>34.4</td>
</tr>
</tbody>
</table>

Figure 23: Average BMI with respect to weight and height in patients of varicose veins.
Table 23: t test analysis between weight, height, and BMI of varicose veins patients and normal individuals.

<table>
<thead>
<tr>
<th>Characteristics of the patient</th>
<th>Reference Group (Normal individuals) (Max N-76) (Mean ± SD)</th>
<th>Patients Group (Max N-180) (Mean ± SD)</th>
<th>P (Student Unpaired t Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>67.68 ± 12.29</td>
<td>87.83 ± 17.98</td>
<td><strong>0.0001</strong></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.45 ± 9.48</td>
<td>208.87 ± 18.6</td>
<td>0.176</td>
</tr>
<tr>
<td>BMI</td>
<td>19.63 ± 1.63</td>
<td>30.03 ± 4.82</td>
<td><strong>0.0001</strong></td>
</tr>
</tbody>
</table>

Max indicates maximum. Values are mean ± SD

** shows the highly significant value (p= 0.0001)

4.6.5 SMOKING AS RISK FACTOR

In present study patients were defined as smokers if they had ever smoked cigarettes, cigar or pipes for longer than one year and those who smoked more than ten cigarettes per day including also as ex-smokers. The prevalence of smokers was 51.5% in out of 127 male patients whereas in this study no female was found who had ever smoked. There were 7.2% of males found with varicose veins who smoked as ex-smokers (Figure- 24). Odds ratio analysis revealed smoking as significant risk factor in comparison to non smokers and ex-smokers with (OR= 1.5121, 95% CI= 0.9207-2.4834) and (OR= 6.1738, 95% CI= 2.8086- 13.571) respectively (Table- 24).

Table 24: Odds ratio comparison between smokers.

<table>
<thead>
<tr>
<th>Categories of smoking</th>
<th>Present</th>
<th>Absent</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>65</td>
<td>62</td>
<td><strong>1.5121</strong></td>
<td>0.9207- 2.4834</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>52</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>65</td>
<td>62</td>
<td><strong>6.1738</strong></td>
<td>2.8086- 13.571</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>09</td>
<td>53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.6.6 CORRELATION AMONG VARIOUS RISK FACTORS

Separate evaluations of risk factors were made for women and men separately as many risk factors for the venous system are sex related. For multivariate evaluation of risk factors, logistic regression enforcing every factor of interest was used to ensure maximal adjustment for potentially confounding variables. Risk factors for varicose veins are summarized in (Table-24, A and B). Prolonged standing and a history of varicose veins in first-degree relatives were the most important risk factors in both sexes (p < 0.0001 (men), p < 0.0004 (women) and p <0.001 (recorded from case history)). In women, height, body mass index and number of pregnancies were positively associated with the presence of varicose veins (p= 0.006, 0.007 and < 0.001 respectively). Lack of sufficient routine exercise (p= 0.77) and long time sitting p= 0.062) were associated with very low risk of varicose veins (Table- 25A). In men, height and body mass index were shown high positively association with the presence of varicose veins (p < 0.0001) as compared to normal persons. Lack of exercise, prolonged sitting and smoking (p= 0.01, 0.02 and 0.03 respectively) were also associated with varicose veins (Table- 25B).
Table 25: Distribution of risk factors of varicose veins among men and women.

<table>
<thead>
<tr>
<th>Risk factors in women</th>
<th>Distribution</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ (n=89)</td>
<td>- (n=62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 1 Pregnancies</td>
<td>69.6</td>
<td>43.5</td>
<td>2.9767</td>
<td>1.514 to 5.892</td>
</tr>
<tr>
<td>Prolonged standing</td>
<td>57.3</td>
<td>27.4</td>
<td>3.5526</td>
<td>1.670 to 7.142</td>
</tr>
<tr>
<td>Prolonged sitting</td>
<td>34.8</td>
<td>37.0</td>
<td>0.9063</td>
<td>0.461 to 1.780</td>
</tr>
<tr>
<td>Exercise less than once a week</td>
<td>26.9</td>
<td>30.6</td>
<td>0.8356</td>
<td>0.408 to 1.707</td>
</tr>
<tr>
<td>Height &gt; 1.65 m</td>
<td>48.3</td>
<td>25.8</td>
<td>2.6875</td>
<td>1.328 to 5.436</td>
</tr>
<tr>
<td>Body mass index &gt; 23 kg/m²</td>
<td>68.5</td>
<td>46.7</td>
<td>2.4791</td>
<td>1.268 to 4.890</td>
</tr>
<tr>
<td>History of venous disease</td>
<td>57.3</td>
<td>30.6</td>
<td>3.0374</td>
<td>1.532 to 6.019</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk factors in men</th>
<th>Distribution</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ (n=89)</td>
<td>- (n=62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prolonged standing</td>
<td>65.3</td>
<td>24.4</td>
<td>3.7119</td>
<td>2.107 to 6.5382</td>
</tr>
<tr>
<td>Prolonged sitting</td>
<td>34.6</td>
<td>14.9</td>
<td>2.0318</td>
<td>1.092 to 3.7984</td>
</tr>
<tr>
<td>Smoking</td>
<td>59.8</td>
<td>45.6</td>
<td>1.7740</td>
<td>1.031 to 3.0515</td>
</tr>
<tr>
<td>Exercise less than once a week</td>
<td>40.1</td>
<td>40.9</td>
<td>0.5162</td>
<td>0.299 to 0.8892</td>
</tr>
<tr>
<td>Height &gt; 1.75 m</td>
<td>67.7</td>
<td>24.4</td>
<td>4.1275</td>
<td>2.333 to 7.3008</td>
</tr>
<tr>
<td>Body mass index &gt; 25 kg/m²</td>
<td>72.4</td>
<td>32.6</td>
<td>5.4324</td>
<td>3.028 to 9.7453</td>
</tr>
<tr>
<td>History of venous disease</td>
<td>63.7</td>
<td>31.5</td>
<td>3.8253</td>
<td>2.164 to 6.7612</td>
</tr>
</tbody>
</table>

Significant ‘p’ values is shown by different color.
4.7 DERMATOGLYPHICS

In present study, dermatoglyphics of two hundred and sixteen patients and control group was analysed. For dermatoglyphics analysis, frequencies of different types of finger tip patterns, atd angles of palm, were calculated from prints taken from palm of patient of varicose veins as well as normal individuals. Frequencies of various patterns of normal individuals were taken as control group (C). Deviation was statistically calculated by Chi-square test and t-test.

4.7.1 FINGERPRINT PATTERNS:

Five types of fingertip patterns were found in present study (Figure-25). Differences in the frequency of fingertip patterns of varicose veins patients and control group was observed. Out of five different patterns, ulnar loop and whorls were of common occurrence in control group as well as in the cases of varicose veins. Rests of the patterns were present even in less than ten percent cases (Figure-26). Frequency of Ulnar loop was 46% and 59.84% in control group and patients respectively. Whorl pattern was present in 51.9% patients of varicose veins and 28% in control group. Simple arch and tented arch were found in 02.94% and 07.91% of patients respectively. Control group had 02.86% and 05.41% of simple and tented arch respectively. Radial loop pattern was observed in 03.66% of patients and 02.28% in control group. $\chi^2$ value revealed the significant variation in fingerprints patterns (For Ulnar loop $p>0.008, df=4$; Whorl $p>0.001, df=4$) (Table- 26).
Figure 25: Finger tip patterns: (a) Radial loop, (b) Ulnar loop, (c) Whorl, (d) Tented arch, (e) Simple arch.
Figure 26: Percentage frequency of various digital patterns (a) Patients of Varicose Veins (b) Control group (Normal individuals).
Table 26: Percentage frequency of different fingertip patterns of patients of Varicose veins (VV) and control group (C).

<table>
<thead>
<tr>
<th></th>
<th>Ulnar loop**</th>
<th>Radial loop</th>
<th>Whorl***</th>
<th>Simple arch</th>
<th>Tented arch</th>
</tr>
</thead>
<tbody>
<tr>
<td>VV</td>
<td>C</td>
<td>VV</td>
<td>C</td>
<td>VV</td>
<td>C</td>
</tr>
<tr>
<td>1.</td>
<td>41.2</td>
<td>58</td>
<td>1.1</td>
<td>0.7</td>
<td>43.6</td>
</tr>
<tr>
<td>2.</td>
<td>56.2</td>
<td>40</td>
<td>4.0</td>
<td>4.3</td>
<td>52.1</td>
</tr>
<tr>
<td>3.</td>
<td>42.3</td>
<td>79.9</td>
<td>9.2</td>
<td>4.7</td>
<td>9.7</td>
</tr>
<tr>
<td>4.</td>
<td>42.7</td>
<td>49.4</td>
<td>2.4</td>
<td>.6</td>
<td>53.6</td>
</tr>
<tr>
<td>5.</td>
<td>47.6</td>
<td>71.9</td>
<td>1.6</td>
<td>1.1</td>
<td>50.5</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>59.84</td>
<td>3.66</td>
<td>2.28</td>
<td>51.9</td>
</tr>
</tbody>
</table>

Ulnar loop** = ($\chi^2 = 13.758$, degree of freedom = 4, $P > 0.008$).

Whorl*** = ($\chi^2 = 17.977$, degree of freedom = 4, $P > 0.001$).

Percentage frequency of five types of fingertip patterns was calculated. Each digit of patients and as well as control group was compared for the percentage frequency of different patterns.

**DIGIT 1:**

Percentage frequency of ulnar loops on first digit was 41.2% in patients and 58% in control group. Whorls were found in 43.6% patients and 47.1% in control group. Radial loops occurred in 01.1% patients and in 0.7% control group. Percentage frequency of simple arch and tented arch in patients was 02.2% and 01.1% respectively. Whereas in control group percentage frequency of simple arch and tented arch was 11.3% and 07.9% respectively (Figure- 27a).
DIGIT 2:

Ulnar loops on first digit were present in 56.2% patients and in 40% control group. Percentage frequency of whorls was 52.1% in patients and 31.2% in control group. Radial loops were found 04.0% in patients and in 04.3% control group. Percentage frequency of simple arch and tented arch in patients was 06.7% and 19.3% respectively. Whereas in control group percentage frequency of simple arch and tented arch was 09.4% and 12.3% respectively (Figure- 27b).

DIGIT 3:

Percentage frequency of ulnar loops on third digit was 42.3% in patients and 79.9% in control group. Whorls were noted in 59.7% patients and in 19% control group. Radial loops were found in 09.2% in patients and in 04.7% control group. Percentage frequency of simple arch and tented arch in patients was 04.3% and 04.2% respectively. Similarly in control group simple arch and tented arch were 02.2% and 05.3% respectively (Figure- 27c).

DIGIT 4:

On the fourth digit percentage frequency of ulnar loops was 42.7% in patients and 49.4% in control group. Whorls were found in 53.6% patients and in 22.3% control group. Percentage frequency of radial loops was 02.4% in patients and 0.6% in control group. Percentage frequency of simple arch and tented arch in patients was 0.7% and 03.6% respectively. Similarly in control group simple arch and tented arch were 01.5% and 01.6% respectively (Figure- 27d).

DIGIT 5:

Percentage frequency of ulnar loops on fifth digit was 47.6% in patients and 71.9% in control. Whorls were found in 50.5% patients and in 20.4% control group. Radial loops in patients and control group were 01.6% and 01.1% respectively. Percentage frequency of simple arch was 0.8% and tented arch was 01.2% in patients. Whereas in control group there was no simple arch and no tented arch (Figure- 27e).
Figure 27: Percentage frequency of various patterns in digits of hands of normal and patients of varicose veins (a) Digit 1 (b) Digit 2 (c) Digit 3 (d) Digit 4 (e) Digit 5.

UL- Ulnar loop, RL- Radial loop, AT-Tented arch, AS-Simple arch
W-Whorl, VV- Varicose veins and C- Control groups
4.7.2 PALM PRINT AND ATD ANGLE:

From the palm prints of patients and control group, atd angle was calculated and grouped into 9 classes from < 30 to >65. In right palm Atd angle between 35-40 was noted in maximum patients i.e 34.6%, whereas atd angle between 40-45 was observed in most (40.6%) of the individuals of controls group. In control group maximum number of persons were between angles 40-45. Angle <30 and >65 was not found in control group (Table- 27). Atd angle of different ranges showed variation between varicose veins patients and control group (Figure- 28).

Atd angle >65 and <30 was not observed in right or left palm of varicose veins patients. In control group also angle < 30 and > 65 was not found in left palm and there were only 0.4% individuals with angle between 60-65 in right palm (Table-27). Atd angle of right hand of varicose veins patients and control group showed non significant values at 5% level (Table-28 and 29). ‘t’ test analysis revealed non significant values between frequency of sum of total atd angles (right +left) of varicose veins patients and control group, p=0.8233 and p= 0.7079 for right and left palm respectively (Table-30).

Figure 28: Palm print of normal person (control group) having atd angle t (<45)
Table 27: Percentage frequency of different atd angle range of Varicose veins (VV) and control group (C)

<table>
<thead>
<tr>
<th>atd angle range</th>
<th>VV (Right)</th>
<th>VV(left)</th>
<th>Control group (Right)</th>
<th>Control group (Left)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30-35</td>
<td>7.6</td>
<td>5.2</td>
<td>8.4</td>
<td>3.2</td>
</tr>
<tr>
<td>35-40</td>
<td>34.6</td>
<td>30.0</td>
<td>33.2</td>
<td>36.0</td>
</tr>
<tr>
<td>40-45</td>
<td>20.4</td>
<td>29.0</td>
<td>40.6</td>
<td>36.0</td>
</tr>
<tr>
<td>45-50</td>
<td>7.4</td>
<td>10.2</td>
<td>6.0</td>
<td>8.6</td>
</tr>
<tr>
<td>50-55</td>
<td>6.4</td>
<td>9.2</td>
<td>9.0</td>
<td>13.6</td>
</tr>
<tr>
<td>55-60</td>
<td>3.4</td>
<td>3.6</td>
<td>2.4</td>
<td>2.6</td>
</tr>
<tr>
<td>60-65</td>
<td>2.1</td>
<td>1.0</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>&gt;65</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 28: Quantitatively analyzed atd angle range of right palms of varicose veins patients and control group.

<table>
<thead>
<tr>
<th>Atd angle</th>
<th>Right (Control group) mean±SD</th>
<th>Right (VV) mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30-35</td>
<td>33.312±1.62</td>
<td>32.56±1.7</td>
</tr>
<tr>
<td>35-40</td>
<td>38.19±1.51</td>
<td>36.61±4.7</td>
</tr>
<tr>
<td>40-45</td>
<td>43.11±3.17</td>
<td>43.09±1.39</td>
</tr>
<tr>
<td>45-50</td>
<td>47.75±1.31</td>
<td>46.133±1.61</td>
</tr>
<tr>
<td>50-55</td>
<td>53.00±1.32</td>
<td>51.76±1.33</td>
</tr>
<tr>
<td>55-60</td>
<td>57.76±1.091</td>
<td>57.09±1.8</td>
</tr>
<tr>
<td>60-65</td>
<td>-</td>
<td>62.5±1.00</td>
</tr>
<tr>
<td>&gt;65</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

P= non significant at 5%
Table 29: Quantitatively analyzed atd angle range of left palms of varicose veins patients and control group.

<table>
<thead>
<tr>
<th>Atd angle</th>
<th>Left (control group) mean±SD</th>
<th>Left (VV) mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30-35</td>
<td>33.47±1.48</td>
<td>32.67±1.09</td>
</tr>
<tr>
<td>35-40</td>
<td>38.21±1.59</td>
<td>38.41±1.644</td>
</tr>
<tr>
<td>40-45</td>
<td>42.898±1.41</td>
<td>42.76±2.03</td>
</tr>
<tr>
<td>45-50</td>
<td>47.82±1.46</td>
<td>46.54±1.34</td>
</tr>
<tr>
<td>50-55</td>
<td>52.69±1.31</td>
<td>54.31±1.01</td>
</tr>
<tr>
<td>55-60</td>
<td>57.75±0.96</td>
<td>56.17±2.42</td>
</tr>
<tr>
<td>60-65</td>
<td>64±1.41</td>
<td>60.33±1.89</td>
</tr>
<tr>
<td>&gt;65</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

P= non significant at 5%

Table 30: Quantitative analysis of atd angle in varicose veins (VV) patients and control group (C).

<table>
<thead>
<tr>
<th>Palm</th>
<th>VV</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>36.63±22.77</td>
<td>34.14±22.44</td>
</tr>
<tr>
<td>Left</td>
<td>41.398±19.16</td>
<td>37.425±23.20</td>
</tr>
</tbody>
</table>

P= 0.8233 (Right) and P= 0.7079 (Left), non significant
In the present study, out of 216 varicose veins cases, 190 cases were subjected to chromosomal investigation. For the remaining 26 cases, samples were not available as the parent’s /guardians were not willing in cytogenetic analysis. Peripheral blood samples of patients of varicose veins were analysed cytogenetically for structural as well as numerical chromosomal anomalies using trypsinization and G banding (GTG banding).

In one hundred ninety patients of varicose veins no gross structural or numerical anomalies could be detected by G banding. These all cases revealed normal karyotype i.e. 46, XX and 46, XY (Plate- 1 and Plate- 2).

Patients who had severe varicose veins with ulcer and other complications had also showed normal karyotype (Plate- 3). There were few cases in this study which were found with recurrence of varicose veins even after vein surgery. Clinical recurrence was progressive from 3 months onward (17 of 124 limbs [13.7%]), with most recurrent varices appearing in 36 of 114 limbs (31.6%) by 1 year. Cytogenetic analysis of these patients was done and revealed no chromosomal anomaly (Plate- 4).

Severe or chronic patients had also normal karyotype (Table- 31). To know any inherited and familial chromosomal anomaly, parents of 26 cases of varicose veins were subjected to cytogenetic investigation using G banding. Here also no anomaly could be detected. These parents were also having the manifestation of varicose veins but G banding did not reveal any gross structural or numerical anomaly. Less than 500 bp structural anomalies could be present in the patients of varicose veins. Molecular cytogenetic analysis of the patients of varicose veins was carried out to know the specific mutations in FOXC2 locus.
Table 31: Chromosomal analysis of patients of varicose veins and normal persons using G banding.

<table>
<thead>
<tr>
<th>SNo.</th>
<th>Status of patients/Persons</th>
<th>No. of patients/Persons (N)</th>
<th>Chromosomal anomalies (Gross)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal persons</td>
<td>73</td>
<td>NIL</td>
</tr>
<tr>
<td>2.</td>
<td>Primary varicose veins patients</td>
<td>24</td>
<td>NIL</td>
</tr>
<tr>
<td>3.</td>
<td>Severe cases of varicose veins</td>
<td>67</td>
<td>NIL</td>
</tr>
<tr>
<td></td>
<td>with ulcer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Multipare varicose veins workers</td>
<td>13</td>
<td>NIL</td>
</tr>
<tr>
<td>5.</td>
<td>Parents of varicose patients</td>
<td>26</td>
<td>NIL</td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>
Plate- 1: Normal karyotype of primary varicose female patient
Plate- 2: Normal karyotype of primary varicose male patient
Plate- 3: Severe varicose veins patient with ulcer and DVT as complication, having normal karyotype.
Plate 4: Female patients with recurrence of varicose vein after 3 months of surgery, showed normal karyotype.
4.9. MOLECULAR CYTOGENETIC ANALYSIS:

Patients of varicose veins were subjected to molecular cytogenetic analysis to identify the mutation in FOXC2 locus. Out of 216 patients of varicose veins, 190 patients were analyzed for mutation detection. Remaining 26 patients were not interested in the molecular analysis. The status of the varicose veins in lower limb was noted with the help of clinicians. Reflux in veins was detected by clinicians with the help of Doppler ultrasound. Peripheral blood sample was collected in EDTA vacutainers carefully by trained lab technicians after the consent of parents and guardians. There were 116 (62%) males and 74 (38%) females. All the 190 patients were subjected to further DNA analysis for FOXC2 gene mutation detection.

4.9.1 DNA EXTRACTION AND PURIFICATION:

DNA was obtained from whole blood using a rapid improvised isolation of mammalian DNA technique (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 (Figure-29 and Table-32). The samples which fluctuated from the reference range were purified again by RNAse and Protienase K treatment. The purified DNA was stored in the TE buffer (pH 7.6) at -20°C. The purified DNA product was also checked by agarose gel electrophoresis (Figure-30(a) and (b)).

![Graph showing absorbance of DNA using spectrophotometer.](image)
Table 32: Calculation of DNA absorbance ratio for checking of purity.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sample Type</th>
<th>Conc. (µg/µl)</th>
<th>Unit</th>
<th>(260 nm)</th>
<th>(280 nm)</th>
<th>260 / 280</th>
</tr>
</thead>
<tbody>
<tr>
<td>VV patients No.</td>
<td>DNA</td>
<td>66.655</td>
<td>ng/µl</td>
<td>1.333</td>
<td>0.741</td>
<td>1.799</td>
</tr>
</tbody>
</table>

Figure 30- DNA amplification whole genome of Normal persons and Patients:

a) Lane 1-8 (DNA samples of normal persons)
b) Lane 1-8 (DNA samples of patients)
4.9.2 ALLELE SPECIFIC AMPLIFICATION OF DNA BY POLYMERASE CHAIN REACTION:

Purified DNA was amplified using polymerised chain reaction. All the 190 selected patients of varicose veins were screened for region I of FOXC2 mutation. Detection of alleles FOXC2 region I was done with PCR product -413 bp of the 5’ regions to +655bp of exon. Amplification of 5’ UTR region I of FOXC2 gene was carried out using 5 µl of DNA in 50 µl reaction using 3 pairs of primers:

1. F1: 5' CCGATTCGCTGGGGGCTTTGGAG 3'
   R1: 5' GCAGGGCTGGTGGTGGTGGTAGG 3’ to amplify fragment of (607 bp)
2. F2: 5’ CCTACCTGAGCGAGCAGAATTACTA3’,
   R2: 5’ GAAGCGGTCCATGATGAACTG 3’ to amplify fragment of (269 bp)
3. ACCTGGTGAAGCGCCTCCCTACAG 3’
   R3: 5’ ACGCCGCCTCGCTCTTTGA 3’ to amplify fragment of (450 bp)

The presence of a high G + C content in the target DNA template had difficulties for in vitro DNA amplification across CG rich triplet repeats. However amplification of highly GC rich sequences of human foxc2 gene was done by improvisation. The designing of the protocol was obtained with certain modifications in standard PCR. PCR was used with certain modifications like adding enhancers (8% DMSO and glycerol) in the reaction buffer to get the amplification in the normal subjects. Several additives were tried, such as formamide and betaine which had been reported to improve PCRs of GC rich structures. However, these did not improve the results. Therefore known enhancer DMSO, as well as PCR master mix from Bio Basic which includes 0.1mg/ml BSA, used for PCR optimization. 15 µl PCR master mix with 3 µl DMSO was used to give a positive amplification. In the present study G+ C content in the FOXC2 gene was between 52% to 70%. Amplification results were compared with the molecular marker for 100 to 1000 bp for FOXC2 gene.
4.9.3 DETECTION OF MUTATION:

In present study, to search for genetic variation among varicose veins patients and normal individuals, -413 bp of upstream to 655 bp of single exon of FOXC2 gene, was amplified. The amplified fragments (I, II and III) were of sizes 607, 269 and 450 bp, respectively. None of these mutations was detected in healthy control subjects. DNA sequencing of these fragments was done to cross check the allele specific amplification and to know the SNP involved.

4.9.3.1 FOXC2 mutation involving fragment 607 bp:

Amplification product range for this mutation varied from -413 to 194bp. Out of 190 patients of varicose veins only 32 were detected with FOXC2 mutation of this fragment. Among 32 patients who showed this mutation number of male patients was 24 and females was 8. In the present study the frequency of FOXC2 mutation was 16.84% among the patients of varicose veins (Figure 31a).

4.9.3.2. FOXC2 mutation involving fragment 269 bp:

PCR product range for this mutation varied from 50 to 318bp. Amplification product range for FOXC2 individuals has minimum of 250-290bp. Among all the 190 patients of varicose veins FOXC2 mutation of fragment 269 was detected in 122 patients. Out of 122 patients there were 68% males and 32% females who showed this mutation. Total frequency of FOXC2 mutation in 190 patients of varicose veins was 64.21%. This mutation was observed in maximum number of patients (Figure- 31b).

4.9.3.3 FOXC2 mutation involving fragment 450 bp:

PCR range for this mutation varied from 206 to 655bp. Amplification product range for FOXC2 individuals has minimum of 410bp. Hundred (52.63%) patients were detected with FOXC2 mutation of fragment 450 bp. Among these there were 48 males and 52 female patients of varicose veins (Figure 31c).
Figure 31: Amplification of FOXC2 gene mutation in varicose vein patients.
   a) Amplified fragment of (607 bp) with mutation in Lane 1, 3 and 5
   b) Amplified fragment of (269 bp) with mutation in Lane 1, 3, 4 and 5
   c) Amplified fragment of (450 bp) with mutation in Lane 1 to 5
   M= Molecular marker (100-1000 bp)
   C= Control (Normal individual)
4.9.4 DNA SEQUENCING OF FOXC2 REGION I

To study genetic variation among varicose veins patients and normal individuals, 655 bp of single exon of FOXC2 gene as well as additional 413 bp of upstream 5’ UTR was sequenced for the confirmation of SNPs involved. Three SNPs were identified in the proximal region I of FOXC2 gene; a -91 C→G transversion was detected in severe cases of varicose veins patients and two SNPs (-41G→A and -41G→T) were also detected in varicose veins patients (Figure- 32 and 33). None of these mutations was present in normal individual. DNA sequencing of amplified fragments using three specific primers confirmed the presence of three mutation i.e. -91C→G, -41G→A and -41G→T with respective amplification of fragments 607 bp, 269 bp and 450 bp. Specific clinical symptoms were found associated with specific mutation (Table- 33).

Figure 32: Representation of FOXC2 gene mutations from varicose veins patients showing the location of (a) - 91 C→G, (b) -41 G→A and (c) -41 G→T SNPs. Base substitution is denoted by arrows.
Figure 33: Base pair sequence with three detected Mutation in FOXC2 gene.

Table 33: Phenotypic association of varicose veins patients with detected FOXC2 mutation.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Nucleotide change</th>
<th>Phenotype</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>-91 C→G (fragment of 607 bp)</td>
<td>Varicose veins, Varicose ulcer, Recurrence of varicose veins and Deep venous insufficiency</td>
<td>32</td>
</tr>
<tr>
<td>2.</td>
<td>-41 G→A (fragment of 269 bp)</td>
<td>Varicose veins and Deep venous insufficiency</td>
<td>122</td>
</tr>
<tr>
<td>3.</td>
<td>-41 G→T (fragment of 450 bp)</td>
<td>Varicose veins and Hemorrhoids</td>
<td>100</td>
</tr>
</tbody>
</table>
4.9.5. SEX RATIO AND FOXC2 MUTATION

Out of 190 varicose veins patients selected for mutation detection, there were 116 males and 74 females. One hundred two male patients and sixty seven female patients have shown at least one mutation in region I of FOXC2 gene. Some varicose veins patients had two or all the three mutations. Sex ratio and mutation of patients revealed prevalence of varicose veins in males. Mutation (-91 C→G) was found in twenty four males (20.68%) and eight (10.81) female cases. There were eighty three (71.55%) males and thirty nine (52.70%) females that were affected with mutation (-41 G→A). However, mutation (-41 G→T) was more frequent in females as compared to males. Fifty two females (70.27%) and forty eight (35.34%) males were having mutation (-41 G→T) (Figure 34). Mutation (-41 G→A) showed highest frequency among all varicose veins cases. $\chi^2$ value revealed a significant association between type of mutation and sex ratio of varicose veins cases (df = 2, p>.002) (Table 34).

<table>
<thead>
<tr>
<th>Different types of mutation</th>
<th>Male N</th>
<th>Female N</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-91 C→G)</td>
<td>24</td>
<td>08</td>
</tr>
<tr>
<td>(-41 G→A)</td>
<td>83</td>
<td>39</td>
</tr>
<tr>
<td>(-41 G→T)</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>Total</td>
<td>155</td>
<td>99</td>
</tr>
</tbody>
</table>

$\chi^2 = 12.279$, d.f. = 2, p>.002

![Figure 34: Percentage frequency of male and female varicose veins patients having FOXC2 mutations in region I.](image)

Table 34: Sex ratio and FOXC2 mutation of region I in varicose veins cases.
4.9.6. CORRELATION OF LIFE STYLE, GENETIC CAUSE AND VARICOSE VEINS

Varicose veins is a complex disease, influenced by a number of genetic and environmental factors. The occurrence of varicose veins in the population is connected with the lifestyle of modern society. The influences of the lifestyle include long-time standing, sitting, lack of dietary fiber and related constipation, increased sitting position on toilets, insufficient movement, and smoking. In the present research the information about various risk factors related to life style was correlated with the mutation present in the patients of varicose veins. FOXC2 mutation analysis in region I showed that frequency of mutation was 57% in heavy work load labors and 31% in light physical labors. Office workers were having mutations in 12% cases. Fisher’s exact analysis revealed a significant association between three working groups sex ratio and FOXC2 mutations (p< 0.05) (Table- 35).

In all three groups of profession heavy physical labors (Group III) were having maximum number of mutational frequency (Figure- 35). The comparison of FOXC2 mutation between all three working groups showed that out of 23 patients of group I, 19 patients were having the mutation (-41G→A) and 4 having the mutation (-41G→T). No one had mutation (-91C→G) or simultaneous mutations together. In group II 59 patients were having one FOXC2 mutation. There were 23 patients who had mutations (-41G→A) and (-41G→T) together and 9 patients had all three mutation. Mutations (-91C→G), (-41G→A) and (-41G→T) were present in 10, 26 and 23 patients respectively in group II. Out of one hundred eight, twenty one patients in group III showed amplification for all three FOXC2 gene mutations. Sixty seven patients were having mutations (-41G→A) and (-41G→T) and nineteen patients were having (-91C→G) and (-41G→A) mutations together. Twenty one patients in group III had mutation (-91C→G), sixty seven with (-41G→A) and twenty patients were showing with mutation (-41G→T). Only one patient in group III had sole mutation otherwise there were simultaneous two or three mutation were present. $\chi^2$ analysis revealed a significant association between types of mutation and professional groups (group I,II and III) of varicose veins cases ($\chi^2 = 16.021$, df = 4 and p>.002) (Table 36 and 37).
Odds ratio analysis revealed that group I was less likely affected with FOXC2 mutation than group II (OR= 0.3058; 95% CI= 0.1794 to 0.5215). Whereas group III was having more mutation as compared to group II (OR=0.692; 95% CI= 0.417 to 1.651). Group I (OR= 0.1046; 95% CI= 0.0621 to 0.1763) was also less frequent than group III (Table- 38). These results had confirmed that environmental and life style factors have a predominant role in the expression of various mutation of FOXC2 locus. These mutations of FOXC2 locus are having a genetic predisposition in the occurrence of varicose veins.

**Table 35: FOXC2 mutation and sex ratio in different professional group.**

<table>
<thead>
<tr>
<th>Professional Groups</th>
<th>% of Males affected with FOXC2 mutation</th>
<th>% of Females affected with FOXC2 mutation</th>
<th>Total % of FOXC2 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Group I</td>
<td>9</td>
<td>8%</td>
<td>14</td>
</tr>
<tr>
<td>Group II</td>
<td>39</td>
<td>30%</td>
<td>20</td>
</tr>
<tr>
<td>Group III</td>
<td>68</td>
<td>62%</td>
<td>40</td>
</tr>
</tbody>
</table>

P< 0.05

**Table 36: Professional group and type of mutation of region I of FOXC2 gene locus**

<table>
<thead>
<tr>
<th>Professional Groups</th>
<th>(-91C→G) Mutation</th>
<th>(-41G→A) Mutation</th>
<th>(-41G→T) Mutation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0</td>
<td>19</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>26</td>
<td>23</td>
<td>59</td>
</tr>
<tr>
<td>Group III</td>
<td>21</td>
<td>67</td>
<td>20</td>
<td>108</td>
</tr>
</tbody>
</table>

χ² = 16.021, d.f. = 4, p =0.002
Table 37: Profession group and occurrence of simultaneous mutation.

<table>
<thead>
<tr>
<th>Professional group</th>
<th>Sole mutation</th>
<th>With simultaneous occurrence (2 mutation)</th>
<th>With simultaneous occurrence (3 mutation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>23</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Group II</td>
<td>27</td>
<td>23</td>
<td>09</td>
</tr>
<tr>
<td>Group III</td>
<td>01</td>
<td>86</td>
<td>21</td>
</tr>
</tbody>
</table>

Table 38: Odds ratio in different professional groups having FOXC2 mutation.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Categories</th>
<th>Present</th>
<th>Absent</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Profession</td>
<td>Group I</td>
<td>23</td>
<td>167</td>
<td>0.3058</td>
<td>0.1794 to 0.5213</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>59</td>
<td>131</td>
<td>0.1046</td>
<td>0.0621 to 0.1763</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>108</td>
<td>82</td>
<td>0.342</td>
<td>0.2246 to 0.5208</td>
</tr>
</tbody>
</table>
Figure 35: Comparison of Foxc2 gene mutation in region I between patients of three working groups. (a) -91C→G, (b) -41G→A and (c) -41G→T

Lane 1- Marker (100 bp ladder)
Lane 2-3 P1 and P2 (patients of group I)
Lane 4-5 P3 and P4 (the patients of group II)
Lane 6-7 P5 and P6 (the patients of group III)
4.9.7. DNA SEQUENCING FOR ANOTHER LOCUS OF FOXC2 GENE

DNA of 78 severe cases (having more than two critical symptoms of varicose veins) were subjected to DNA sequencing for detecting the mutation in another locus of FOXC2 gene. Since the FOXC2 gene has >60% GC content, all PCR reactions were performed using the GC-rich PCR kit according to the manufacturer’s recommendations with primers located in the 5’ and 3’-UTR of the gene FOXC2 (GenBank NG_012025.1) sequence analysis was performed.

(FOXC2F 5’-TGGCTCTCTCGCTCTCTC-3’, 
FOXC2R 5’-CGTCTCTGCAGCCCCCTAATTG-3’).

4.9.7.1 Sequence analysis

Sequence analysis of 78 patients revealed four mutation in 69 patients in the region II of FOXC2 gene, of which two missense (c.1205C>T and c.1331A>G) and two were frameshift mutations (c.902-920dup19 and c.876-877delCG) (Table- 39 and Figure- 36). None of the changes was found in healthy people. Out of 78 selected patients there were 52 males and 26 females. Forty eight males and twenty one females showed mutation in FOXC2 gene (Table- 40). 78% of males who showed mutations were working as of heavier physical laborers including walking, whole body movement and heavy load handling. Remaining 22 % males were light physical laborers, office workers or those who do less work did not show these mutation. Out of 21 women with mutation, 15 (73%) were those who had three or more pregnancies, 19% were secundipare and remaining 8% were nullipare.
Figure 36: Representative FOXC2 gene sequencing from VV patients showing the location of (a) c.876-877delCG (b) c.902-920dup19 (c) c.1331A>G and (d) c.1205C>T mutations detected.
Table 39: FOXC2 mutations and associated phenotypes in varicose veins patients.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Nucleotide change</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>c.876-877delCG</td>
<td>Swelling of ankles, venous Insufficiency includes superficial and deep venous insufficiency.</td>
</tr>
<tr>
<td>2.</td>
<td>c.902-920dup19</td>
<td>Varicose veins, Deep venous insufficiency and Hemorrhoids</td>
</tr>
<tr>
<td>3.</td>
<td>c.1331A&gt;G</td>
<td>Varicose veins with Swelling and Ulcer</td>
</tr>
<tr>
<td>4.</td>
<td>c.1205C&gt;T</td>
<td>Varicose veins, insufficient deep venous system with thrombosis right</td>
</tr>
</tbody>
</table>

Table 40: FOXC2 mutation in region II and patients of varicose veins.

<table>
<thead>
<tr>
<th>TYPE OF MUTATION</th>
<th>PATIENTS showing mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.876-877delCG</td>
<td>13 09</td>
</tr>
<tr>
<td>c.902-920dup19</td>
<td>18 07</td>
</tr>
<tr>
<td>c.1331A&gt;G</td>
<td>10 05</td>
</tr>
<tr>
<td>c.1205C&gt;T</td>
<td>07 -</td>
</tr>
</tbody>
</table>
4.10. FAMILY HISTORY AND VARICOSE VEINS

The occurrence of varicose veins is more frequent in patients with a positive family history mainly in the case of father affection. On the basis of questionnaire studied, one hundred thirty two patients (61.11%) had family history of close relatives suffering from varicose veins. The occurrence of varicose veins in several members of the same family suggest that hereditary factors may be important in causation of varicose veins. Fifty eight patients had no family history of varicose veins and twenty six patients were not interested to giving the family history (Table- 41).

In the present study it was found that Individuals were more affected by varicose veins when parents and sibling had varicose veins. Family history screening revealed 63.63% cases development of varicose veins, when both parents were affected, whereas the risk was only 19% for individual who had unaffected parents. There were 50% patients whose sibling had varicose veins or complained to have symptoms of varicose veins. Only 1.5% patients were those whose had cousin or nonfamily members affected with varicose veins (Table- 42).

Out of 132 patients with family history of varicose veins, only twelve (09.09%) patients had at least one person from their family affected with varicose veins. Sixty eight (51.51%) patients were those who had two affected person in their family members and fifty three (40.15%) were with three or more affected person (Table- 43).

The prevalence of VV increased with age and even greater among those with a family history of the condition. In age group 21-30 out of seventy eight patients, forty six patients had the positive family history and thirty one cases had both affected parents (Table- 44).

According to prevalence, family history indicated a high risk of varicose veins. Age and body mass index adjusted OR was 1.7920 (95% CI: .5119-6.273). The prevalence of varicose veins in those with positive family history was 61.11%, 39.81% in men and 21.29% in women. Adjusted OR was 6.375 (95% CI: 0.7508-54.126) in men and 0.319 (95% CI: 0.0319-3.181) in women (Table- 45).
Table 41: Occurrence of the varicose veins in the family of patients

<table>
<thead>
<tr>
<th>Occurrence of VV in the family</th>
<th>N (216)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>132</td>
<td>61.11</td>
</tr>
<tr>
<td>NO</td>
<td>58</td>
<td>26.85</td>
</tr>
<tr>
<td>Not specified</td>
<td>26</td>
<td>12.03</td>
</tr>
</tbody>
</table>

Table 42: Relatives having varicose veins in families of patients.

<table>
<thead>
<tr>
<th>Relationship of persons</th>
<th>N (216)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>76</td>
<td>57.57</td>
</tr>
<tr>
<td>Grand mother</td>
<td>51</td>
<td>38.63</td>
</tr>
<tr>
<td>Father</td>
<td>91</td>
<td>68.93</td>
</tr>
<tr>
<td>Grand father</td>
<td>55</td>
<td>41.66</td>
</tr>
<tr>
<td>Both patents</td>
<td>84</td>
<td>63.63</td>
</tr>
<tr>
<td>Sister or brother</td>
<td>26</td>
<td>19.69</td>
</tr>
<tr>
<td>Next generation</td>
<td>66</td>
<td>50.00</td>
</tr>
<tr>
<td>Others</td>
<td>13</td>
<td>09.84</td>
</tr>
<tr>
<td>Aunt</td>
<td>08</td>
<td>06.06</td>
</tr>
<tr>
<td>Cousins</td>
<td>02</td>
<td>01.51</td>
</tr>
<tr>
<td>Without (VV) in the family</td>
<td>02</td>
<td>01.51</td>
</tr>
</tbody>
</table>

Table 43: Number of persons with varicose veins in the family.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Affected persons with VV in the family</th>
<th>N (132)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1 Person</td>
<td>12</td>
<td>09.09</td>
</tr>
<tr>
<td>2.</td>
<td>2 Persons</td>
<td>68</td>
<td>51.51</td>
</tr>
<tr>
<td>3.</td>
<td>3 Persons or more</td>
<td>53</td>
<td>40.15</td>
</tr>
</tbody>
</table>

Table 44: Number of patients with Positive family history in each age group
### AGE GROUPS

<table>
<thead>
<tr>
<th>AGE GROUPS</th>
<th>Number of patients in each age group</th>
<th>Number of patients with Positive family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>02</td>
<td>01</td>
</tr>
<tr>
<td>11-20</td>
<td>08</td>
<td>02</td>
</tr>
<tr>
<td>21-30</td>
<td>78</td>
<td>46</td>
</tr>
<tr>
<td>31-40</td>
<td>53</td>
<td>41</td>
</tr>
<tr>
<td>41-50</td>
<td>37</td>
<td>18</td>
</tr>
<tr>
<td>51-60</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>&gt;60</td>
<td>08</td>
<td>04</td>
</tr>
<tr>
<td>Total</td>
<td>216</td>
<td>132</td>
</tr>
</tbody>
</table>

Table 45: Adjusted odds ratios with 95% confidence intervals (CI) for prevalence (OR) of varicose veins by family history.

<table>
<thead>
<tr>
<th>Family History</th>
<th>Prevalence of Varicose veins</th>
<th>Adj*OR (CI),</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (Cases)</td>
<td>(%)</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>58</td>
<td>26.85</td>
</tr>
<tr>
<td>Yes</td>
<td>132</td>
<td>61.11</td>
</tr>
<tr>
<td>Uncertain</td>
<td>26</td>
<td>12.03</td>
</tr>
<tr>
<td>TOTAL</td>
<td>216</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>28</td>
<td>22.04</td>
</tr>
<tr>
<td>Yes</td>
<td>86</td>
<td>67.71</td>
</tr>
<tr>
<td>Uncertain</td>
<td>13</td>
<td>10.23</td>
</tr>
<tr>
<td>TOTAL</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>30</td>
<td>33.70</td>
</tr>
<tr>
<td>Yes</td>
<td>46</td>
<td>51.68</td>
</tr>
<tr>
<td>Uncertain</td>
<td>13</td>
<td>14.60</td>
</tr>
<tr>
<td>TOTAL</td>
<td>89</td>
<td></td>
</tr>
</tbody>
</table>

*= adjusted for age, body mass index (BMI as a dichotomous variable, with 25 kg/m² as a cut-off point), in all-group also with sex. **= significant, ***= highly significant
4.10.1 FAMILIAL MUTATION DETECTION

Out of 216 cases, 132 cases showed a family history of varicose veins. 72 families of varicose veins have agreed to take part in this research and provided their blood sample for mutation detection. Out of these, sixty one patients had severe varicose veins. Other had primary varicose veins. Family members of these patients were examined and blood samples taken from both parents, affected offspring and even from unaffected family members. The common features in these families included varicose veins in childhood and pubertal or later onset of varying severity. As mutations in FOXC2 were found in all selected families, it appears likely that there must be genetic cause in the occurrence of varicose veins.

The presence and absence of mutation was checked in all family members. DNA was extracted from peripheral venous blood with the help of rapid improvised isolation of mammalian DNA technique (Sambrook 2002). Purified DNA was amplified using polymerised chain reaction. Allele specific PCR was performed to detect the mutation in region I of FOXC2 gene. Pedigree of 216 patients of varicose veins revealed only 132 cases of family history of varicose veins. Maternal age varied from 30 to 65 years. Whereas paternal age was between 34 to 70 years. The detail information of pedigree of few selected families has been described as under. The generations have been shown by roman numbers (I,II and III). Affected individuals in dark and proband have been shown by arrowing. Male and females had usual depictions of square and circles respectively.

4.10.1.1. PEDIGREE AND MOLECULAR ANALYSIS

A molecular and pedigree analysis of patient’s family members was done. Mutation of affected and unaffected family member was detected with polymerase chain reaction using allele specific three designed primers set. For each pedigree amplification results were shown as (a, b and c) for (-91C→G), (-41 G→ A) and (-41 G→ T) mutation in FOXC2 gene respectively.

The presence of FOXC2 mutation in family members was detected by amplifying their DNA samples and running the sample on agarose gel. Seventy two parents, forty six grandparents, thirty nine siblings and fifty four next progeny of patients of varicose veins were subjected to mutational analysis. Out of total 72
mothers, 51 were found with at least one FOXC2 mutation out of three studied mutations. Father of 63 patients were affected with these mutations. Out of 46 grandparents, 19 grandfather and 14 grandmothers have shown the mutation in FOXC2 gene. Siblings of 24 patients were found with FOXC2 mutation and had the varicose veins symptoms whereas rest of siblings were normal. Next progeny of 39 Patients, who were above the 20 years of age, were found with FOXC2 mutation. Seventy two pedigrees were made and their family members were subjected to detailed molecular analysis.

**Pedigree 1:**

The first and second generation had no patients of varicose veins. The grandmother’s age of patient of varicose veins was 71 years where as grandfather’s age was 74 years. The mother’s age of patient of varicose veins was 46 years where as father’s age was 49 years. The index female patient of varicose veins had two brothers and they had no varicose vein symptoms. This patient was identified as first proband or first affected family member who sought medical attention for varicose veins. Age of affected female was 27 years she was 2nd time pregnant at the time of diagnosis and having severe stage of varicose veins with ulcer in one limb. At the time of 1st pregnancy she was diagnosed with varicose veins. In two generations there was neither history of varicose veins nor symptoms. No mutation was detected in family members (Pedigree- 37a and Figure- 38). Whereas mutation was detected in two FOXC2 region in the proband i.e. (-91C→G) and (-41G→A). In the present study there were five families who had proband as affected female and the main cause of these females may be 2nd or 3rd time pregnancy as neither symptoms nor any mutation could be detected in the family.

**Pedigree 2:**

The first generation had no case of varicose veins. In the second generation there was one female case with varicose veins. The female in second generation was married with an affected male. In the third generation index patient was male with varicose veins and DVT. Patient had two normal female siblings who had neither symptoms nor any FOXC2 mutation. The mother’s age of patient of varicose veins
was 54 years where as father’s age was 58 years. The patient age at the time of diagnosis was 32 years (Pedigree- 37b and Figure- 39). The affected individual was grouped under the heavy physical laborer. This index patient had (-41G→A) and (-41G→T) mutations whereas both mother and father were having only (-41G→A) mutation and mother of patient had visibly varicose veins also. Role of genetic predisposition along with environmental factors was revealed for the occurrence of varicose veins.

**Pedigree 3:**

First generation had a male (grandfather, 82 years age) with varicose veins. In second generation one male had varicose veins whereas rest two were normal. The affected male was married with an unaffected female. Third generation had one female (index patient) suffering with varicose veins. The age of index female in third generation was 34 years (Pedigree- 38c). The mother’s age of patient of varicose veins was 52 years where as affected father’s age was 59 years. Mutation analysis revealed (-91C→G) and (-41G→A) mutations in both grandfather as well as patient’s father. Patient had all three FOXC2 gene mutation and showed severe varicose veins symptoms at the time of examination (Pedigree- 37c and Figure- 40). These results indicated strong genetic predisposition of varicose veins.

**Pedigree 4:**

First generation and second generation were normal and did not show any symptoms of varicose veins. The index male patient in third generation had varicose vein. His one brother also had varicose vein whereas female of 2nd generation had no symptoms. The age of patient at the time of diagnosis was 27 years and his brother age was 22 years. Patient had the (-41G→A) and (-41G→T) mutations whereas his brother had only (-41G→A) FOXC2 mutation. In present study there were six cases of unaffected parents and affected offspring with mutation as well as symptoms of varicose veins (Pedigree- 37d and Figure- 41). In these six cases the age of individuals was between 19-27 years. Varicose veins might have developed due to their lifestyle factors, however role of genetic predisposition cannot be ignored.
Pedigree 5:

In the three generation pedigree, the first generation had one affected female (age 69 years) mother of patient. In the second generation there was one female case (index patient) of varicose veins. The age of affected female patient in second generation was 47 (Pedigree- 37e and Figure- 42). The female patient was married with normal male. The index female had symptoms of varicose veins at the time of pregnancy. The female was the case of nullipare who had varicose veins from 1st pregnancy and still present at the time of diagnosis. In third generation one male had varicose vein symptoms, mutation detection revealed that he had (-91C→G) FOXC2 mutation. He was undergoing medical treatment from two year. The mother of index patient had (-91C→G) mutation whereas the patient had (-91C→G) as well as (-41G→A) FOXC2 mutation.

Pedigree 6:

This pedigree is the typical manifestation of early appearance of varicose veins. The first generation had a female (age 53 years) with of varicose veins. In the second generation there were one male and one female with varicose veins and other one male sibling was normal. The age of affected female was 27 years and of male was 34 years. Blood sample of female patient was not available for mutation detection but she had varicose vein on his right limb confirmed from Doppler examination by clinicians. Male in second generation was married to normal female but the time of 1st pregnancy she got varicose veins. The third generation male child was affected with varicose veins at the age of only 9 years (index patient) (Pedigree- 37f and Figure- 43). This child was the youngest one of this study and showed the all three FOXC2 mutation. Mother of patient detected with (-41G→A) and (-41G→T) mutations whereas his father was having all three FOXC2 mutation. Grandmother in first generation was having the (-41G→A) mutation.

Present study revealed that out of seventy two families, twelve families had both affected parents and 70% affected offspring. Genetic mutation in FOXC2 locus was detected in these patients. In present study it was found that out of 72 studied families there were eleven families who had varicose veins in all three generations.
with at least one FOXC2 mutation. This strongly suggests genetic predisposition and involvement of FOXC2 locus in etiopathogenesis of varicose veins.

Evaluation of patients of varicose veins and their family members have highlighted strong genetic predisposition in occurrence of varicose veins. Role of various risk factors as well as mutation in FOXC2 locus have been depicted as well as validated in the present study.

Screening of parents and family members revealed that history of varicose veins in first degree relatives was the most important cause for varicose veins in both sexes. Along with family history, environmental change, lifestyle, pathological and gene mutation led to physiological alteration in vein system. All these factors may have contributed to venous insufficiency and finally varicose veins formation.

Symptoms in early varicose veins are minimal and they become severe with time. One must know early symptoms and diagnosis should be made early to avoid painful and dangerous complications. The present information will be a great help to the clinician to evaluate and diagnose the varicose vein disorder in early stage. Eventually symptoms and suffering from the disease can be prevented by early intervention and taking precaution as per advice of clinician.
Figure 37 (a-f): Pedigree of patients of varicose veins
Figure 38: PCR product of the varicose veins patient and family members of pedigree 37 a.

A. Amplified product of mutation (-91C→G)
B. Amplified product of mutation (-41 G→A)
C. Amplified product of mutation (-41G→T)
• Lane 1 is molecular marker in both gels (M)
• Lane 2 sample of patient,
• Lane 3 sample of Patient’s mother,
• Lane 4 sample of patient’s father,
• Lane 5 and 6 samples of patient’s grandmother and grandfather respectively.
A. Lane 2 shows amplification for mutation (-91C→G)
B. Lane 2 Shows amplification for mutation (-41 G→A)
C. Lane 2-6 shows no amplification for mutation (-41G→T)
Figure 39: PCR product of the varicose veins patient and family members of Pedigree-37b.

A. Amplified product of mutation (-91C→G)
B. Amplified product of mutation (-41 G→A)
C. Amplified product of mutation (-41G→T)
   • Lane 1 is molecular marker in all three gel (M)
   • Lane 2 sample of patient,
   • Lane 3 sample of Patient’s mother,
   • Lane 4 sample of patient’s father,
   • Lane 5 and 6 samples of patient’s grandmother and grandfather respectively.
A. Lane 2-6 shows no amplification for mutation (-91C→G)
B. Lane 2, 3 and 4 shows amplification for mutation (-41 G→A)
C. Lane 2 shows amplification for mutation (-41G→T)
Figure 40: PCR product of the varicose veins patient and family members of Pedigree-37c.

A. Amplified product of mutation (-91C→G)
B. Amplified product of mutation (-41 G→A)
C. Amplified product of mutation (-41G→T)
   • Lane 1 is molecular marker in all three (M),
   • Lane 2 sample of patient,
   • Lane 3 sample of Patient’s mother,
   • Lane 4 sample of patient’s father,
   • Lane 5 and 6 samples of patient’s grandmother and grandfather respectively.

A. Lane 2, 4 and 6 shows amplification for mutation(-91C→G)
B. Lane 2, 4 and 6 shows amplification for mutation (-41 G→A)
C. Lane 2 shows amplification for mutation (-41G→T)
Figure 41: PCR product of the varicose veins patient and family members of Pedigree-37d.

A. Amplified product of mutation (-91C→G)
B. Amplified product of mutation (-41 G→A)
C. Amplified product of mutation (-41G→T)
   • Lane 1 is molecular marker in all three (M)
   • Lane 2 sample of patient,
   • Lane 3 sample of Patient’s mother,
   • Lane 4 sample of patient’s father,
   • Lane 5 and 6 sample of patient’s one male and one female siblings respectively.
A. Lane 2-6 shows no amplification for mutation (-91C→G)
B. Lane 2 and 5 shows amplification for mutation (-41 G→A)
C. Lane2 shows amplification for mutation (-41G→T)
Figure 42: PCR product of the varicose veins patient and family members of Pedigree-37e.

A. Amplified product of mutation (-91C→G)
B. Amplified product of mutation (-41 G→A)
C. Amplified product of mutation (-41G→T)
   • Lane 1 is molecular marker in all three (M)
   • Lane 2 sample of patient,
   • Lane 3 sample of Patient’s mother,
   • Lane 4 sample of patient’s father
   • Lane 5 and 6 samples of patient’s next progeny.
A. Lane 2, 3 and 5 shows no amplification for mutation(-91C→G)
B. Lane 2 shows amplification for mutation (-41 G→A)
C. Lane2-6 shows no amplification for mutation (-41G→T)
Figure 43: PCR product of the varicose veins patient and family members of Pedigree-37f.

A. Amplified product of mutation (-91C→G)
B. Amplified product of mutation (-41 G→A)
C. Amplified product of mutation (-41G→T)
  • Lane 1 is molecular marker in all three gel (M)
  • Lane 2 sample of patients,
  • Lane 3 sample of Patient’s mother,
  • Lane 4 sample of patient’s father,
  • Lane 5 and 6 sample of patient’s grandmother and grandfather respectively.
A. Lane 2 and 4 shows amplification for mutation(-91C→G)
B. Lane 2-5 shows amplification for mutation (-41 G→A)
C. Lane2-4 shows amplification for mutation (-41G→T)