APPENDIX- I
PERFORMA FOR STUDY OF VARICOSE VEIN PATIENTS

Date:

1. HISTORY

a) Patient Name:
   Card No.:

b) Father’s Name:

c) Mother’s Name:

d) Clinical Diagnosis

e) Age at Diagnosis:

f) Sex: Male/Female

g) Height:

h) Weight:

i) Education

j) Address:

k) Occupation:

l) Socioeconomic status:
   Caste/Religion

2. Which type of the following varicose veins do you have?
   Saphenous Type
   Segment Type
   Reticular Type
   Web Type

3. Limb involvement
   Right Limb
   Left Limb
   Both Limbs
4. Manifestations of Varicose veins

5. Associated Complications

6. Smoking/ Alcohol

7. Do you have Diabetes

8. Family History:

8.1 FAMILY History of Disease

Varicose veins: No/Yes  No/Yes

8.2 Environmental Factors:

Type:

8.3 Drugs: No/Yes  No/Yes

Type:

8.4 Mutation Present No/Yes  No/Yes

9. Cytogenetic Analysis

9.1 Karyotype: Normal/Abnormal

9.2 If Abnormal Type of Abnormality: Structural/ Numerical/ Both
10. PROTOCOL USED FOR DIAGNOSIS AND TREATMENT:

10.1 Doppler Ultrasound:

10.2 Local Varicose veins removal/excision:
   Laser:
   Operation:

10.3 Therapy Used:
   Sclerotherapy:

10.4 Medicine used
   Response:

11. Recurrence of Varicose after treatment:  
   Yes  No
   ☐  ☐

12. Dermatoglyphics

   I  II  III  IV  V

   | Whorl loop | ☐  ☐  ☐  ☐  ☐ |
   | Whorl     | ☐  ☐  ☐  ☐  ☐ |

   | Radial | ☐  ☐  ☐  ☐  ☐ |

   | Atd angle | < 45  45-56 >56 |
Patient’s Consent Form

Statement: I have been explained all about the nature and purpose of the study. I have been asked to clear any question regarding this protocol. I hereby give consent to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Government agencies, and ethics committee. My identity will be kept confidential if my data are publicly presented. I have decided to be in the research study and agree to cooperate with the investigator.

Name of patient ______________________

Patients / Parents of the patients / Guardians.

Signature__________  Thumb Impression
APPENDIX- II
PRECAUTIONS

➢ Glasswares were washed properly.
➢ Proper sterilization was used for culture technique. Fumigation should be done to maintain sterile conditions in lab for performing all Molecular Genetics experiments.
➢ Disposal of all culture wastes was done as per rules.
➢ All the specimens were handled as biohazardous. Nitrile gloves were worn throughout the procedure and were discarded with care. The blood vaccutainers were also discarded separately in a discard bag and sent to incinerators nearby. Hypochloride solution was used to discard any blood contaminations.
➢ Laboratory coat and gloves were worn for all steps up to slide making.
➢ EtBr contaminants were taken care of and were disposed separately.
➢ While viewing the Agarose gel in a gel Documentation machine, proper care was taken to avoid any kind of exposure to the UV.
➢ All the EDTA and acids that are used to make buffers should be used with caution.
APPENDIX- III
PEDIGREE SYMBOLS

- Male
- Female
- Sex unspecified
- Number of children of sex indicated
- Affected
- Obligate carrier, will not manifest disease
- Proband
- Deceased individual
- Stillbirth
- Adopted into family
- Adopted out of family
- Heterozygote (autosomal recessive trait)
- Heterozygote (x-linked trait)
- Marriage or union
- Divorced
- Consanguinity
- Monozygotic twins
- Dizygotic twins
- Twins of unknown zygosity
- No offspring
- Multiple unions
- Pedigree with generations (Roman numerals) and individuals numbered (Arabic numerals)
- Abortion (Spontaneous or induced)
APPENDIX- IV
REQUIREMENTS

1. FOR CYTOGENETICS:
   1. Coplin jar
   2. Filteration assembly
   3. Culture vials (10 ml)
   4. Vaccutainers (heparinised)
   5. Slides
   6. Bunsen burner
   7. Phase contrast Microscope
   8. Centrifuge
   9. Oven
   10. Incubator
   11. Water bath
   12. Laminar air flow
   13. Vacuum pump

2. FOR DNA EXTRACTION:
   1. Microfuge tubes 2 ml.
   2. Microtips 1ml, 100ul, 10 ul.
   3. Micropipette
   4. Discard bottle.
   5. Incubator

   6. Refrigerator

3. FOR PCR:
   1. PCR tubes 250 Ul.
   2. Microtips 1ml, 100ul, 10 ul.
   3. Micropipette
   4. PCR thermocyler
5. Mini coolers

4. FOR AGAROSE GEL ELECTROPHORESIS:

   1. Gel tank with safety lid
   2. Gel tray Comb
   3. Gel tray
   4. Gel caster (optional)
   5. Power supply capable of at least 100 V, 100 mA.
   6. Microwave oven, hot plate or heater.
   7. UV transilluminator and gel documentation system.

5. FOR DNA SEQUENCING

   1. PCR tubes
   2. Mini coolers
APPENDIX-V

REAGENT PREPARATION

- **Colchicine**
  - 3 mg in 50 ml distilled water (stored for 4-5 month at 4°C)

- **Fixative**
  - 3:1 (Methanol: Acetic acid) prepared fresh and chilled before use.

- **Giemsa stain**
  - Giemsa- 5g
  - Methanol- 500ml
  - Incubated in water bath at 60°C for 5-6 hrs.

- **Hank’s solution**
  - KCl- 0.40g
  - KH₂PO₄⁻ 0.60g
    - NaCl- 8.0g
    - NaHCO₃- 0.35g
    - Na₂HPO₄₂H₂O₂H₂O- 0.060g
    - Glucose- 1.0g
    - Phenol red- 0.01
    - D,W- 1000ml

- **Heparin**
  - 167µl/10ml blood
  - 50µl/3-4ml blood

- **Hypotonic solution**
  - (0.075M) 0.56g KCl and 100 ml distilled water (Prepared fresh and kept in incubator an hour prior to harvesting).

- **NP-40 in 0.4 X SSC wash solution (0.3%)**
  - Distilled water - 47.5 ml
  - 20X SSC- 1 ml
  - N P-40 - 0.15 ml

- **NP-40 in 2X SSC wash solution (0.1%)**
- Distilled water - 42-45 ml
- 20X SSC - 5 ml
- NP-40 - 0.5 ml

- **Pepsin solution (stock solution) (10%)**
  - Pepsin - 1 g
  - 0.01 N HCl - 10 ml

- **Pepsin working solution**
  - 0.01 N HCl - 40 ml
  - 10% pepsin - 20 ml

- **Phosphate buffer**
  - 2.5 ml KH₂PO₄ (2.966 in 50 ml distilled water)
  - 2.5 ml Na₂HPO₄ (2.268 in 50 ml distilled water)
  - 45 ml distilled water
  - pH - 6.8.

- **Phosphate buffered saline**
  - NaCl - 4 g
  - KCl - 0.1 g
  - Na₂HPO₄·H₂O - 0.7
  - KH₂PO₄ - 0.1 g
  - Make volume 500 ml (pH-7.4)

- **Phytohaemagglutin**
  - 10 mg/10 ml distilled water (stock solution),
  - 1 ml of stock solution in 100 ml media (working solution).

- **Sodium saline citrate (20X) (pH-7)**
  - NaCl - 17.53 g
  - Trisodium citrate - 8.82 g
  - Distilled water - 100 ml
  - pH - 7.

- **Sodium saline citrate (2X)**
  - Distilled water - 45 ml
  - 20X SSC - 5 ml

- **Trypsin solution**
  - Phosphate buffer - 50 ml
• EDTA - 0.0400 g
• Trypsin - 0.0250 g

➤ FOR DNA EXTRACTION:
• Cell lysis buffer: 10 mM Tris Cl (PH 8.0)
• 1mM EDTA (PH 8.0)
• 0.1% w/v SDS
• Ethanol
• Isopropanol
• Potassium Accetate solution: 5M : Potassium acetate
• Glacial acetic acid
• Double distilled water
• Red Blood cell lysis buffer: 20 mM Tris HCl (PH 7.6)
• TRIS EDTA (PH 7.6)

➤ FOR PCR:
• d NTP mixture
• Reaction Buffer (manufacturer composition)
• Dimethyl sulfoxide
• Primers
• PCR master mix (Bio Basic)
• Nuclease free milliQ

➤ FOR AGAROSE GEL ELECTROPHORESIS:
• Powdered agarose.
• Electrophoresis buffer.
• 10X Gel loading buffer: The loading buffer contained 0.25% bromphenol blue (BMB) and 0.25% xylene cyanol as tracking dyes and 40% W/V glycerol.
• Ethidium bromide solution was prepared as a stock solution at a concentration of 10 mg/ml in water.
APPENDIX- VI

STATISTICAL ANALYSIS

1. Percentage frequency

\[ \frac{\text{No. of single pattern}}{\text{Total no. of patterns}} \times 100 \]

2. Arithmetic mean (\( \bar{X} \))

\[ \bar{X} = \frac{\sum X}{N} \]

\( \sum X \) = Sum of values

\( N \) = Number of individuals

3. Standard deviation

\[ \sigma = \frac{\sum f(X - \bar{X})^2}{\Sigma} \]

\( \Sigma f \) = Total frequency

\( \sum f(X - \bar{X})^2 \) = Sum of the products of squares of deviations from Arithmetic mean with corresponding frequency.

4. Chi-square test (\( \chi^2 \)-test)

\[ E = \frac{\sum (O - E)^2}{E} \]

\( O \) = Observed frequency

\( E \) = Expected frequency

5. Student t-test

\[ \frac{(\bar{X} - \mu)^2}{S\bar{X}} \]

\( S\bar{X} \) = Standard error of mean
\[ \mu = \text{Population mean} \]

\[ \bar{X} = \text{Sample mean} \]

6. Partial correlation coefficient

\[ r = \frac{r_{12} - r_{13} \cdot r_{23}}{\sqrt{1 - r_{13}^2} \cdot \sqrt{1 - r_{23}^2}} \]

\[ r = \frac{\sum dx \cdot dy - \sum dx \cdot \sum dy}{\sqrt{\sum fdx^2 - \left(\frac{\sum dx}{N}\right)^2} \cdot \sqrt{\sum fdy^2 - \left(\frac{\sum dy}{N}\right)^2}} \]

\[ r = \text{Coefficient of correlation} \]

\[ dx = \text{Deviation of x series from assume drive} \]

\[ dy = \text{Deviation of x series from assume drive} \]

\[ N = \text{No. of pairs of observation} \]

7. Analysis of variance (ANOVA)

Calculation of sum of squares between columns (SSC) and sum of squares between rows (SSR).

\[ \frac{\sum (X1)^2}{N} + \frac{\sum (X2)^2}{N} + \frac{\sum (X3)^2}{N} + \frac{\sum (X4)^2}{N} - \frac{T^2}{N} \]

Sum of squares due to errors = SSC + SSR.

Mean squares of columns (MSC) and rows (MSR).

\[ \text{MSC} = \frac{\text{SSC}}{c - 1} \]

\[ \text{MSR} = \frac{\text{SSR}}{r - 1} \]
Mean square error (MSE) = \frac{\text{SSE}}{(c-1)(r-1)}

8. ODDS RATIO

\[ \frac{p_1}{1-p_1} = \frac{p_1}{q_1} = \frac{p_1q_2}{p_2q_1} \]

\[ \frac{p_2}{1-p_2} = \frac{p_2}{q_2} = \frac{p_2q_1}{p_1q_2} \]

\( p_1 \) (first group) and \( p_2 \) (second group)

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

9. Fisher’s Exact Test

\[ P = \frac{(a+b)(c+d)}{a} = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{a!b!c!d!n!} \]

\( P = \) Probability

\( a, b, c \) and \( d \) = Set of frequencies \( a, b, c \) and \( d \) in a 2 x 2 contingency table, given fixed row and column marginal totals and sample size

\( n = \) Sample Size
APPENDIX- VII
STASTICAL SOFTWARES

1. WINDOW 2007 EXCEL
2. BIOSTAT V2.0
3. SPSS V 17
4. MEGASTAT

APPENDIX- VIII
STASTISTICAL CALCULATIONS

Frequency of various parameters were calculated in VV and Normal population and the data was analyzed using standard software like SPSS V 17.0, MEGASTAT 9, Microsoft word Excel 2007 and STATS V 2.0 for assessment of various values and test scores. Various other Non parametric tests like chi square and Fisher’s exact analysis were used to evaluate the different parameters in the study. Odds ratio analysis to understand the correlation between the various risk factors in aetiology of varicose veins was used. Calculation of one way and two way ANOVA was done on the data of varicose veins individuals and normal subjects under different age groups and sex ratio.
CURRICULUM VITAE

SHIKSHA SHARMA

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Deptt. Of Genetics,  
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Career Objective

- Aim for progressive and innovative growth as a professional and add value to a team, an organization and the society.

Qualification

- M.Sc (Genetics) from M.D.U. Rohtak (Hr) in year (2008).

Technical Skills

- Computer : Knowledge of SPSS Package.  
- MS Office, Internet.

Cytogenetic, Mol. Biology & Biotechnology Techniques:

- 15/02/2008-25/02/2008: Training on Molecular Biology & Biotechnology Techniques from Chemind Diagnosis & Biosolutions, Jaipur 302019, (Rajasthan) India  
- DNA isolation from Blood, Plants & Plasmid  
- Competent cell preparation & Transformation  
- Agarose Gel Electrophoresis & PAGE  
- Real Time PCR/ RT-PCR and RNA Isolation from Blood  
- Lymphocyte Culture & Karyotype Preparation  
- RAPD Analysis and ELISA Test

Professional Experience

- 01/12/2008-13/12/2008: Faculty Development Program on Entrepreneurship Development & Small Business Management, Deptt. Of Science & Technology, Govt.of India conducted by Hardicon Limited at Vaish Technical Institute, Rohtak (Haryana).
**Personal Interest**

- Interests in creative arts, crafts and technical skills.
- Participated in quizzes, exhibitions and debates, drama & other cultural activities in School & College.

**References**

- Dr. Minakshi Vashist, Professor and Head, Deptt. Of Genetics, MDU, Rohtak.
- Prof. J.P. Yadav, Deptt. Of Genetics, MDU, Rohtak.
- Prof. Ravi Prakash, Deptt. Of Genetics, MDU, Rohtak.

**Personal Details**

- Nationality : Indian
- Date of Birth : 28.10.1986
- Father’s Name : Sh. Randhir Sharma
- Mother’s Name : Smt. Nirmala Devi
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- Gender : Female
- Marital Status : Married
- Languages Known : English & Hindi

I hereby declare that all above entries furnished by me are true as per my knowledge in case of any doubt I shall be responsible for that.

Date :

Place : (Shiksha Sharma)
Publications

Paper Published:


- Minakshi Vashist., Ritu Yadav, **Shiksha Sharma** 2011. Study of prevalence, sex ratio and different level of intellectual disability in Haryana. Indian journal of public health research & development. (Accepted) (No.-2619/IJPHRD/2011).
