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Research Paper

Genetic Polymorphism of TMPRSS6 (RS855791) Gene and Its Association with Anaemia in Tribal Population of Madhya Pradesh.

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ARTICLE DETAILS

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ABSTRACT

Anemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. This problem is the result of a wide variety of causes that can be isolated, but\ more often coexist. Iron-Deficiency Anemia (IDA) is the condition in which the hemoglobin concentration is abnormally low due to iron deficiency, which occurs when there is a long period of negative balance between the amount of biologically available iron and the need for this trace element. On the other hand, iron deficiency or iron depletion is characterized by the decreased iron stores, but the amount of functional iron is not affected. Thus, even though both are related to the same pathological condition, they are characterized by different degrees of deficiency. In our findings, Anaemia patients had markedly reduced levels of weight of men (P=0.0026**) and women (P= P<0.0001***) thus BMI of Women (P=0.0042**) and Men (P=0.0080**) was also significantly different between anaemia patient and healthy tribal population. PCR amplification with specific primers gave 473-bp product which was digested with *StuI* enzyme (New England Biolabs, Boverly, MA) for 16 h at 37˚C. The wild-type genotype (CC) was not digested and gives single 249 bp intact band, whereas the mutated homozygous genotype (TT) was given one band of 125bp (after enzymatic digestion of T allele, gel picture showing single band because of approx same molecular weighted product generated). significant level of change has been seen in overall distribution of TMPRSS6 genotypes in Healthy control group as compared to disease group although Healthy control group showed little increase in 'TT' genotype as compared to Patients of Anaemia (69.44% *vs* 56.25%). Similarly, mutant type 'CC' genotype was present in low frequency in Anaemia patients group 3.75% and also in control group 4.44% (χ^2 = 0.0243*), P= 7.436). '*TT*' genotype is higher in control group and may be protective in our population and statistically significantly different between both groups.

1. INTRODUCTION

Iron deficiency is the most common nutritional disorder worldwide. As well as affecting a large number of children and women in non-industrialized countries, it is the only nutrient deficiency which is also significant prevalent in many industrialized nations. Menstruating women have been recognized to be among the most likely individuals to develop iron deficiency anemia (IDA) and its prevalence is 6–22% in developed countries **[1,3].** Even though menorrhagia serves as a major contributing factor of IDA in this population, it is not uncommon that women with heavy menstrual loss have normal hemoglobin (Hb) levels and adequate iron stores. Individual susceptibility to IDA varies. However, whether genetic variants play a role in IDA pathogenesis remains unclear. Hepcidin is the core of iron metabolism and is tightly regulated by several mediators. Matriptase-2 is an important one and down regulates hepcidin expression through cleaving membrane-bound hemojuvelin, which can enhance hepcidin transcription. Complete loss of function mutation of matriptase-2 leads to a rare disease, iron-refractory iron deficiency anemia (IRIDA) **[2,4].** Patients with IRIDA have high serum hepcidin levels and cannot absorb iron from intestine. It is of interest that whether genetic variants with incomplete

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loss of function of matriptase-2 will be associated with IDA in general population. Recently, several genome-wide association studies identified several single nucleotide polymorphisms (SNPs) that influence blood cell phenotypes. Among these SNPs, rs855791 has the strongest association with red blood cell indices and iron parameters in general population. This SNP is located in the functional part of *TMPRSS6* and the rs855791 (2321 C>T) causes a nonsynonymous substitution that reduces the ability of the enzyme to inhibit hepcidin transcription. To investigate the role of this genetic variant rs855791 on the susceptibility of IDA in menstruating women, we conducted this case-control study **[1-5].**

The tribal population groups of India are known to be the autochthonous people of the land. Madhya Pradesh (undivided) is the largest state of the country. The state has a total population of 603.48 lakhs as per 2001 census and ranks sixth in the country, with a total tribal population of 120.69 lakhs. There is about 23% of the total tribal population of the country. Nutritional status of the population largely depends on the consumption of food in relation to their needs; which in turn is influenced by the availability of food and purchasing power. The literature on the tribal nutrition is very scanty **[6,8].** We concentrate mainly on the studies carried out among the seven primitive tribes and also the other regional health problems studied by the centre India is a vast, ethnically diverse country and the people inhabiting it are as diverse as the land itself. The large Indian population is multi-ethnic and divided into subgroups. Tribal communities in India constitute the largest tribal population in the world. Most of them have been practising endogamy for a long period of time, due to which tribal communities are highly vulnerable to various hereditary diseases. Because of their remote and isolated living, tribal groups are difficult to reach **[7,9].** Iron deficiency anaemia (IDA) is a major public health problem in the Indian subcontinent. As a result, most children presenting with microcytic hypochromic anemia end up receiving oral iron trial without systematic investigation. This has led to physicians now having to tackle oral iron refractoriness more often. Moreover, the recent recognition of iron refractory iron deficiency anaemia (IRIDA) as a new genetically linked entity, the same, now needs to be considered in any systematic approach to diagnosis **[6-9].**

The IRIDA genotype involves mutation in the TMPRSS6 gene on chromosome 22qc, encoding the type II serine protease, transmembrane protease, serine 6 (TMPRSS6; also termed matriptase-2) a negative regulator of hepcidin transcription. Hepcidin is a circulatory hormone that is responsible for the inhibition of duodenal iron absorption and macrophage iron recycling when body iron is replete **[11,14].** TMPRSS6 is responsible for the repression of hepcidin when body iron stores are depleted. Any loss of function mutation in TMPRSS6 leads to elevated hepcidin levels and hence iron absorption is hampered. This is also the reason why patients with IRIDA do not respond to oral iron supplements. However, key features of the IRIDA phenotype include varying degrees of anaemia, moderate-severe microcytosis, oral iron refractoriness, low serum iron, low-normal (lower end of normal range) to normal serum ferritin and inappropriately high hepcidin levels for the degree of anaemia **[10,12].** The frequency of IRIDA phenotype and the underlying gene variations is currently not known but is likely to have been under diagnosed or mis-diagnosed until now, especially in our setting. We believe that it is important to systematically evaluate and define this phenotype in an iron deficient endemic cohort so that genetic screening for TMPRSS6 gene variations can be cost effectively undertaken n a resource constraint setting. axonal damage followed by patient death occur. Exact etiology of these disorders has not been established yet. It is commonly accepted that MS is an autoimmune disease, and CD4 + T cells being responsible for the synthesis of interferon and interleukin 17 play a significant role in its pathogenesis. Both processes are closely related to the iron balance in a human body **[10-14].**

2. MATERIALS AND METHODS:

2.1 Study Population:

Our tribal population consisted of 340 unrelated subjects comprising of 160 Aneamia patients and 180 ethnically matched controls of central Indian population were included in this study. In this tribal population, most of people economically weak are mainly living in Rewa Sidhi, Satna and Sahdol district.

2.2 Inclusion and Exclusion criteria for Cases:

Cases included consecutive patients who attended the Department of Medicine, Shyam Shah Medical College and Sanjay Gandhi Memorial Hospital, Rewa, Ayurveda Medical College, Rewa, Ranbaxy pathology regional collection centre Rewa, District hospital Satna, Shahdol, Sidhi. Aneamia was diagnosed in accordance with World Health Organization (WHO Expert committee 2003) criteria. Pregnant women, children under age of 18 years and any patients with Aneamia were excluded from the study.

2.3 Inclusion and Exclusion criteria for Controls:

Control group composed of healthy individuals that were collected during “Aneamia Awareness Camps” organized in urban regions in and around SSMC Rewa and many volunteers were also included to collect control sample. The control subjects were recruited from the regions that from homogenous cluster in Vindhyan region India in accordance with a recent report of genetic landscape of the people of India. (Indian Genome Variation Consortium 2008)

2.4 Anthropometry:

Height and Weight were measured in light clothes and without shoes in standing position as per standard guidelines. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in standing position midway between iliac crest and lower costal margin and hip circumference was measured at its maximum waist to hip ratio (WHR) was calculated using waist and hip circumferences. Systolic and diastolic blood

pressures were measured twice in the right arm in sitting position after resting for at least 5 minutes using a standard sphygmomanometer and the average of the two reading was used.

2.5 Biochemical Analysis:

Biochemical parameters related to Aneamia were estimated for both cases and controls subjects. Measurement of Serum levels of Post-Prandial Glucose (mg/Dl), HbA1C(%), HDL-C(mmol/L), LDL-C (mg/dL), TG(mg/dL), Systolic BP (mmHg, Diastolic BP (mmHg), Ferritin (ng/ml) Plasma Iron levels, (lmol/l), Serum calcium (mg/dl) , Serum Iron (mg/dL), Plasma Hepcidin levels (ng/ml), CRP [mg/L] and AGP [mg/L] were measured based on spectrophotometric method using automated clinical chemistry analyzer Cobas Integra 400 plus (Roche Diagnostics, Mannheim, Germany).

2.6 Blood collection and plasma/serum separation:

Venous blood samples were obtained from the subjects after 12 hours of overnight fasting in vacutainers with and without appropriate anti-coagulants. Immediately, plasma and serum from the respective vacutainers were separated by centrifuging the tubes at 1000 rpm for 10 min. at 4˚C

2.7 Method for DNA isolation:

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller and coworkers (Miller *et al*. 1988). Frozen blood sample was thawed at room temperature. 0.5 ml. of whole blood sample was suspended in 1.0 ml. of lysis buffer (0.32 M Sucrose, 1 mM Mgcl2, 12 mM Tris and 1% Triton-X-100) in a 1.5 ml. microcentrifuge tubes. This mixture was mixed gently by inverting the tube upside down for 1 min. The mixture was then allowed to stand for 10 min. at room temperature to ensure proper lysis of cells. The mixture was centrifuged at 11,000 rpm for 5 min. at 4˚C to pellet the nuclei. The supernatant was discarded carefully in a jar containing disinfectant, as pellet formed is loosely adhered to the bottom of centrifuge tube. The pellet was resuspended in 0.2 ml. of lysis buffer and recentrifuge at 11,000 rpm for 5 min. The pellet was than dissolved in 0.2 ml. of deionized autoclaved water and mixed thoroughly on vortexer. The mixture was centrifuged at 14,000 rpm for 1 min. at 4˚C. Supernatant was discarded to gain an intact pellet. To the above pellet, 80 µl. of proteinase K buffer (0.375 M Nacl, 0.12 M EDTA, pH 8.0) and 10 µl. of 10% SDS (10% w/v SDS, pH 7.2) was added. Mixture was well frothed with the help of micro tip to allow proper lysis of pelleted nuclei. After digestion was complete, 100 µl. of saturated cold 5M Nacl was added and shaken vigorously for 15 sec. To the above mixture 0.2 ml. of deionized, autoclaved water and 0.4 ml. of phenol-chloroform (4:1 v/v) was added to remove most of the non-nucleic acid organic molecules. Microcentrifuge tube was inverted upside down until the solution turned milky. Phases were separated by centrifuging the above mixture at 12,000 rpm for 10 min. at 4˚C. Aqueous (top) layer was saved and transferred in another microcentrifuge tube. Transferring of any interface layer was avoided. To the aqueous layer, 1 ml. chilled absolute ethanol was added and the tube was inverted several times until the DNA precipitated. DNA precipitates like thread. This was centrifuged at 14,000 rpm for 4 min. at 4˚C to pellet the DNA thread. Supernatant was discarded. The pellet was washed twice with 1 ml. of 70% alcohol. The mixture was again centrifuged at 14,000 rpm for 1 min. 4˚C. Supernatant was discarded and pellet was air dried for 10-20 min. The pelleted DNA was rehydrated in 100-200 µl. of TE buffer pH 7.4 (10 mM Tris-HCL pH 7.4, 1mM EDTA, pH 8.0). DNA was allowed to dissolve overnight at 37˚C before quantization.

2.8 Determination of quality and quantity of isolated DNA:

The isolated DNA is to be used for PCR based study. Therefore, its suitability for PCR along with its size heterogeneity is among the most important criterion for purity. As a matter of general practice all DNA preparations were tested for quality and quantity measures, as described in the following paragraphs.

2.9 Quantitation by UV spectrophotometry:

The isolated genomic DNAs were then tested for purity by measuring their absorbance values at 230 nm, 260 nm, 280 nm and 300 nm using a UV visible spectrophotometer (Systronic, India). A DNA preparation was considered to be good if it had A 260 nm / A 280 nm ratio as approximately 1.8 and A 300 nm was 0.1 or lesser. The absorbance at 260 nm was used to calculate the amount of DNA, using the relationship that double stranded DNA at 50µg/ml concentration has an absorbance= 1.0 at 260 nm.

2.10 Agarose Gel Electrophoresis:

Gel electrophoresis of the genomic DNAs was carried out for qualitative estimation of samples prepared. A good DNA preparation appears as single band. A horizontal agarose slab gel electrophoresis apparatus (Bangalore Genei, Bangaore, India) was used. In brief, 4-5 µl of each genomic DNA was loaded on 0.8 agarose (0.8 % w/v, Sigma) containing ethidium bromide solution (0.5 µg/ml) and electrophoresis was done at 80 V in 1x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA). Lambda DNA *Eco*RI / *Hind* III double digest (Bangalore Genei, Bangalore, India) was used as molecular weight marker after completion of electrophoresis, the DNA bands were visualized and photographed using an UV transilluminator (312 nm) and gel documentation system (Vilber Lourmate, Cedex 1, France) respectively.

2.11 Polymorphism screening:

In general, the genomic DNA extracted from peripheral blood of healthy individuals and diseased individuals was subjected to PCR followed by restriction digestion and electrophoresis to genotype both the groups for relevant gene of interest. All the PCRs were carried out in a PTC 200 thermal cycler (MJ Research Inc. USA). PCR is a rapid, inexpensive and

simple mean of producing relatively large copy number of DNA molecules from the small amounts of source DNA material, even when the source DNA is of relatively poor quality. Due to the extreme sensitivity, precautions were taken against contamination of the reaction mixture with the trace amounts of DNA, which could serve as an unwanted template. Appropriate negative control was included in each PCR run carried out for each gene, to monitor this contamination of PCR mix to avoid any false positive results. The negative control used for PCR contained whole PCR reaction mix except target DNA which was replaced by HPLC purified water free of RNase, DNase, and any contamination from any other source resembling the gene sequence.

Subsequently restriction enzyme digestion was performed by incubating the double stranded DNA with appropriate amount of restriction enzyme, in its respective buffer as recommended by the supplier and at optimal temperature for that specific enzyme. A typical digestion includes one unit of enzyme per microgram of starting DNA. One enzyme unit is usually defined as the amount of enzyme needed to completely digest one microgram of double stranded DNA in one hour at the appropriate temperature. Their biochemical activity of the restriction enzyme is the hydrolysis of phosphodiester backbone at specific sites in a DNA sequence. Precaution was taken to avoid star activity of restriction enzymes. When DNA is digested with certain restriction enzymes under non-standard conditions, cleavage can occur at sites different from the normal recognition sequence. Such aberrant cutting is called "star activity" which can be due to high pH (>8.0) or low ionic strength, extremely high concentration of enzyme (>100 U/µg of DNA) and presence of organic solvents in the reaction (e.g. ethanol, DMSO). The PCR and restriction digestion conditions were optimized for specific locus of relevant segment of the gene to be studied. The PCR products as well as the digested products were separated on either agarose gel or polyacrylamide gel depending on their size. Gels were stained with ethidium bromide solution (0.5 µg/ml) and subsequently visualized and photographed under UV transilluminator.

2.12 Detection of TMPRSS6 (Rs855791) Single Nucleotide Polymorphism:

The **TMPRSS6** Gene has been amplified by PCR. The TMPRSS6 gene encodes the transmembrane serine protease 6 (TMPRSS6), a key regulator of iron metabolism. TMPRSS6 polymorphisms importantly rs855791 is found to play an essential role in iron homeostasis in the human body. The rs855791 (T > C) polymorphism is highly associated with iron levels, and multiple blood parameters, leading to IDA. The oligonucleotide sequence (primers) was designed to amplify the gene wild type gene is 249bp having restriction site for *StuI* enzyme cleaves in to 125 and 124bp fragment.

2.13 Primer sequence: The oligonucleotides sequences (primers) used were those described by F Yukcu ***(Yukcu F et. al. 2015).***

Forward primer: 5'- AAGCATACGATGGCCAAAACTTCTGCA-3'
Reverse Primer: 5' -GAACTAGCATTGGAACTTTTCCCAACC-3'

2.14 PCR Mix:

The PCR was carried out in a final volume of 25 µl, containing 50-100 ng of genomic DNA(4-5 µl), 2.5 µl of 10X *Taq* polymerase buffer (10 mM Tris HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concentration 1X; Genetix Biotech Asia Pvt. Ltd.,India), 1 µl of 10 mM dNTPs (Banglore Genei, Bangalore, India), 1 µl of 25 pmol/µl of forward and reverse primers specific for and 1 µl of unit of 1U/ µl Red Taq DNA polymerase (Bangalore genei).

2.15 PCR Thermal Program:

After an initial denaturation of 5 min at 95˚C, the samples were subjected to 35 cycles at 95˚C for 1 min, at 58˚C for 45 s, and 72˚C for 45 s, with a final extension of 10 min at 72˚C in a thermal cycler. A 100bp ladder with amplified product has been run under 2.5 % agarose gel electrophoresis.

2.16 Restriction digestion:

The amplified product size of 249 base pairs (bp) was digested by the specific restriction enzyme, *Stu1.* for 16 h at 37˚C. The mutated genotype was digested, in to 125 bp and 124 bp. The wild-type genotype (CC) was not digested, whereas the mutated homozygous genotype (TT) was cut as a doublet of 125 and 124 bp. The heterozygous genotype (TC) was represented as 2 fragments of 249 and 125 bp whereas 124 bp. The digestion products were then separated by electrophoresis on a 2.5% agarose gel. The results were documented by digital camera and further saved by gel documentation system.

3. RESULTS:

3.1 Anthropometric results:

The descriptive data and comparison of anthropometric and biochemical parameters of Anemia patients versus controls are presented in Table no. 4.1. The age, sex, BMI, WHR were the parameters. As expected, the Anaemia patients had markedly reduced levels of weight of men (P=0.0026**) and women (P= P<0.0001***) thus BMI of Women (P=0.0042**) and Men (P=0.0080**) was also significantly different between anaemia patient and healthy tribal population. Thus WHR in Women (P=0.1741) and Men (P=0.0973) were not found significantly different between case and control group **(See Table No. 1).**

Table 1. Comparison of anthropometric parameters of Anaemia patients and healthy controls

Characteristics	Cases (160)	Controls(180)	P-value
n(Men/Women)	160(104/56)	180(118/62)	
Age(years)	52.5±12.5	52.6±12.4	0.9411,ns
Height(m)	162.50±11.3	161.2±12.4	0.3152,ns
Weight (Kg)			
Women	52.5 ±4.7	61.6 ± 4.5	P<0.0001***
Men	65.90±5.60	67.80±5.90	0.0026**
BMI (kg/m^2)			
Women	24.6±3.1	26.1 ± 4.3	0.0042**
Men	24.6±3.7	25.8± 5.1	0.0080**
Waist circumference (cm)			
Women	92.5±6.2	93.6±6.7	0.1186,ns
Men	90.0±7.0	89.0±6.0	0.1571,ns
Hip (cm)			
Women	95.9±2.4	96.1±2.2	0.4233,ns
Men	90.8±4.3	91.2±1.5	0.2426,ns
WHR			
Women	0.97±0.05	0.98±0.08	0.1741,ns
Men	0.98±0.08	0.99±0.01	0.0973,ns

(*denotes level of significant change between case and control)

3.2 Biochemical and Clinical Findings:

Biochemical test performed in the blood sample for following clinical parameters and the findings were tabulated. Statistical analysis was done by using student's t-test and p value suggests the level of significant were changed here. The descriptive data and comparison of biochemical parameters of Anaemia patients versus healthy controls are presented in Table no. 4.2. As expected, the Anaemia patients had markedly reduced levels of Ferritin (P<0.0001), Plasma Iron levels (P<0.0001) and Serum Iron (P<0.0001) compared to that of control subject. Whereas rest of all parameters were not significantly different between patient and healthy population**.**

Table -2. Comparison of Biochemical and clinical findings of Anaemia patients and healthy controls.

Characteristics	Cases (160)	Controls(180)	P-value
Post-Prandial Glucose (mg/Dl)	118.7±12.4	119.4±11.6	0.5912,ns
HbA1C(%)	5.9±0.7	5.8±0.8	0.2235,ns
HDL-C(mmol/L)	108.8±12.2	109.3±11.6	0.6989,ns
LDL-C (mg/dL)	42.1±2.6	41.8±3.7	0.3932,ns
TG(mg/dL)	125.9±13.2	126.2±12.2	0.8278,ns
Systolic BP (mmHg)	125.4±8.1	124.8±5.7	0.4263,ns
Diastolic BP (mmHg)	87.1±5.8	86.5±6.2	0.3593,ns
Ferritin (ng/ml)	86.5±5.6	160.8±8.4	P<0.0001***
Plasma Iron levels, lmol/l	9.58±4.96	22.62±3.57	P<0.0001***
Serum Iron (mg/dL)	54.45±3.47	96.71±5.26	P<0.0001***
Serum calcium (mg/dl)	9.42±0.32	9.46±0.38	0.2978,ns
Plasma Hepcidin levels (ng/ml)	147.91±2.9	148.51±3.1	0.0672,ns
CRP [mg/L]	7.9±1.47	8.1±1.26	0.1777,ns
AGP [mg/L]	634.91±3.9	635.51±4.1	0.1691,ns

(* denotes the level of significant change between case and control)

3.3 Detection of Genetic Polymorphism In TMPRSS6 (Rs855791) Gene:

PCR amplification with specific primers gave 473-bp product which was digested with *StuI* enzyme (New England Biolabs, Boverly, MA) for 16 h at 37˚C. The wild-type genotype (CC) was not digested and gives single 249 bp intact band, whereas the mutated homozygous genotype (TT) was give one band of 125bp (after enzymatic digestion of T allele, gel picture showing single band because of approx same molecular weighted product generated). The heterozygous genotype (TC) was represented as 2 fragments of 249bp and 125bp.

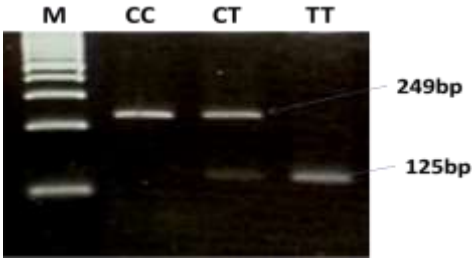


Fig .-1: Representative gel picture of TMPRSS6 polymorphism. Lane M represents 50 bp molecular marker, Lane CC Wild type genotype, Lane TC heterozygous genotype and Lane TT variant genotype.

The distribution of the polymorphisms of TMPRSS6 was consistent with Hardy- Weinberg equilibrium (HWE) in healthy controls. The observed genotype frequencies, allele frequencies and carriage rates for TMPRSS6 polymorphism are depicted in table 4.5 and table 4.6 and Graph 1, 2, 3. significant level of change has been seen in overall distribution of TMPRSS6 genotypes in Healthy control group as compared to disease group although Healthy control group showed little increase in 'TT' genotype as compared to Patients of Anaemia (69.44% *vs* 56.25%). Similarly, mutant type 'CC' genotype was present in low frequency in Anaemia patients group 3.75% and also in control group 4.44% (χ^2 = 0.0243*), P= 7.436). '*TT*' genotype is higher in control group and may be protective in our population and statistically significantly different between both groups. An odds ratio of TT genotype is 0.5657 which indicates little protective effect whereas an odds ratio of TC genotype is 1.887 of Anaemia patients group respectively indicate little or no effect and association of this mutant genotype with the Anaemia susceptibility. Overall allele 'C' was found little lower frequency in disease group as compared to HC group whereas allele '*T*' was present in little high frequency in the disease group but the difference is nominal and was not significant (χ^2 = 0.0437*, P=4.069). Carriage rate of allele 'T' was slightly high in Anaemia group as compared to healthy control (75.77% Vs 68.75%) whereas carriage rate of allele '*C*' was approximately similar in both control and disease group and no significant level of change has been seen. The pattern of genotype distribution, allele frequency and carriage rate in disease and control group suggests **TMPRSS6** polymorphism is significantly associated with Anaemia in our population.

Table 3. Frequency distribution and association of Genotype, allele frequency and carriage rate of TMPRSS6 gene polymorphism in population of Vindhyan region using Chi Square Test

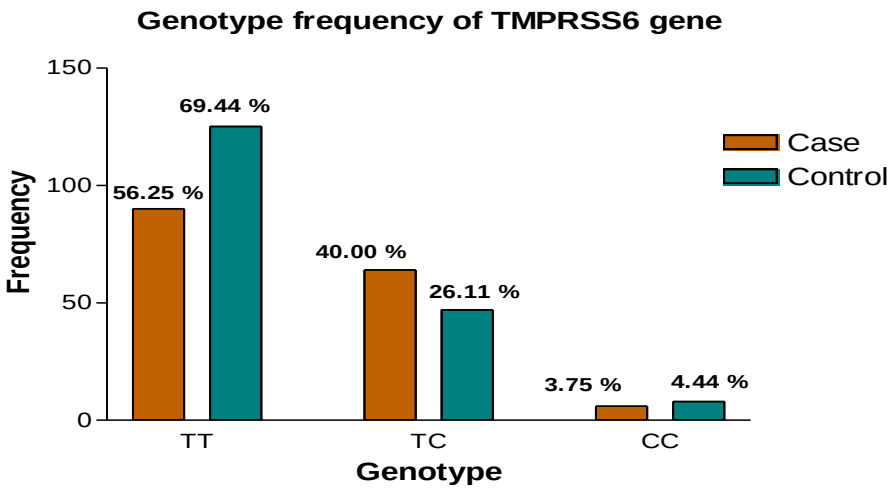
TMPRSS6 GENE	**CASE N= 160**		**CONTROL N=180**		**CHI SQUARE VALUE χ^2 (P Value)**
	N	**%**	**N**	**%**	
Genotype					
CC	90	56.25	125	69.44	
CT	64	40.00	47	26.11	
TT	6	3.75	8	4.44	7.436 (0.0243*)
Allele					
C	244	76.25	297	82.50	
T	76	23.75	63	17.50	4.069 (0.0437*)
Carriage Rate					
C	154	68.75	172	75.77	
T	70	31.25	55	24.22	2.774 (0.0958ns)

(- denotes the level of significant association between case and control.)*
(N – Number of individuals in study group.)
(% - Genotype allele frequency and carriage rate expressed in percentage.)

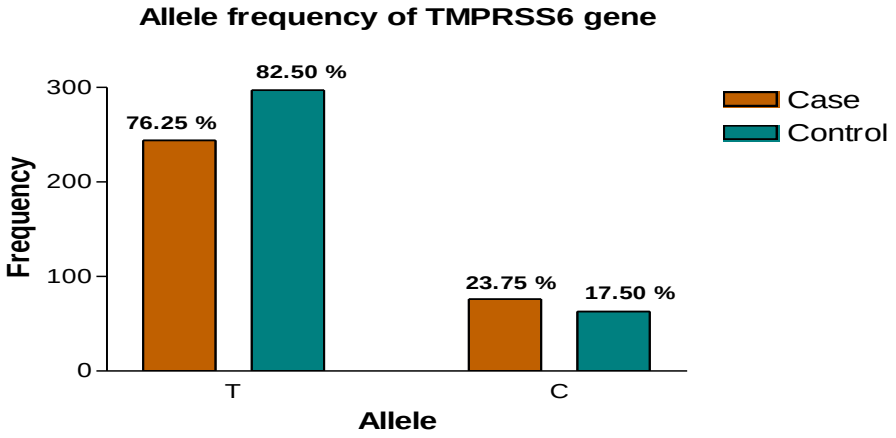
Table 4. Fisher Exact Test values of TMPRSS6 gene polymorphism

TMPRSS6 GENE	**CASE N= 160**		**CONTROL N=180**		**P Value**	**Odds Ratio (95% confidence interval)**
	N	**%**	**N**	**%**		
Genotype						
CC	90	56.25	125	69.44	0.0133*	0.5657 (0.3624 to 0.8830)
CT	64	40.00	47	26.11	0.0077**	1.887 (1.192 to 2.986)
TT	6	3.75	8	4.44	0.7914ns	0.8377 (0.2842 to 2.469)
Allele						
C	244	76.25	297	82.50		0.6810 (0.4683 to 0.9904)
T	76	23.75	63	17.50	0.0459*	1.468 (1.010 to 2.135)
Carriage Rate						
C	154	68.75	172	75.77	0.1144ns	0.7035 (0.4646 to 1.065)
T	70	31.25	55	24.22		1.421 (0.9388 to 2.152)

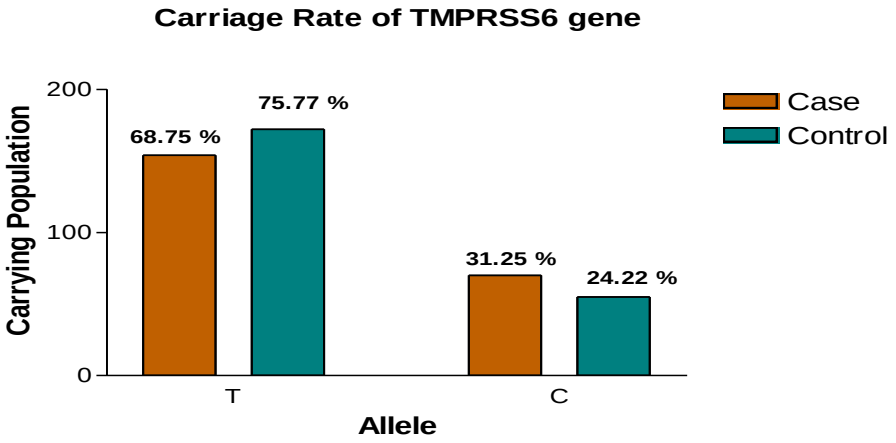
(* - denotes the level of significant association between case and control.) (N – Number of individuals in study group.)
(% - Genotype allele frequency and carriage rate expressed in percentage.)



Graph No.-1: Genotype Frequency of TMPRSS6 gene.



Graph No.-2: Allele Frequency of TMPRSS6 gene.



Graph No.-3: Carriage rate of TMPRSS6 gene.

4. DISCUSSION:

Iron deficiency is the most common nutritional disorder worldwide and accounts for approximately one-half of anemia cases. The diagnosis of iron deficiency anemia is confirmed by the findings of low iron stores and a hemoglobin level two standard deviations below normal. Women should be screened during pregnancy, and children screened at one year of age. Men and postmenopausal women should not be screened, but should be evaluated with gastrointestinal endoscopy if diagnosed with iron deficiency anemia. The underlying cause should be treated, and oral iron therapy can be initiated to replenish iron stores **[3,14,].** Parenteral therapy may be used in patients who cannot tolerate or absorb oral preparations**.** Iron is an essential trace element for various cellular proteins and for biological processes in all cells. Severe iron deficiency (ID) impairs haem synthesis, reduces erythropoiesis and causes iron deficiency anaemia (IDA). Iron restriction in anaemia of inflammation is mainly due to retention of iron in macrophages. This condition is known as 'functional iron deficiency'. A review of studies performed in Europe shows that the prevalence of ID and IDA in young children varies by region. It is more common in eastern than western European countries **[15,17].** This overview summarizes information on the need for iron supplementation in children, and the current understanding of the regulatory mechanisms of iron homeostasis and iron restricted erythropoiesis. The causes of anaemia during infection and the usefulness of classical and new indicators to distinguish absolute from functional iron deficiency are discussed **[15-18].**

In Central Indian tribal population on revealed different causes of aneamia which constitutes mainly of Madhya Pradesh, Chhattisgarh and its adjoining area, holds 23% of the total population of the country. Madhya Pradesh and Chhattisgarh has about 46 Scheduled Tribes of which 7 of them are declared as primitive tribes. The primitive tribes are Saharias of Chambal division, Bharias of Patalkot, Baigas of Baigachak area, Hill Korbas and Birhors of Sarguja, Kamars of Raipur and Abujhmarias of Bastar **[21].** Due to different socio-cultural milieu, different diseases are prevailing among them. However, some of the diseases are common among all these primitive tribes: like acute respiratory infections, sexually transmitted diseases, diarrhoeal diseases and nutritional disorders are common among all these tribes. Some of the genetic disorders like sickle cell anaemia, thalassaemia are restricted to their clan because of consanguineous marriages **[19].** Baiga tribe has highest prevalence (22%) of Sickle cell disease, followed by Abujhmaria tribe (17%) and Bharia tribe (13.7%). Sickle cell anaemia was absent among Saharia, Hill Korba, Kamar and Birhor tribes. Thalassaemia was commonly seen among the Hill Korba (10%), Saharia (8.7%) and Kamar (7 %) tribe, while thalassaemia was absent among Abujhmaria, Baiga and Bharia tribe. G6PD deficiency was seen among all these tribes except Birhors. About 21% of the Birhor pre-school children were severely malnourished followed by Kamar (10%), Saharia and Bharia (9%), Abujhmaria (8%) and Baiga (7%) children. Hookworm infestation was 27% among Abujhmarias followed by Baigas 13% and 7% among the Bharias. Nutritional anaemia ranged from 30% to 100% among 6-14 years children of these tribes **[16,20].** Recent study carried out in the Bijadandi block revealed that Iron deficiency anaemia was 94% among the tribal adolescent girls. Some of the tribes also had different diseases, which are restricted to some specific geographical area. Yaws was restricted to Abujhmarias of Bastar only. Intervention study with IFA supplementation among tribal adolescent girls of Bijadandi block, Mandla district, Madhya Pradesh, India. Girls (n 274) from twelve villages randomly selected out of 100 potential villages received daily tablets containing iron (III) hydroxide polymaltose complex equivalent to 100mg of elemental Fe and 350mg of folic acid **[19-22].**

The potential use of anthropometric parameters and their optimal cut-off value for predicting the anemia status of adolescent girls. This cross-sectional study analyzed data from 2,184 adolescent girls aged 15–19 years from West Bandung and Sumedang districts of Indonesia who participated in the Better Investment for Stunting Alleviation Program (BISA). Anthropometric parameters studied were Body Weight (BW), BMI-for-Age z-Score (BAZ), Waist Circumference (WC), Waist-to-Hip Ratio (WHR), and Waist-to-Height Ratio (WHtR) and all were measured according to WHO procedure **[1].** Receiver Operating Characteristics (ROC) was used to analyze the potential of anthropometric parameter to predict anemia status. Based on multivariate analysis, a significant correlation was found between age (OR=0.88; 95% CI:0.79–0.97) and WHtR (OR=0.12; 95% CI:0.02–0.63) with anemia status. The ROC analysis revealed that the WHtR parameter had the highest Area Under the Curve (AUC) for predicting anemia, although with a very low accuracy (AUC=0.529). The optimal cut-off with value of WHtR for adolescent girls was ≤0.44. This study suggests that WHtR is a potential parameter for early detection of anemia status among adolescent girls and needs to be confirmed with further studies **[21].** Our statistical data from anthropometric analysis suggested that body mass parameter such as BMI was significantly associated with aneamia in tribal population. Iron deficiency aneamia causes loss of weight. The age, sex, BMI, WHR were anthropometric parameters. As expected, the Anaemia patients had markedly reduced levels of weight of men (P=0.0026**) and women (P= P<0.0001***) thus BMI of Women (P=0.0042**) and Men (P=0.0080**) was also significantly different between anaemia patient and healthy tribal population. Thus, WHR in Women (P=0.1741) and Men (P=0.0973) were not found significantly different between case and control group.

The clinical symptoms and physical characteristics between normal and anemic group of middle school girls in the Ulsan metropolitan area. It was carried out with 237 subjects (normal 190, anemic subject 47). They were evaluated with a questionnaire and measurement of hematological indices. BMI (kg/m2) of the two groups were 19.54 ± 2.44 (normal girls) and 19.22 ± 2.27 (anemic girls). The hemoglobin concentration of the anemic girls was 10.84 ± 1.17g/dl and the serum iron of the anemic girls represent 35.15 ± 27.47 g/100 ml. The TIBC (Total Iron Binding Capacity) of the anemic girls showed significantly high to 449.30 ± 64.87 g/100 ml. The serum ferritin of the anemic girls was 20.53 ± 42.29 g/ℓ, it represented significantly low **[5,22].** Various laboratory parameters are used to guide the treatment of anemic patients; however, their interpretation may be complicated by the etiology of the anemia. They compared anemia-related laboratory values obtained from 261 patients (174 female) screened prior to participation in a clinical trial for cancer and chemotherapy related anemia (ACD), with those from a group of 50 otherwise-healthy women referred for treatment of anemia related to heavy menses (IDA). Pairwise correlations were explored graphically and analyzed using Pearson's correlation coefficients **[12,23].** Highly skewed data were log transformed. Relationships between Hb and laboratory values associated with anemia were explored using multiple linear regression models; the most parsimonious model was arrived at by stepwise regression. For the ACD group, laboratory values tested in the regression analyses included: ferritin, transferrin saturation (TSAT), reticulocyte Hb content, folate, vitamin B12, transferrin, iron, and albumin. For the IDA group, regression analyses included: ferritin, iron binding capacity (TIBC), iron, albumin, BUN, Creatinine, WBC, C-reactive protein, and endogenous EPO. Complete set of lab values was not available for all patients. In the IDA group, a strong correlation was identified between markers of iron status, serum ferritin and TSAT, but this association was much weaker among the ACD group **[22-24].**

5. CONCLUSION:

Our finding from biochemical studies were associated with Iron deficiency aneamia. Biochemical test performed in the blood sample for following clinical parameters and the findings were tabulated. Statistical analysis was done by using student's t-test and p value suggests the level of significant were changed here**.** The descriptive data and comparison of

biochemical parameters of Anaemia patients versus healthy controls are presented in Table no. 4.2. As expected, the Anaemia patients had markedly reduced levels of Ferritin (P<0.0001), Plasma Iron levels (P<0.0001) and Serum Iron (P<0.0001) compared to that of control subject. Whereas rest of all parameters were not significantly different between patient and healthy population**.** The anemia in pregnancy may not only be associated with maternal morbidity and mortality but can also be detrimental to the fetus. A definitive diagnosis of anemia is a pre-requisite to unravelling possible cause(s), to allow appropriate treatment intervention. It is hypothesized that measured hemoglobin (HGB), complemented by biochemical and other hematological parameters would enhance anemia diagnosis. Venous blood was collected and hemoglobin genotype, complete blood count and biochemical parameters [ferritin, iron, total iron binding capacity (TIBC), transferrin saturation (TfS), C-reactive protein (CRP) and bilirubin] were determined**.** Thick blood films were prepared for malaria parasitemia, while early morning stool and midstream urine samples were examined for enteric and urogenital parasites, respectively **[25].**

REFERENCES:

1. McLean, E.; Cogswell, M.; Egli, I.;Wojdyla, D.; de Benoist, B.Worldwide prevalence of anaemia,WHO vitamin and mineral nutrition information system, 1993–2005. Public Health Nutr. **2009**, 12, 444–454.
2. Tanaka, T.; Roy, C.N.; Yao, W.; Matteini, A.; Semba, R.D.; Arking, D.; Walston, J.D.; Fried, L.P.; Singleton, A.; Guralnik, J.; et al. A genome-wide association analysis of serum iron concentrations. Blood J. **2010**, 115, 94–96.
3. Gichohi-Wainaina,W.N.; Towers, G.W.; Swinkels, D.W.; Zimmermann, M.B.; Feskens, E.J.; Melse-Boonstra, A. Inter-ethnic di_erences in genetic variants within the transmembrane protease, serine 6 (TMPRSS6) gene associated with iron status indicators: A systematic review with meta-analyses. Genes Nutr. **2014**, 10, 442.
4. Chambers, J.C.; Zhang,W.; Li, Y.; Sehmi, J.;Wass, M.N.; Zabaneh, D.; Hoggart, C.; Bayele, H.; McCarthy, M.I.; Peltonen, L.; et al. Genome-wide association study identifies variants in TMPRSS6 associated with hemoglobin levels. Nat. Genet. **2009**, 41, 1170–1172.
5. Tatala, S.; Ndossi, G.; Ash, D. Impact of dietary iron intake on anaemia in Tanzanian schoolchildren. Sajcn **2004**, 17, 94–100.
6. Cook, J.D.; Skikne, B.; Baynes, R. The use of transferrin receptor for the assessment of iron status. In Iron Nutrition in Health and Disease; Hallberg, L.A., Asp, N.-G., Eds.; John Libbey& Co.: London, UK, 1996; pp. 91–99.
7. Thurnham, D.I.; Mburu, A.S.W.; Mwaniki, D.L.; Wagt, A.D. Micronutrients in childhood and the influence of subclinical inflammation. Proc. Nutr. Soc. **2005**, 64, 502–509.
8. Åkesson, A.; Bjellerup, P.; Berglund, M.; Bremme, K.; Vahter, M. Serum transferrin receptor: A specific marker of iron deficiency in pregnancy. Am. J. Clin. Nutr. **1998**, 68, 1241–1246.
9. Gan, W.; Guan, Y.; Wu, Q.; An, P.; Zhu, J.; Lu, L.; Jing, L.; Yu, Y.; Ruan, S.; Xie, D.; et al. Association of TMPRSS6 polymorphisms with ferritin, hemoglobin, and type 2 diabetes risk in a Chinese Han population. Am. J. Clin. Nutr. **2012**, 95, 626–632.
10. Gichohi-Wainaina,W.N.; Melse-Boonstra, A.; Swinkels, D.W.; Zimmermann, M.B.; Feskens, E.J.; Towers, G.W. Common variants and haplotypes in the TF, TNF-alpha, and TMPRSS6 genes are associated with iron status in a female black South. J. Nutr. **2015**, 145, 945–953.
11. Guo, X.; Zhou, D.; An, P.; Wu, Q.; Wang, H.; Wu, A.; Mu, M.; Zhang, D.; Zhang, Z.; Wang, H.; et al. Associations between serum hepcidin, ferritin and Hb concentrations and type 2 diabetes risks in a Han Chinese population. Br. J. Nutr. **2017**, 110, 2180–2185.
12. Finberg KE, Heeney MM, Campagna DR, Aydinok Y, Pearson HA, Hartman KR, et al. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). Nat Genet. 2008; 40: 569-71.
13. Kullo IJ, Ding K, Jouni H, Smith CY, Chute CG. A genome-wide association study of red blood cell traits using the electronic medical record. PLoS One. 2010; 5: e13011.
14. Soranzo N, Spector TD, Mangino M, Kuhnel B, Rendon A, Teumer A, et al. A genome-wide meta-analysis identifies 22 loci associated with eight hematological parameters in the HaemGen consortium. Nat Genet. 2009; 41:1182-90.
15. Ganesh SK, Zakai NA, van Rooij FJ, Soranzo N, Smith AV, Nalls MA, et al. Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. Nat Genet. 2009; 41: 1191-8.
16. Chambers JC, Zhang W, Li Y, Sehmi J, Wass MN, Zabaneh D, et al. Genome-wide association study identifies variants in TMPRSS6 associated with hemoglobin levels. Nat Genet. 2009; 41: 1170-2.
17. Benyamin B, Ferreira MA, Willemsen G, Gordon S, Middelberg RP, McEvoy BP, et al. Common variants in TMPRSS6 are associated with iron status and erythrocyte volume. Nat Genet. 2009; 41: 1173-5.
18. Andrews NC. Iron deficiency and related disorders. Wintrobe's Clinical Hematology 12th ed. Philadelphia, PA: Lippincott Williams & Wilkins. 2009: 810-34.
19. Higham JM, O'Brien PM, Shaw RW. Assessment of menstrual blood loss using a pictorial chart. British journal of obstetrics and gynaecology. 1990; 97: 734-9.
20. Donnez J, Tatarchuk TF, Bouchard P, Puscasiu L, Zakharenko NF, Ivanova T, et al. Ulipristal acetate versus placebo for fibroid treatment before surgery. N Engl J Med. 2012; 366: 409-20.
21. Philipp CS, Faiz A, Dowling N, Dilley A, Michaels LA, Ayers C, et al. Age and\ the prevalence of bleeding disorders in women with menorrhagia. Obstet Gynecol. 2005; 105: 61-6.
22. Oehler MK, Rees MC. Menorrhagia: an update. Acta obstetricia et gynecologica Scandinavica. 2003; 82: 405-22.
23. Marret H, Fauconnier A, Chabbert-Buffet N, Cravello L, Golfier F, Gondry J, et al. Clinical practice guidelines on menorrhagia: management of abnormal uterine bleeding before menopause. European journal of obstetrics, gynecology, and reproductive biology. 2010; 152: 133-7.

24. Nai A, Pagani A, Silvestri L, Campostrini N, Corbella M, Girelli D, et al. TMPRSS6 rs855791 modulates hepcidin transcription in vitro and serum hepcidin levels in normal individuals. Blood. 2011; 118: 4459-62.
25. An P, Wu Q, Wang H, Guan Y, Mu M, Liao Y, et al. TMPRSS6, but not TF, TFR2 or BMP2 variants are associated with increased risk of iron-deficiency anemia. Human Molecular Genetics. 2012; 21: 2124-31.