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"ASSOCIATION OF TMPRSS6 (TRANSMEMBRANE PROTEASE SERINE6) GENE POLYMORPHISM IN PREGNANT WOMEN WITH IRON DEFICIENCY ANAEMIA"

Anatomy

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ABSTRACT

Introduction: Anaemia is a blood disorder affecting about one fourth of the world's population, particularly in pregnant women due to the high iron requirement. Among all types of anaemia, iron deficiency anaemia (IDA) is the most common. Recently, several genome studies identified many single nucleotide polymorphisms that influence blood cell phenotypes and participate in the causing anaemia. Particularly, TMPRSS6 (Trans-membrane protease, serine 6) gene SNPS rs 855791 polymorphism has a strongest association with red blood cell indices in general population. Methods: This study was conducted in 80 pregnant women with IDA and 80 healthy pregnant women without anaemia and investigated blood cell indices and TMPRSS6 rs855791 genotypes. Results: There were significantly fewer rs855791 C homozyogotes in the IDA group than in the healthy group (8.75% vs 26.25%, p=0.00362). The odds ratio (OR) of C homozygotes in IDA vs CT+TT genotypes in IDA was 3.71. Conclusion: Present study showed that TMPRSS6 gene SNP rs855791 CC genotype is less frequent in pregnant women with iron deficiency anaemia than in healthy mothers. This study recommends C homozogocity genotype for TMPRSS6 rs899791 has a protective role against IDA in pregnant women.

KEYWORDS:

Iron deficiency anaemia (IDA), Trans-membrane protease serine 6 (TMPRSS6), single nucleotide polymorphism (SNP), rs 855791, Pregnant women.

Introduction

Anaemia is a fatal blood disorder affecting the world's population; particularly more than 40 percent of pregnant women are suffering from anaemia due to the high iron requirement (World Health Organization (WHO, 2008). In all types of anaemia, iron deficiency anaemia (IDA) is the most common type of anaemia which may occur due to the deficiency of nutrition or absorption, blood loss, infections and genetic mutations (WHO, 2004). Among these factors some genetic mutations also have been known to cause hereditary anaemia (Peng et. al., 2012). Recently, several genome based studies have been identified including single nucleotide polymorphisms (SNPs). There are many genes involved which influence blood cell phenotypes and participate in the causing anaemia (Kullo et al., 2009). Among these genes, TMPRSS6 (Trans-membrane protease, serine 6) gene SNPS rs 855791 has a strongest association with red blood cell indices in general population (Chambers et. al., 2009; Benyamin et. al., 2009). TMPRSS6 gene is located on the long (q) arm of chromosome 22 at position 12.3 (22q12.3) (National Library of Medicine 2015).

TMPRSS6 gene provides instructions to RNA for making a protein called metriptase-2. This protein is part of a signaling pathway that controls the levels of a protein called hepcidin, which is a key regulator of iron balance in the human body (National Library of Medicine 2015).When iron levels are decreased, this signaling pathway reduces hepcidin production, allowing more iron absorption from the diet through the intestine and transported out of storage sites into the blood stream. TMPRSS6 gene mutations decrease or eliminate functional matripatase-2, which interrupts iron regulation and leads to a shortage of iron in the blood stream. Mutations in the TMPRSS6 gene cause iron-refractory iron deficiency anaemia (IRIDA) (National Library of Medicine 2015). IRIDA has been described as "iron-refractory" because the condition is totally resistant (refractory) to treat with oral iron treatment and partially resistant to iron given in other ways, such as intra-venous. The aim of the present study was to evaluate the association of TMPRSS6 gene SNP rs855791 polymorphism with iron deficiency anaemia in pregnant Indian women.

Materials and Methods

Study population: This study was conducted in 160 pregnant women. Among this, 80 pregnant women with iron deficiency anaemia (cases) and 80 pregnant women without anaemia (healthy; controls) were attending regularly and admitted in Department of Obs & Gynae at Rama Medical College, Hospital & Research Centre, Mandhana, Kanpur (India). Inclusion criteria: Pregnant women with Iron deficiency anaemia included as IDA group. IDA defined as hemoglobin level <11g/dl and serum ferritin level <10ng/ml and pregnant women with normal hemoglobin and serum ferritin levels included as control group. Exclusive criteria: Gastrointestinal bleeding, renal insufficiency and history of gastrointestinal resection were considered. Before the conduction of this study, permission has taken from the institution ethical committee as well as written consent from the cases and controls.

Assessment of blood cell indices: 3ml of venous blood was collected in plain and EDTA vacutainer. Hemoglobin, RBC count, Hematocrit, Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration were estimated by Sysmex XS-800 autoanalyzer as well as with slide method. Serum ferritin was determined by Latex Enhanced Immunoturbidimetry (AGAPPE). Serum transferrin was measured by Turbidimetric Immunoassay (AGAPPE). Serum iron levels were assessed by ferrozine method (AGAPPE).

Genotyping: Genomic DNA was extracted from the blood using by the Qiagen genomic DNA isolation Kit (Germany). The TMPRSS6 SNP rs855791 gene polymorphism was determined by polymerase chain reaction (PCR; T100 Bio-Rad).Primers were got synthesized from the GCC Biotech, Kolkata. Used primers sequence was as follows; forward primer: 5'-TAGAGAACA GGGGCTCCAGG-3' and reverse primer; 5'-ATGTGGGCAGCATCCTTTC-3'. Primers were dissolved with sterile double distilled water and TAE buffer based on the manufacturer's instruction. The PCR conditions were 95oC for 3min, 35 cycles of 95oC for 30sec, 52oC for 30sec, 72oC for 1.20 min and final extension was at 72oC for 5min.Then PCR product was run with 1% agarose gel containing ethidium bromide. Half of the amount of

PCR products was digested with the restriction endonuclease Stu I and remaining half reaction volume was directly run in agarose gel. Genotype was determined by fragment size under UV light in gel documentation system (Bio-Rad). The PCR product (unpurified) was directly submitted for sequencing to Chromous Biotech Pvt. Ltd situated at Bangaluru.

Results

In the present study, all the pregnant women in two groups were belong to same age group (p = 0.7191). All the red blood cell indices were differed statistically significant with 0.01 between two groups (Table 1).

Table 1: List of blood components estimated by auto analyzer as well as with slide method

BLOOD CELL INDICES	CONTROL(n=80)	IDA (n=80)	P-VALUE
Age (Yrs)	26.25+/-3.30	26.02+/-4.66	0.7191
Haemoglobin (g/dl)	12.13+/-0.8	8.97+/-1.2	0.0001
RBC (million/dl)	3.95+/-0.2	3.48+/-0.34	0.0001
Haematocrit (%)	35.25+/-3.06	25.22+/-3.14	0.0001
Mean corpuscular volume (fl)	89.04+/-3.83	72.32+/-4.53	0.0001
Mean Corpuscular Haemoglobin (pg/µl)	30.68+/-1.06	25.75+/-2.52	0.0001
Mean Corpuscular Haemoglobin concentration (g/dl)	34.48+/-1.16	35.60+/-2.60	0.0006
Serum Ferritin (ng/dl)	66.15+/-26.92	6.17+/-1.81	0.0001
Serum iron (µg/dl)	86.16+/-28.03	23.62+/-6.19	0.0001
Serum Transferrin (mg/dl)	278.55+/-23.77	164.78+/-31.55	0.0001
Transferrin Saturation (%)	21.65+/-5.71	10.19+/-2.08	0.0001

80 IDA cases and 80 controls samples DNA were amplified with PCR for the screening of TMPRSS6 SNP rs 855791 gene polymorphism. C allele was observed at 249bp and T allele was observed at 125bp.There are 3 types of genotypes are known for TMPRSS6 gene polymorphism: CC homozygous, CT heterozygous and TT homozygous.

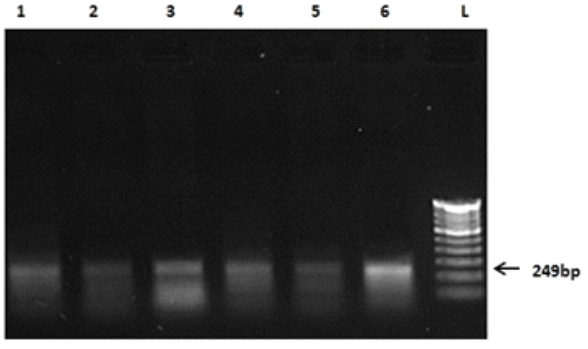


Fig 1; Agarose gel picture before restriction digestion

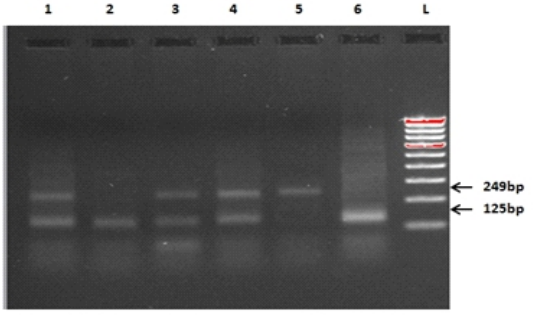


Fig 2: Agarose gel picture after restriction digestion. Single band at 249bp was represented as C homozygous; single band at 125bp was denoted as T homozygous and two bands denoted as heterozygous

Observations:

The frequency distribution of the TMPRSS6 gene rs855791 CC, CT and TT in both groups were differed statistically significant at the level of ≤0.05 (Table 2). However the proportions of the C homozygous are found lower and T homozygous proportions were higher in IDA with respect to controls (8.75% vs 26.25%. p=0.00362).

Table 2: TMPRSS6 rs855791 genotypes distribution between control and IDA groups

	CC	CT	TT	P value
CONTROL(N=80)	21(26.25%)	37(46.25%)	22(27.5%)	<0.05
IDA (N=80)	7(8.75%)	41(51.25%)	32(40%)	

The odd's ratio (OR) of C homozygous versus those with CT + TT genotypes was 3.71. The Odd's ratio represented C homozygous genotype has found 3 times more protective role than CT+TT genotypes (Table 3).

Table 3: List of odd's ratio of C homozygous versus with CT + TT genotypes

	CONTROL(N=80)	IDA (N=80)	Odd's ratio
CC	21(26.25%)	7 (8.75%)	3.71
CT+TT	59 (73.75%)	73 (91.25%)	

TAGAGAACAGGGGCTCTAGGCTCCTGAGATCTCACTTCTG
CCCTTGACCACGGACAGGCCCCATCAGCAACGCTCTGCAG
AAAGTGGATGTGCAGTTGATCCCACAGGACCTGTGCAGCG
AGGTCTATCGCTACCAGGTGACGCCACGCATGCTGTGTGCC
GGCTACCGCAAGGGCAAGAAGGATGCCTGTCAGGTGAGTC
CCCCGGGCATGGGAGGGAGAGAGGAGGGAGAAAGGATGC
TGCCCACAT

Fig 3: The obtained gene sequence from gene sequencing

Query 1
TAGAGAACAGGGGCTCTAGGCTCCTGAGATCTCACTTCTG
CCCTTGACCACGGACAGGCC 60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 36762
TAGAGAACAGGGGCTCCAGGCTCCTGAGATCTCACTTCTG
CCCTTGACCACGGACAGGCC 36821

Query 61
CCATCAGCAACGCTCTGCAGAAAGTGGATGTGCAGTTGAT
CCCACAGGACCTGTGCAGCG 120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 36822
CCATCAGCAACGCTCTGCAGAAAGTGGATGTGCAGTTGAT
CCCACAGGACCTGTGCAGCG 36881

Query 121
AGGTCTATCGCTACCAGGTGACGCCACGCATGCTGTGTGCC
GGCTACCGCAAGGGCAAGA 180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 36882
AGGTCTATCGCTACCAGGTGACGCCACGCATGCTGTGTGCC
GGCTACCGCAAGGGCAAGA 36941

Query 181
AGGATGCCTGTCAGGTGAGTCCCCCGGGCATGGGAGGGAG
AGAGGAGGGAGAAAGGATGC 240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 36942
AGGATGCCTGTCAGGTGAGTCCCCCGGGCATGGGAGGGAG
AGAGGAGGGAGAAAGGATGC 37001

Query 241 TGCCCACAT 249
|||||||||
Sbjct 37002 TGCCCACAT 37010

Fig 4: Gene sequence homology with known sequence showed 99 % identical and C is replaced by T at position

Discussion

This is the rare study for the detection of IDA frequency rate in the north Indian population. Melis et al., 2008 has been conducted the sequencing analysis of TMPRSS6 gene polymorphism which revealed a homozygous causal mutation, may be predicting by a splicing error and a truncated TMPRSS6 protein in affected members. Nai et al., 2010 found that SNP rs855791 is located in the functional part of TMPRSS6 and mutation in the rs855791 reduces the ability of the enzyme to inhibit hepcidin transcription. According to Nai et al., 2010 study, C homozygous has lower hepcidin levels and higher transferrin saturation level than T homozygous in general population. The present study was also having higher amount of transferrin saturation level as found by other coworkers in this field which is seen in C homozygous than T homozygous. In the study of Pei et al., 2014 it has been mentioned that the association between menstrual blood loss and iron deficiency anaemia was no more significant in women with CC genotype and suggested that homozygosity for TMPRSS6 rs855791 C genotype has a protective role against IDA in women at reproductive age, especially in those with menorrhagia. In the present study the presence of C homzygous genotype shows protective role. This study results are correlated with the studies of Nai et al., and Pei et al.

Conclusion

In this study the TMPRSS6 gene SNP rs855791 polymorphism has been analyzed in north Indian population. This is first report generated for this region and not available in literature so far. Present study has showed that TMPRSS6 gene SNP rs855791 CC genotype is less frequent in pregnant women with iron deficiency anaemia than in healthy mothers. Maternal iron deficiency anemia during pregnancy and the perinatal periods have devastating effects on both the mother and child. In addition to the direct effects of anemia there are other defects also being involved such as reduces fetal brain maturation and pediatric cognitive defects in child. In the cases of IRIDA, iron should be administered through the intravenously to prevent the complications. For more accurate results study should be done with large population size in Indian ethnicity to prevent bias.

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