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Effect of Tmprss6 Gene Polymorphism on Morphometry of Placenta and Foetal Outcome

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ABSTRACT

Most of the pregnant women are suffering from iron deficiency anaemia (IDA) which may also be due to genetic mutations. There are many genes involved which influence blood cell phenotypes and are responsible for the causing anaemia. Among these genes, TMPRSS6 gene SNP rs 855791 have the strongest association with red blood cell indices in general population. The placenta is a membranous vascular organ that develops in female mammals and mediates materno-foetal exchange. Careful examination of the placenta can give information, which can be useful in the management of complications in mother and the newborn. The aim of our study to find out the influence of TMPRSS6 *gene* SNP rs855791 polymorphism in the development of iron deficiency which results in abnormal morphometrical changes of placenta leads to foetal outcome.

The material for study consists of venous blood and placenta of 88 IDA pregnant women and 88 Healthy pregnant women from the department of obstetrics and gynecology at Rama hospital with permission from the institution ethical committee.

Among the study of TMPRSS6 gene SNP rs855791 polymorphism, TT genotype shows the pathogenic role which cause iron deficiency anaemia. Morphometrical changes like placental weight, diameter, thickness, cord insertion ratio, surface area, volume, number of maternal cotyledons were decreased in TT genotype whereas placental index was increased in TT genotype. Intrauterine growth retardation and preterm birth of neonates were more noticed among TT genotype. Foetal weight was reduced among TT genotype mothers. Low birth weight neonates and perinatal death were more noticed among TT genotype.

TT genotype of TMPRSS6 gene SNP rs 855791 causes development of iron deficiency anaemia which may lead to abnormal morphometrical changes of placenta resulting into poor outcome of pregnancy in Indian population.

KEY WORDS: Placenta, TMPRSS6 gene, IUGR, Low birth weight.

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INTRODUCTION

Anaemia is a fatal blood disorder affecting the world's population; particularly due to the high iron requirement, pregnant women are suffering from anaemia. World Health Organization (WHO, 1992) has defined for anaemia in pregnant women is Hemoglobin (Hb) <11g/dl. Anaemia is classified as mild, moderate and severe. The Hemoglobin (Hb) level for each class of anaemia in pregnancy are 9.0-10.9g/dl (mild), 7.1-8.9g/dl (moderate) and <7.0g/dl (severe)[1]. Toteja GS (2001) stated that Indian Council of Medical Research in sixteen districts of eleven states has indicated an overall prevalence of anaemia was 84.9 percent among pregnant women[2]. NFHS -3 (2005-06) survey stated that the prevalence of 52% among women in central India[3]. In all types of anaemia, iron deficiency anaemia (IDA) is the most common nutritional disorder. Bansal et al., (2016) observed that prevalence of IDA in a tertiary care hospital, Uttar Pradesh is 71.4%[4]. IDA is characterized by a shortage of healthy red blood cells results from the insufficient amount of iron in the blood stream due to the deficiency of nutrition or absorption, blood loss, infections and genetic mutations[5]. Among these factors, some genetic mutations also have been known to cause hereditary anaemia. Recently, several genome-based studies have been identified including single nucleotide polymorphisms (SNPs). There are many genes involved which influence blood cell phenotypes and are responsible for the causing anaemia[6]. Among these genes, TMPRSS6 (Trans-membrane protease, serine 6) gene SNP rs 855791 has the strongest association with red blood cell indices in general population[7,8].

The placenta is a membranous vascular organ that develops in female mammals and mediates materno-foetal exchange. In humans, it is developed from two sources – foetal and maternal. Survival and growth of foetus are essentially dependent on the formation, full development and functions of the placenta. It undergoes different changes in weight, volume, structure, shape and function continuously throughout the gestation to support the prenatal life. The examination of the placenta during the period of pregnancy and post-partum provides valuable information about the state of foetus wellbeing. The aim of this study to know the effect of TMPRSS6 gene SNP rs855791 polymorphism on morphometry of placenta and foetal outcome.

MATERIALS AND METHODS:

Placentae and 5ml of venous blood was drawn from peripheral vein using sterilized Di sodium EDTA vial from Department of Obstetrics and Gynaecology, Rama Medical College, Hospital and Research centre. All the blood samples were stored at -80^{o}C until tested. The laboratory work was carried out in the central research laboratory, Rama Medical College, Hospital & Research Centre.

In this study, 88 samples of iron deficiency anaemia were taken by screened for Hematocrit (<30%), MCV (<79fl), serum iron (<35microgram/dl), serum ferritin (<10ng/dl) and serum transferrin (>370mg/dl) levels to diagnose the iron deficiency anaemia. 88 control and 88 IDA samples were screened for the TMPRSS6 gene SNP rs855791 sequence located on chromosome 22q12.3 by the simple PCR and RFLP.

Genotyping: Genomic DNA was extracted from the blood using by theQiagen genomic DNA isolation Kit (Germany). The TMPRSS6 SNPrs855791 gene polymorphism was determined by polymerase chain reaction (PCR; T100 Bio-Rad).Primers were got synthesized from theGCC Biotech, Kolkata. Used primers sequence was as follows: forward primer: 5'-TAGAGAACAGGGGCTCCAGG-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 95^{o}C for 3min,35 cycles of 95^{o}C for 30sec, 52^{o}C for 30sec, 72^{o}C for 1.20 min and final extension was at 72^{o}C for 5min.Then PCR product was run with1% agarose gel containing ethidium bromide. Half of the amount of PCR products was digested with the restriction end nuclease 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. For the study of placentae, the placentae were washed under running tap water to remove the blood clots and examined the placentae for the following morphometrical data: Weight, diameter, the number of maternal cotyledons. Placental weight measured in grams by weighing machine. The diameter of the placenta (d) was taken by the mean of the two maximum diameters in centimeters right angles to each other by metallic scale. The number of maternal cotyledons has counted on the maternal surface. Fetal weight also examined in grams by weighing machine.

RESULTS

According to the sequence we obtained C allele was observed at 249bp and T allele was observed at 125bp.Single band at 249 bp represented C homozygous; single band at 125 bp denoted as T homozygous, and two bands represented as CT heterozygous. There are 3 types of genotypes are known for TMPRSS6 gene polymorphism: CC homozygous, CT heterozygous and TT homozygous.

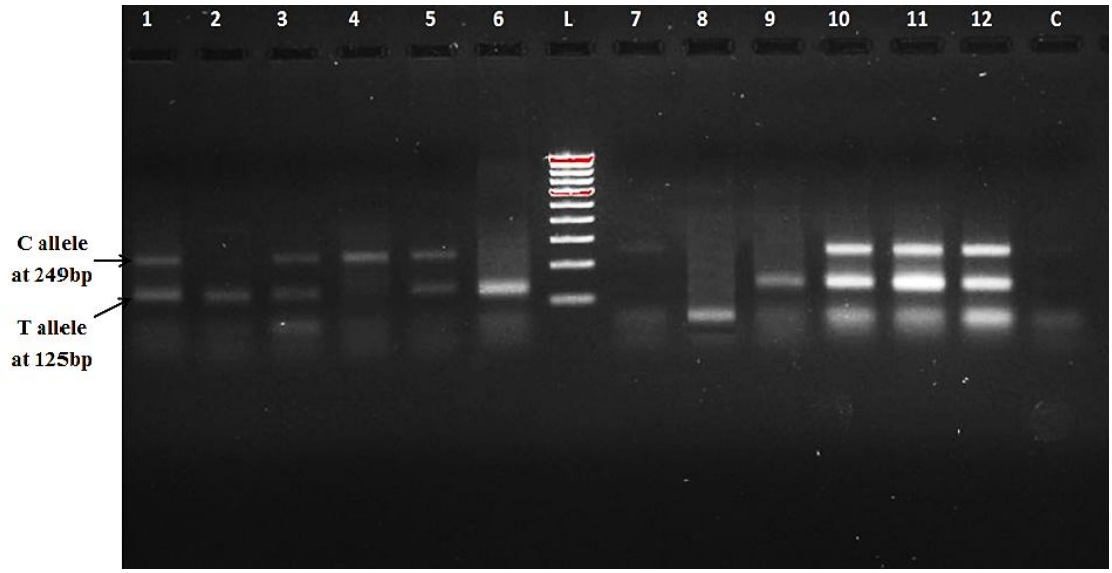

Fig.1: PCR product after restriction fragment length polymorphism (RFLP). The PCR products were digested with Stu I endonuclease. L: 1000bp ladder, C- control.

Table.1:TMPRSS6 gene SNP rs855791 genotypes distribution between IDA and Healthy groups.

Genotype	IDA (N=88)	Healthy (N=88)	Chi-square Value	p-Value*
TT	35 (39.8%)	22 (25%)	10.2188	0.00604
CT	41 (46.6%)	37 (42%)		
CC	12 (13.6%)	29 (33%)		

***Chi-square test; p<0.05 indicates statistically significant**

In this study, it was analyzed that CT genotype distribution was common in both groups (IDA and Healthy group) whereas TT genotype distribution was more noticed in IDA group than Healthy group while CC genotype distribution was more observed in Healthy group than IDA group. The difference was statistically significant (x^2 = 10.2188; p=0.00604) as shown in Table.1.

Logistic Regression Analysis for iron deficiency anaemia (IDA):

Table.2: Odd's Ratio between TT genotype and CC genotype

Genotype	IDA	Healthy	OR (95% CI)	p-Value
TT	35	22	3.84 (1.63-9.07)	0.0021
CC	12	29		

p<0.05 indicates statistically significant

With comparison of the individual frequencies of two homozygous genotypes it was found that TT genotype was at least 1.63 times [OR (95%CI): 3.84 (1.63-9.07)] more prone to develop iron deficiency anaemia than CC genotype which was statistically significant (p=0.0021) as shown in Table.2.

Table.3: Odd's Ratio between TT genotype and CT genotype

Genotype	IDA	Healthy	OR (95% CI)	p-Value
TT	35	22	1.44 (0.72-2.87)	0.3072
CT	41	37		

p<0.05 indicates statistically significant

With comparison of the individual frequencies of TT and CT genotypes it was found that TT genotype was at least 0.72 times [OR(95%CI): 1.44 (0.72-2.87)] more prone to develop iron deficiency anaemia than CT genotype which was statistically not significant (p=0.3072) as shown in Table.3.

Table.4: Odd's Ratio between TT genotype and CX (CC+CT) genotype.

Genotype	IDA	Healthy	OR (95% CI)	p-Value
TT	35 (39.8%)	22 (25%)	1.98 (1.04-3.77)	0.0375
CX(CC+CT)	53 (60.22%)	66 (75%)		

p<0.05 indicates statistically significant

With comparison of the frequencies of TT and CX(CC+CT) genotypes it was found that TT genotype was at least 1.04 times [OR(95%CI): 1.98 (1.04-3.77)] more prone to develop iron deficiency anaemia than CX(CC+CT) genotype which was statistically significant (p=0.0375) as shown in Table.4.

Table.5: Odd's Ratio between CC genotype and CT genotype.

Genotype	IDA	Healthy	OR (95% CI)	p-Value
CC	29	12	2.68 (1.19-5.99)	0.0166
CT	37	41		

p<0.05 indicates statistically significant

With comparison of the frequencies of CC and CT genotypes it was found that CC genotype was at least 1.19 times [OR(95%CI): 2.68 (1.19-5.99)] more protective from iron deficiency anaemia than CT genotype which was statistically significant (p=0.0166) as shown in Table.5.

Table.6: Odd's Ratio between CC genotype and TX(CT+TT) genotype

Genotype	IDA	Healthy	OR (95% CI)	p-Value
CC	29 (33%)	12 (13.6%)	3.113 (1.46-6.61)	0.0032
TX(CT+TT)	59 (67%)	76 (86.3%)		

p<0.05 indicates statistically significant

With comparison of the frequencies of CC and TX (CT+TT) genotypes, it was found that CC genotype was at least 1.46 times [OR (95%CI): 3.113 (1.46-6.61)] more protective from iron

deficiency anaemia than TX (CT+TT) genotype which was statistically significant (p=0.0032) as shown in Table.6.

With the above findings, it was noticed that TT genotype has the pathogenic role and CC genotype has the protective role in the development of iron deficiency anaemia whereas CT genotype has the intermediate role.

In the present study, Hematological findings also observed between genotypes of IDA, Healthy and Combined (IDA+Healthy) groups.

Hematological findings among genotypes of IDA Group:

Table.7: Difference of Hematological findings between genotypes in IDA group.

Hematological findings	**CC(N=12) Mean±SD**	**CT(N=41) Mean±SD**	**TT(N=35) Mean±SD**	**p-value***
Hemoglobin (g/dl)	9.73±0.62	9.50±0.79	8.55±1.59	0.001
Red Blood Cell (RBC) Count (million/dl)	3.69±0.19	3.58±0.26	3.38±0.49	0.015
Hematocrit (%)	27.50±1.97	26.19±2.58	24.45±3.79	0.006
Mean Corpuscular Volume (MCV) (fl)	74.50±3.09	73.00±4.09	71.05±4.59	0.028
Mean Corpuscular Hemoglobin (MCH) (pg)	26.41±1.78	26.53±1.84	25.37±3.21	0.114
Serum Ferritin (ng/dl)	7.40±1.54	6.61±2.05	5.45±1.92	0.004
Serum Iron (µg/dl)	23.25±5.93	24.85±6.59	21.63±5.00	0.066
Serum Transferrin (mg/dl)	389.33±13.65	397.68±26.04	414.63±29.59	0.004
Tsaturation(%)	4.24±1.11	4.46±1.27	3.72±0.94	0.020

***One way Anova test; p<0.05 indicates statistically significant**

Hematological findings among genotypes of Healthy Group:

Table.8: Difference of Hematological findings between genotypes in Healthy group

Hematological findings	**CC(N=29) Mean±SD**	**CT(N=37) Mean±SD**	**TT(N=22) Mean±SD**	**p-value***
Hemoglobin (g/dl)	12.19±0.85	12.16±0.71	11.41±0.39	0.0001
Red Blood Cell (RBC) Count (million/dl)	3.95±0.27	3.87±0.23	3.78±0.12	0.034
Hematocrit (%)	36.62±2.65	35.02±2.55	34.40±2.03	0.005
Mean Corpuscular Volume (MCV) (fl)	89.93±3.50	89.21±4.06	85.81±3.28	0.0001
Mean Corpuscular Hemoglobin (MCH) (pg)	30.96±2.02	31.54±2.53	30.09±1.30	0.045
Serum Ferritin (ng/dl)	78.20±28.84	70.39±16.86	39.29±15.29	0.0001
Serum Iron (µg/dl)	100.55±27.14	91.38±21.25	59.36±13.47	0.0001
SerumTransferrin (mg/dl)	241.79±28.21	234.97±28.80	263.09±37.67	0.004
Tsaturation(%)	29.81±8.91	27.75±6.34	16.12±3.71	0.0001

***One way Anova test; p<0.05 indicates statistically significant**

Hematological findings among genotypes of combined (IDA+Healthy) Group

Table.9: Difference of Hematological findings between genotypes in combined group

Hematological findings	CC(N=41) Mean±SD	CT(N=78) Mean±SD	TT(N=57) Mean±SD	P-value*
Hemoglobin (g/dl)	11.47±1.37	10.76±1.53	9.65±1.88	0.0001
Red Blood Cell Count (RBC) (million/dl)	3.88±0.28	3.72±0.28	3.53±0.43	0.0001
Hematocrit (%)	33.95±4.86	30.38±5.13	28.29±5.84	0.0001
Mean Corpuscular Volume (MCV)(fl)	85.41±7.85	80.69±9.10	76.75±8.33	0.0001
Mean Corpuscular Hemoglobin (MCH) (pg)	29.63±2.85	28.91±3.33	27.19±3.50	0.001
Serum Ferritin (ng/dl)	57.48±40.58	36.87±34.09	18.51±19.13	0.0001
Serum Iron (µg/dl)	77.93±42.35	56.41±36.76	36.19±20.66	0.0001
SerumTransferrin (mg/dl)	284.97±72.30	320.50±86.18	356.14±81.26	0.0001
Tsaturation(%)	22.32±13.95	15.50±12.51	8.50±6.54	0.0001

***One way Anova test; p<0.05 indicates statistically significant**

In the IDA group, hematological findings observed that hemoglobin, RBC count, Hematocrit, Mean Corpuscular Volume, serum Ferritin and Tsaturation was lower in levels than the CT and CC genotypes which were statistically significant whereas Mean Corpuscular hemoglobin, Serum iron in TT genotype were also lower in level but it was statistically not significant and Serum Transferrin level in TT genotype was higher than CT and CC genotypes which was statistically highly significant.

In the Healthy group, hematological findings observed that Hemoglobin, RBC count, Hematocrit, Mean Corpuscular Volume, Mean Corpuscular hemoglobin, Serum Ferritin, Serum iron levels and Tsaturation % were lower than the CT and CC genotypes which were statistically significant and Serum Transferrin level in TT genotype was higher than CT and CC genotypes which was also statistically highly significant.

However, In the combined group, hematological findings observed that Hemoglobin, RBC count, Hematocrit, Mean corpuscular volume, Mean corpuscular hemoglobin, serum Ferritin, Serum iron levels and Tsaturation % were lower in TT genotype than the CT and CC genotypes which were statistically significant except Serum Transferrin level in TT genotype was higher than CT and CC genotypes which was also statistically highly significant.

In the present study, it was also observed the difference of all hematological findings between genotypes. In IDA group, the difference between CC and CT genotypes was statistically not significant ($p>0.05$); the difference between TT and CT genotypes was statistically significant except RBC count, Mean corpuscular volume, Mean corpuscular hemoglobin and serum iron which was showing not significant ($p>0.05$) and difference between TT and CC group was statistically

significant ($p<0.05$) except serum iron and Tsaturation which were not significant ($p>0.05$) whereas in Healthy group, the difference between CC and CT genotypes was statistically not significant ($p>0.05$) except hematocrit which were showing statistically significant ($p<0.05$); the difference between TT and CT group was statistically significant ($p<0.05$) and difference between TT and CC group was also statistically significant ($p<0.05$) except RBC count, hematocrit which was statistically not significant ($p>0.05$).

However, in combined group, the difference between CC and CT genotypes was statistically significant ($p<0.05$) except Hb, RBC count, MCH, Serum Transferrin; the difference between TT and CT genotypes was statistically significant ($p<0.05$) except hematocrit and also difference between TT and CC group was statistically significant ($p<0.05$).

Morphometric changes of placenta and foetal weight with different genotypes:

In the present study, morphometrical findings of placenta was observed among mothers with different TMPRSS6SNPrs 855791 genotypes of IDA, Healthy and Combined groups.

Placental weight:

Table.8 showing placental weight in relation with genotypes in all groups

Groups	CC Mean±SD	CT Mean±SD	TT Mean±SD	P-value*
IDA	461.58±70.60	421.83±67.59	388.94±70.56	0.006
Healthy	480.00±89.08	495.78±77.71	434.59±71.17	0.020
Combined	474.61±83.65	456.91±81.11	406.56±73.65	0.0001

***One way Anova test; p<0.05 indicates statistically significant**

Table.9: Statistical significance (p-value) of placental weight between genotypes

Groups	CCvsCT	CTvsTT	CCvsTT
IDA	0.193	0.103	0.007
Healthy	0.708	0.016	0.117
Combined	0.481	0.001	0.0001

Tukey HSD test; p<0.05 indicates statistically significant

Placental weight (gm) in TT genotypes was reduced in all groups than CC, CT genotypes in all groups which were statistically highly significant as shown in Table.8. Table.9 revealed that the placental weight between CCvsCT and CTvsTT were insignificant in all groups except combined group whereas between CCvsTT were statistically significant in all the groups except in Healthy group.

Placental Diameter:

Table.10 showing placental diameter in relation with genotypes in all groups

Groups	CC Mean±SD	CT Mean±SD	TT Mean±SD	p-value*
IDA	16.28±1.16	15.94±1.57	14.73±1.93	0.003
Healthy	16.92±1.41	17.28±1.62	16.74±1.54	0.382
Combined	16.73±1.36	16.58±1.73	15.51±2.03	0.0001

***One way Anova test; p<0.05 indicates statistically significant**

Table.11: Statistical significance (p-value) of placental diameter between genotypes

Groups	CCvsCT	CTvsTT	CCvsTT
IDA	0.812	0.007	0.020
Healthy	0.611	0.389	0.906
Combined	0.888	0.002	0.002

Tukey HSD test; p<0.05 indicates statistically significant

Table.10 revealed that Diameter of placenta (cm) was decreased in TT genotypes when compared with CC and CT genotypes among the all the groups which were statistically significant. Table.11 shown that the placental diameter between CCvsCT was statistically insignificant in all groups whereas between CTvsTT and CCvsTT were statistically significant in all the groups except in Healthy group.

Placental Thickness:

Table.12 showing placental thickness in relation with genotypes in all groups

	CC Mean±SD	CT Mean±SD	TT Mean±SD	p-value
IDA	2.20±0.41	1.98±0.36	1.75±0.39	0.001
Healthy	2.23±0.45	2.12±0.25	1.97±0.27	0.030
Combined	2.22±0.44	2.05±0.32	1.84±0.36	0.0001

***One way Anova Test; p<0.05 indicates statistically significant**

Table.13: Statistical significance (p-value) of placental thickness between genotypes

	CCvsCT	CTvsTT	CCvsTT
IDA	0.172	0.030	0.002
Healthy	0.433	0.213	0.022
Combined	0.038	0.003	0.0001

Tukey HSD test; p<0.05 indicates statistically significant

Thickness of placenta (cm) was reduced in TT genotypes when compared with CC and CT genotypes among the all the groups which were statistically significant as shown in Table.12.

Table.13 revealed that the placental thickness between CCvsCT was statistically insignificant in all groups whereas between CTvsTT and CCvsTT were statistically significant in all the groups except between CTvsTT in Healthy group.

Cord insertion ratio:

Table.14 showing cord insertion ratio in relation with genotypes in all groups

Groups	CC Mean±SD	CT Mean±SD	TT Mean±SD	p-value*
IDA	0.30±0.17	0.28±0.14	0.27±0.14	0.814
Healthy	0.42±0.09	0.38±0.08	0.19±0.12	0.0001
Combined	0.38±0.13	0.33±0.13	0.23±0.13	0.0001

***One way Anova test; p<0.05 indicates statistically significant**

Table.15: Statistical significance (p-value) of cord insertion ratio between genotypes

Groups	CCvsCT	CTvsTT	CCvsTT
IDA	0.919	0.933	0.805
Healthy	0.477	0.0001	0.0001
Combined	0.118	0.0001	0.0001

Tukey HSD test; p<0.05 indicates statistically significant

Umbilical cord insertion ratio of placenta was decreased in TT genotypes when compared with CC and CT genotypes among the all the groups. But, it was statistically insignificant in IDA group whereas it was statistically significant in Healthy and Combined groups as shown in Table.14. Table.15 shown that the cord insertion ratio between CCvsCT was statistically insignificant in all groups whereas between CTvsTT and CCvsTT were statistically significant in all the groups except in IDA group.

Surface area of placenta:

Table.16 showing Surface area of placenta in relation with genotypes in all groups

Groups	CC Mean±SD	CT Mean±SD	TT Mean±SD	p-value*
IDA	209.10±29.77	201.43±38.74	173.35±46.08	0.004
Healthy	226.42±38.01	236.64±44.61	221.83±40.62	0.372
Combined	221.35±36.31	218.13±44.98	192.06±49.75	0.001

***One way Anova Test; p<0.05 indicates statistically significant**

Table.17: Statistical significance (p-value) of placental surface area between genotypes

Groups	CCvsCT	CTvsTT	CCvsTT
IDA	0.836	0.010	0.028
Healthy	0.584	0.386	0.919
Combined	0.926	0.003	0.005

Tukey HSD test; p<0.05 indicates statistically significant

Surface area of placenta (cm^2) was decreased in TT genotypes when compared with CC and CT genotypes among the all the groups. But, it was statistically insignificant in Healthy group whereas it was statistically significant in IDA and Combined groups as shown in Table.16. Table 17 shown that the placental surface area between CCvsCT was statistically insignificant in all groups whereas between CTvsTT and CCvsTT were statistically significant in all the groups except in Healthy group.

Placental Volume:

Table.18 showing placental volume relation with genotypes in all groups

Groups	CC Mean±SD	CT Mean±SD	TT Mean±SD	p-value*
IDA	477.17±136.70	396.12±97.68	310.31±124.70	0.0001
Healthy	501.60±119.96	497.74±84.30	435.56±89.69	0.036
Combined	494.45±123.85	444.32±104.34	358.65±127.44	0.0001

***One way Anova Test; p<0.05 indicates statistically significant**

Table.19: Statistical significance (p-value) of placental volume between genotypes

Groups	CCvsCT	CTvsTT	CCvsTT
IDA	0.085	0.005	0.0001
Healthy	0.986	0.056	0.052
Combined	0.070	0.0001	0.0001

Tukey HSD test; p<0.05 indicates statistically significant

Table.18 revealed that Volume of placenta (cm) was decreased in TT genotypes when compared with CC and CT genotypes among the all the groups which were statistically significant. Table 19 revealed that the placental volume between CCvsCT was statistically insignificant in all groups whereas between CTvsTT and CCvsTT were statistically significant in all the groups except in Healthy group.

Maternal cotyledons:

Table.20: Number of maternal cotyledons relation with genotypes in all groups

Groups	CC Mean±SD	CT Mean±SD	TT Mean±SD	p-value*
IDA	21.25±4.65	18.22±4.40	17.26±3.71	0.020
Healthy	24.97±4.17	21.97±5.69	22.27±6.04	0.063
Combined	23.88±4.59	20.00±5.36	19.19±5.30	0.0001

One way Anova Test; p<0.05 indicates statistically significant

Table.21: Statistical significance (p-value) of number of maternal cotyledons between genotypes

Groups	CCvsCT	CTvsTT	CCvsTT
IDA	0.075	0.578	0.015
Healthy	0.067	0.976	0.181
Combined	0.000	0.644	0.0001

Tukey HSD test; p<0.05 indicates statistically significant

Table.20 revealed that number of maternal cotyledons was decreased in TT genotypes when compared with CC and CT genotypes among the all the groups except in CT genotype among Healthy group which was slightly lower than TT genotype. The difference was statistically insignificant in Healthy group. However, the difference was statistically significant in IDA and Combined groups. Table.21 revealed that the number of maternal cotyledons between CCvsCT and CTvsTT was statistically insignificant in all groups except CCvsCT in combined group whereas between CCvsTT were statistically significant in all the groups except in Healthy group.

Foetal weight:

Table.22 revealed that Foetal weight (gm) was decreased in TT genotypes when compared with CC and CT genotypes among the all the groups which were statistically significant. Table.23 was shown that foetal weight was statistically significant between all genotypes of all the groups except CC vs CT in Healthy group.

Table.22 showing foetal weight in relation with genotypes in all groups

Groups	CC Mean±SD	CT Mean±SD	TT Mean±SD	p-value*
IDA	2847.42±517.03	2539.76±208.97	2200.00±261.09	0.0001
Healthy	2994.14±399.78	2923.92±362.32	2432.50±187.14	0.0001
Combined	2951.20±435.84	2721.99±348.34	2289.74±259.92	0.0001

***One way Anova Test; p<0.05 indicates statistically significant**

Table.23: Statistical significance (p-value) of foetal weight between genotypes

Groups	CCvsCT	CTvsTT	CCvsTT
IDA	0.004	0.000	0.000
Healthy	0.687	0.000	0.000
Combined	0.002	0.000	0.000

Tukey HSD test; p<0.05 indicates statistically significant

Placental Index:

Table.24 showing placental index relation with genotypes in all groups

Groups	CC Mean±SD	CT Mean±SD	TT Mean±SD	p-value*
IDA	0.17±0.03	0.17±0.02	0.18±0.04	0.117
Healthy	0.16±0.03	0.17±0.02	0.18±0.03	0.093
Combined	0.16±0.031	0.16±0.02	0.18±0.03	0.014

***One way Anova Test; p<0.05 indicates statistically significant**

Table.25: Statistical significance (p-value) of placental index between genotypes

Group	CCvsCT	CTvsTT	CCvsTT
IDA	1.000	0.124	0.353
Healthy	0.427	0.480	0.077
Combined	0.658	0.063	0.018

Tukey HSD test; p<0.05 indicates statistically significant

Placental index was increased in TT genotypes when compared with CC and CT genotypes among the all the groups as shown in Table.7.84 and Fig.6.77. But it was statistically insignificant in IDA and Healthy group whereas in combined group it was statistically significant. Table.25 revealed that placental index was statistically insignificant between the all the genotypes of all groups except CCvsTT in combined group.

Foetal Outcome:

Intrauterine Growth Retardation (IUGR):

In this study, intrauterine growth retardation was observed in the foetuses of IDA group, Healthy group and combined groups among CC, CT and TT genotypes. But in IDA group it was insignificant. In the Healthy group among genotypes, foetuses of TT genotype were at least 2.5 times [OR, 95%CI: 13.500(2.562-71.126)] more prone for the intrauterine growth retardation which was statistically significant while in the combined group among genotypes, foetuses of TT genotype were at least 4.5 times [OR, 95%CI: 16.213(4.478-58.698)]more prone for the intrauterine growth retardation which was statistically highly significant as shown in Table.26.

Table.26 showing IUGR relation with different genotypes

Groups	Genotype	Yes (%)	No (%)	OR(95% CI)	p-Value*
IDA	CC	1(8.3%)	11(91.7%)	1.000(Reference)	
	CT	6(14.6%)	35(85.4%)	1.886(0.204-17.410)	0.576
	TT	21(60%)	14(40%)	16.500(1.911-142.441)	0.11
Healthy	CC	2(6.9%)	27(93.1%)	1.000(Reference)	
	CT	0(0%)	37(100%)	0.000(0.000)	0.998
	TT	11(50%)	11(50%)	13.500(2.562-71.126)	0.002
Combined	CC	3(7.3%)	38(92.7%)	1.000(Reference)	
	CT	6(7.7%)	72(92.3%)	1.056(0.250-4.458)	0.941
	TT	32(56.1%)	25(43.9%)	16.213(4.478-58.698)	0.000

***Logistic regression analysis; p<0.05 indicates statistically significant**

Preterm Birth:

Table.27 showing preterm birth in relation with different genotypes

Groups	Genotype	Yes (%)	No (%)	OR(95% CI)	p-Value*
IDA	CC	3(25%)	9(75%)	1.000(Reference)	
	CT	7(17.1%)	34(82.9%)	0.618(0.133-2.879)	0.540
	TT	27(77.1%)	8(22.9%)	10.125(2.200-46.589)	0.003
Healthy	CC	6(20.7%)	23(79.3%)	1.000(Reference)	
	CT	3(8.1%)	34(91.9%)	0.338(0.077-1.491)	0.152
	TT	8(36.4%)	14(63.6%)	2.190(0.628-7.643)	0.219
Combined	CC	9(22%)	32(78%)	1.000(Reference)	
	CT	10(12.8%)	68(87.2%)	0.523(0.194-1.412)	0.201
	TT	35(61.4%)	22(38.6%)	5.657(2.273-14.077)	0.000

***Logistic regression analysis; p<0.05 indicates statistically significant**

In this study gestational period of new born baby was observed in the IDA group, Healthy group and combined groups among CC, CT and TT genotypes. Preterm birth in Healthy group was insignificant whereas in IDA group and combined group, TT genotype was at least 2.2 times [OR, 95%CI: 10.125(2.200-46.589)] and also 2.2 times [OR, 95%CI: 5.657(2.273-14.077)] more prone for the preterm birth which were statistically highly significant as shown in Table.27.

Low Birth Weight (LBW):

Table.28 showing LBW in relation with different genotypes

Groups	Genotype	Yes (%)	No (%)	OR(95% CI)	p-Value*
IDA	CC	2(16.7%)	10(83.3%)	1.000(Reference)	
	CT	11(26.8%)	30(73.2%)	1.833(0.346-9.719)	0.476
	TT	28(80.0%)	7(20%)	20.000(3.548-112.746)	0.009
Healthy	CC	2(6.9%)	37(100%)	1.000(Reference)	
	CT	0(0%)	37(100%)	0.000(0.000)	0.998
	TT	10(45.5%)	12(54.5%)	11.250(2.132-59.375)	0.004
Combined	CC	4(9.8%)	37(90.2%)	1.000(Reference)	
	CT	11(14.1%)	67(85.9%)	1.519(0.452-5.107)	0.500
	TT	38(66.7%)	19(33.3%)	18.500(5.745-59.570)	0.000

***Logistic regression analysis; p<0.05 indicates statistically significant**

In this study low birth weight was observed in IDA, Healthy and combined group among genotypes, TT genotype was at least 3.5 times [OR, 95%CI: 20.000(3.548-112.746)], 2.1 times [OR, 95%CI: 11.250(2.132-59.375)] and 5.7 times [OR, 95%CI: 18.500(5.745-59.570)] more lead to low birth weight of neonates respectively which was statistically highly significant (Table.28).

Perinatal Death:

Table.29 showing perinatal death in relation with different genotypes

Groups	Genotype	Yes (%)	No (%)	OR(95% CI)	p-Value*
IDA	CC	1(8.3%)	11(91.7%)	1.000(Reference)	
	CT	2(4.9%)	39(95.1%)	0.564(0.047-6.817)	0.652
	TT	8(23.5%)	26(76.5%)	3.385(0.377-30.398)	0.276
Healthy	CC	0(0%)	29(100%)	1.000(Reference)	
	CT	1(2.7%)	36(97.3%)	44874301.22	0.998
	TT	2(9.1%)	20(90.9%)	161547491.6	0.998
Combined	CC	1(2.4%)	40(97.6%)	1.000(Reference)	
	CT	3(3.8%)	75(96.2%)	1.600(0.161-15.887)	0.688
	TT	10(17.9%)	46(82.1%)	8.696(1.066-70.928)	0.043

***Logistic regression analysis; p<0.05 indicates statistically significant**

Table.29 revealed that perinatal deaths were observed in all groups among genotypes, but in IDA and Healthy group which was also statistically insignificant (p>0.05) whereas in combined group, among TT genotype 1 time [OR (95% CI): 8.696(1.066-70.928)] more prone to perinatal deaths of babies.

DISCUSSION

With the above findings, we noticed that TT genotype has the pathogenic role and CC genotype has the protective role in the development of iron deficiency anaemia whereas CT genotype has the intermediate role.

In the study of Gonclaves L et al., (2014) the genotypic and allelic frequency determination among IDA and normal groups, it was revealed that the CC genotype and C allele were significantly less frequent in the IDA group whereas the frequency of the TT genotype and of the T allele was significantly higher in the IDA group among Portuguese women[9].

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. In their study, it was also noticed that C homozygous has lower hepcidin levels and higher transferrin saturation level than T homozygous in general population[10]. 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[11]. Melis et al., (2008) had conducted the sequencing analysis of TMPRSS6 gene polymorphism which revealed a homozygous causal mutation[12].

Morphometric changes of placenta with different genotypes:

In the present study, morphometrical findings of placenta was observed among mothers with different TMPRSS6 rs 855791 genotypes of IDA and Healthy groups.

Placental weight (gm) in TT genotypes was reduced in all groups than CC, CT genotypes in both groups which were statistically highly significant ($P<0.0001$).

Regarding the Diameter and thickness of placenta, it was decreased in TT genotypes when compared with CC and CT genotypes among the both the groups which were statistically significant ($p<0.05$). Umbilical cord insertion ratio of placenta was decreased in TT genotypes when compared with CC and CT genotypes among both the groups. But, it was statistically insignificant ($p>0.05$) in IDA group. However, overall observation among both groups it was statistically significant. Surface area of placenta (cm2) was decreased in TT genotypes when compared with CC and CT genotypes in both the groups. But, it was statistically insignificant in Healthy group ($p>0.05$). However, it was significant ($p<0.05$) in collective observation. Volume of placenta was decreased in TT genotypes when compared with CC and CT genotypes among both the groups which were statistically significant ($p<0.05$). The number of maternal cotyledons was decreased in TT genotype when compared with CC and CT genotypes among the all the groups except in CT genotype among

healthy group which was slightly lower than TT genotype. The difference was statistically insignificant (p>0.05) in Healthy group. However, according to overall observation of both groups together, it was decreased in TT genotype than CT and CC genotypes which was statistically significant (p<0.05). Regarding placental index, it was increased in TT genotype when compared with CC and CT genotypes among both the groups. But it was statistically insignificant (p>0.05). However, overall observation of both groups together, it was statistically significant (p<0.05).

In this study, morphometrical changes of placenta like placental weight, thickness, diameter, cord insertion ratio, surface area and volume were decreased and placental index was increased in mothers with TT genotype than the CC and CT genotypes

Foetal Outcome:

In this study, intrauterine growth retardation was observed in the foetuses of IDA group and healthy group among all the three genotypes. But in IDA group it was insignificant (p>0.05). Although, it was observed that among both the groups collectively, foetuses of TT genotype were at least 4.5 times [OR, 95%CI: 16.213(4.478-58.698)] more prone for the intrauterine growth retardation which was statistically highly significant (p<0.0001).

In this study gestational period of new born baby, preterm birth was observed in the IDA group, healthy group and combined groups among all the three (CC, CT and TT) genotypes. Preterm birth in healthy group was insignificant (p>0.05). However, overall observation of both groups together, TT genotype was at least 2.2 times [OR, 95%CI: 5.657(2.273-14.077)] more prone for the preterm birth which were statistically highly significant (p<0.0001).

Foetal weight (gm) was decreased in TT genotypes when compared with CC and CT genotypes among all the groups which were statistically significant (p<0.05).

In this study low birth weight was observed in IDA and healthy group among genotypes. Overall observation of both groups together, TT genotype was at least 5.7 times [OR, 95%CI: 18.500(5.745-59.570)] more led to low birth weight of neonates respectively which were statistically significant (p<0.05).

In this study, Perinatal deaths were also observed in all groups among genotypes, but in IDA and healthy group which was also statistically insignificant (p>0.05) whereas overall observation of both groups together, among TT genotype 1 time [OR (95% CI): 8.696(1.066-70.928)] more prone to perinatal deaths of babies.

According to above findings foetal complications like intrauterine growth retardation and preterm birth of neonates, low birth weight neonates and perinatal death were more noticed among

TT genotype. All these changes may also be due to hypoxia which is caused by iron deficiency anaemia of pregnant women which lead to histo-morphological changes of placenta.

CONCLUSION

- Among the study of TMPRSS6 gene SNP rs855791 polymorphism, TT genotype shows the pathogenic role which cause iron deficiency anaemia.
- Morphometrical changes like placental weight, diameter, thickness, cord insertion ratio, surface area, volume, number of maternal cotyledons were decreased in TT genotype whereas placental index was increased in TT genotype.
- Intrauterine growth retardation and preterm birth of neonates were more noticed among TT genotype. Foetal weight was reduced among TT genotype mothers.
- Low birth weight neonates and perinatal death were more noticed among TT genotype.
- According to above findings, this study concluded that TT genotype of TMPRSS6 gene SNP rs 855791 causes development of iron deficiency anaemia which may lead to abnormal changes of placenta resulting into poor outcome of pregnancy in Indian population.
- This study suggests that Regular antenatal care is mandatory to take all the sufficient supplements and taking of intravenous iron therapy for the mothers who have TMPRSS6 gene SNP rs855791 polymorphism in order to prevent the adverse outcome of pregnancy.
- Due to development and modern life style, frequency of precious pregnancies is increasing. It is suggested that all the precious pregnancies should be screened for TMPRSS6 gene SNP rs 855791 polymorphism in order to prevent the adverse outcome of pregnancy.

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