Role of TMPRSS6 rs855791 (T > C) polymorphism with iron and ferritin in Iraqi adult patients with iron deficiency anemia

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Abstract. Our study aimed to investigate the association between TMPRSS6 polymorphism and risk of iron deficiency anemia. In this analytical (case and control) study, venous blood samples were taken from (100) subjects, (50) patients with iron deficiency anemia and (50) healthy volunteers as a control group. Hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), iron, and ferritin were measured. Genomic DNA was extracted by salting out method and the TMPRSS6 C/T polymorphism and AQP1 were analyzed using polymerase chain reaction (PCR). The results showed that the frequency of TT genotype of TMPRSS6 C/T polymorphism was higher in IDA patients. The results also showed significantly low Hb concentration, MCV, MCHC, MCH, iron, and ferritin in IDA patients when compared with normal individuals (P≤0.00). The well gene and mutant gene within the IDA patients were shown to have no interaction with the CBC parameters (Hb, MCV, MCHC, MCH and RBC). The frequency of TT genotype of AQP1 polymorphism was significantly higher with mutant gene in IDA patients in comparison with the controls. In conclusion, there were statistically significant association between TMPRSS6 C/T polymorphism and risk of IDA and the same as of AQP1gene among Iraqi patients in Baghdad state. Also, There were interactions observed between TMPRSS6 C/T genotypes and AQP1 with means of Hb, MCV, MCHC, MCH, and iron in IDA patients group when compared with normal individuals. But there were no statistically significant differences between well gene and mutant gene in IDA patients.

1 Introduction

Iron deficiency (ID) is among the most common type of nutritional deficiencies, both in developed and developing countries and reported as the primary cause of anemia in women and children under 5 years [1]. More than 50% of reproductive age women (15–49) years have been reported as anemic [2]. Iron deficiency occurs due to disturbance of balance among iron uptake, utilization and storage in the body. Iron is important for a large number of different processes in the body, most important of which is erythropoiesis as iron is a component of hemoglobin, found in red blood cells [3]. Reduced iron level due to decreased

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absorption from food or excessive blood loss lead to insufficient red blood cells formation subsequently leading to IDA [4]. Iron deficiency anemia (IDA) occurs due to multiple factors including micronutrient deficiency, genetics, infectious diseases and blood loss caused by internal or external injuries [5].

Homozygous inactivation of the TMPRSS6 gene leads to excessive Hepcidin Anti-Microbial Peptide production, impaired dietary iron absorption and microcytic anemia in mice and iron-refractory iron deficiency anemia (IRIDA) in humans [6]. Iron refractory iron deficiency anemia is a recently recognized recessive disorder that causes microcytic hypochromic anemia. It is due to mutations of the trans-membrane protease serine 6 (TMPRSS6) gene, which encodes matriptase-2, a type of II trans-membrane serine protease mainly expressed by hepatocytes [7].

2 Materials and methods

This study was designed as a case-control study conducted on 100 subjects those divided into two groups: the first group included (50) patients with Iron deficiency anemia, and the second one included (50) individuals as healthy control group. Venous blood samples (5 ml) were obtained from the study groups, (2) ml of blood was put in EDTA tubes and mixed carefully, and used for CBC tests and DNA extraction.

The rest of blood was drawn gradually into dispensable tubes containing isolating gel for serum iron and ferritin investigations. Genomic DNA was extracted by salting out method and the TMPRSS6 C/T polymorphism and AQP1 were analyzed using polymerase chain reaction (PCR). Amplified fragments were separated on 2% agarose gel and stained with ethidium bromide then demonstrated by gel documentation system, which produce single band at 249 bp representing C homozygous (CC) and produce single band at 192bp representing C homozygous. Patients with known other inherited microcytic anemia were excluded from the study, while persons with IDA, aged 20 to 50 years were included. The ELISA kit for diagnostics was employed to calculate the serum ferritin, while the serum iron was measured using Abbott method.

3 Results

This study is conducted to find out the potential association between IDA susceptibility and TMPRSS6 gene polymorphism at rs855791. A total of 100 subjects were enrolled in the study. Table (1) showed that the mean levels and SD of Hb, RBC, MCV, MCH, MCHC, Iron, and Ferritin within the IDA patients were (11.11±0.73), (4.15 ±0.44), (72.39±7.31), (23.7±2.36), (29.39±2.61), (50.50±29.76), and (19.48) respectively when compared with their mean levels and SD within the control group (13.89±1.26), (4.66±0.69), (81.36±5.45), (30.73±6.86), (33.07±3.00), (97.06±26.61), and (43.78) respectively, with highly significant differences ($p<0.001$).

Table 1. Distribution of mean and SD of hematologic parameters among the study groups (IDA patients and controls)

p value	Study groups			
	Patients	Control		
<0.001**	11.11	13.89	Mean	Hb
	0.73	1.26	SD	
<0.001**	4.15	4.66	Mean	RBC
	0.44	0.69	SD	
<0.001**	72.39	81.36	Mean	MCV
	7.31	5.45	SD	

<0.001**	23.07	30.73	Mean	MCH
	2.36	6.86	SD	
<0.001**	29.39	33.07	Mean	MCHC
	2.61	3.00	SD	
<0.001**	50.50	97.06	Mean	Iron
	29.76	26.61	SD	
<0.001**	19.48	43.78	Mean	Ferritin

Results in table (2) revealed that the rs855791 (V736A) genotype frequencies were in HWE for both cases and controls. In our study, the association of rs855791 with the pathogenicity of IDA in co-dominant, additive, dominant, and recessive models was assessed. It was found that rs855791 (V736A) is significantly associated with IDA as observed in codominant model ($P < 0.05$, OR: 1.4 and 95% CI: 1.08- 2.8).

Similarly, significant association was found in Additive model ($P < 0.05$, OR: 1.7 and 95% CI: 1.1- 3.01). However, non-significant results were obtained in Dominant and Recessive models ($P > 0.05$). Among the four studied genetic model's, significant P-values (0.038, and 0.041 respectively) were observed in co-dominance and additive models, confirming that rs855791 is significantly associated with IDA. We found no significant correlation between blood and Fe parameters with TMPRSS6 rs855791 genotypes, while a highly significant correlation was found between blood and Ascorbic acid with TMPRSS6 rs855791 genotypes.

Table 2. Different genetic models for TMPRSS6 rs85791 (T > C) association with IDA

	rs855791				
	Genotype			Allele	
	CC	CT	TT	C	T
Hb	11.25±0.67	11.22±0.79	10.98±0.67	11.23±0.74	11.05±0.72
p value	0.235 ^{NS}			0.255 ^{NS}	
RBC	4.15±0.16	4.15±0.51	4.16±0.43	4.15±0.42	4.16±0.45
p value	0.998 ^{NS}			0.968 ^{NS}	
MCV	68.82±9.26	72.09±6.30	73.60±7.44	70.90±7.58	73.12±7.11
p value	0.123 ^{NS}			0.164 ^{NS}	
MCH	22.48±2.06	23.00±2.27	23.29±2.53	22.81±2.20	23.20±2.44
p value	0.563 ^{NS}			0.446 ^{NS}	
MCHC	28.97±1.93	28.99±1.79	29.87±3.28	28.98±1.83	29.59±2.91
p value	0.246 ^{NS}			0.275 ^{NS}	

Genotyping was performed using PCR–RFLP technique. The PCR product (249 bp) and (192bp) were digested with stu1 restriction enzyme to detect T > C polymorphism as shown in figure (1a). The genotype frequencies of patients and control subjects were in Hardy–Weinberg equilibrium for both SNPs.

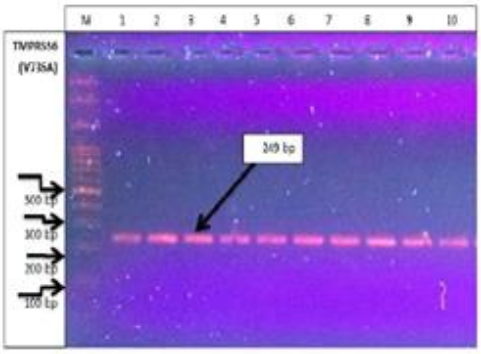

Fig. 1. **(a):** Genotyping of TMPRSS6 by PCR (PCR product = 249 bp).

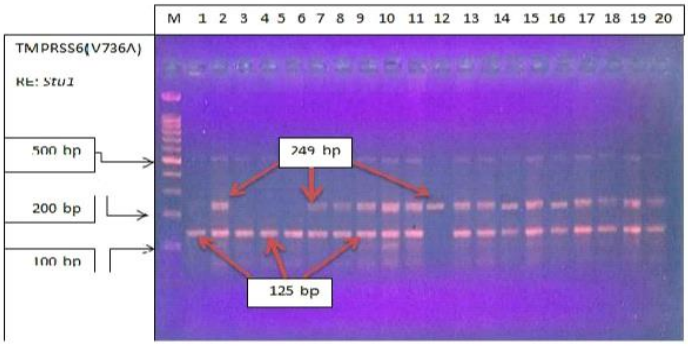

Fig. 1. (b): Genotyping of TMPRSS6 by RFLP. PCR products were digested by stu1. Single band 249 bp represented homozygous C and single band at 125 bp represented homozygous T. Two bands represented heterozygous TC

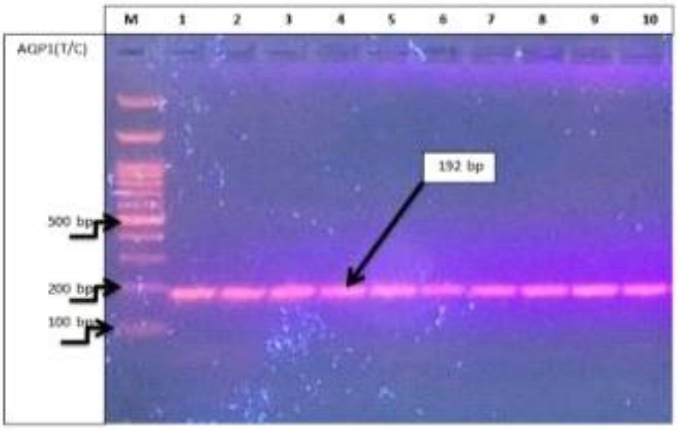

Fig. 1. (c): Genotyping of AQ1 by PCR (PCR product = 129 bp)

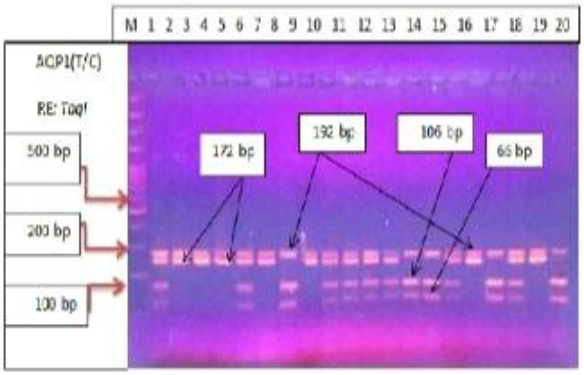

Fig. 1. (d): Genotyping of AQ1 by RFLP. PCR products were digested by stu1. Single band 192 bp represented homozygous C and single band at 172 bp represented homozygous T. Two bands represented heterozygous TC

4 Discussion

Iraqi Adults patients are at high risk of developing IDA. The GWAS studies in European and Indian Asian populations and in Chianti (Italy) and Baltimore (Washington DC) populations proved that TMPRSS6 SNPs are strongly associated with low hemoglobin and lower serum iron concentrations [8]. This study was designed to investigate the role of TMPRSS6 gene rs855791 mutation in adult patients affected with IDA in Iraq. We found that TMPRSS6 rs855791 is significantly associated with IDA in our study subjects. TMPRSS6 gene was investigated by PCR, which produce Single band at 249 bp represented well gene (C homozygous CC). The frequency of mutant (TT) genotype of TMPRSS6 rs855791 polymorphism was higher in IDA patients.

There were statistically significant association between TMPRSS6 C/T polymorphism genotypes and risk of IDA (P≤0.038). Also, our results found that the means of Hb, MCV, MCH and MCHC in IDA patients and were significantly lower than their means in the control group (P≤0.00). The interpretation of low Hb concentration, MCV, MCH and MCHC values is that TMPRSS6 polymorphisms is due to imbalance in iron hemostasis (low heam decrease Hb synthesis) which lead to IRIDA. The present results are in agreement with Sung et al (2014) who reported (8.5 g/dl, 69 fl and 18%) values for Hb concentration, MCV and RDW respectively in IDA patients [9].

An et al (2012) conducted a study on Chinese population, and reported that TMPRSS6 polymorphisms are significantly associated with decreased iron status which is associated with lower hemoglobin levels and there were a common variants in TMPRSS6 as being a genetic risk factor for IDA (P≤ 0.00) [10]. Women of higher reproductive age are mostly anemic due to increasing gynecological issues related to pregnancy, postpartum bleeding and menorrhagia . TMPRSS6 rs855791 SNP is crucial in the pathogenicity of IDA as shown in our study as well as globally inferring that genetics is an important factor in IDA pathogenesis. Consistent with their associations to increased iron deficiency and anemia risk which agree with current result, a study conducted on Italian population, observed that TMPRSS6 mutation leads to overproduction of hepcidin and defective iron absorption and utilization, which is a high risk factor for iron deficiency anemia [11].

TMPRSS6 homozygous mutation increases the risk of iron deficiency anemia by inappropriately elevated hepcidin expression in Tmprss6-/- , which results in chronically impaired uptake of dietary iron, reflected in decreased hepatic iron stores [12]. Significantly

fewer C homozygotes in the IDA group compared to the healthy group have been reported, suggesting that homozygosis for TMPRSS6 C genotype has a protective role against IDA [13].

5 Conclusion

There were statistically significant association between TMPRSS6 polymorphic genotypes and IDA risk. There were interactions observed between TMPRSS6 C/T genotypes with means of Hb, MCV, MCHC and MCH with IDA patients group when compere with normal individuals.

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