Genetic Polymorphisms of AQP1 rs10244884 Associated with Iron Deficiency Anemia in Baghdad City

Assist. Lect. Sawsan Hashim Hoshe;
Prof. Dr. Moead E. AL Gazally
and Assist. Prof. Dr. Hussein Naji AL-Shammari

Department of Chemistry and Biochemistry, College of Medicine,
University of Babylon, Babylon / Iraq
Iris2008hashim@gmail.com; moaedalgazally@yahoo.com; dhussein013@gmail.com

تعدد الاشكال الجينية للبروتين اكيوابورين 1rs10244884 المرتبط بفقر الدم الناجم عن نقص الحديد في مدينة بغداد

م. م. سوسن هاشم حوشي، أ. د. مؤيد عمران الغزالي
و أ. م . د. حسين ناجي الشمري

قسم الكيمياء و الكيمياء الحياتية،كلية الطب - جامعه بابل، بابل \ العراق

Abstract

Most people throughout the world suffer from iron deficiency anemia (IDA). The morbidity rate is relatively high for such a complicated illness. Importantly, it has been identified that the aquaporin-1 polymorphism rs10244884 plays a critical role in iron homeostasis in the human body. The rs10244884 (T > C) polymorphism is significantly associated with iron levels and a variety of blood factors, leading to iron deficiency anemia (IDA). Venous blood samples were collected from (100) people in this analytical (case and control) investigation, including (50) patients with iron deficiency anemia and (50) healthy volunteers as a control group. The following parameters were measured: hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH). The AQP1 C/T polymorphism was identified by polymerase chain reaction (PCR) after genomic DNA was extracted through the salting-out procedure.

Finally, the AQP1 C/T polymorphism was shown to have a significant association with IDA prevalence among Iraqi patients in Baghdad city. Furthermore, we found significant associations between AQP1 C/T genotypes and the medians of Hb, MCV, MCHC, and MCH in IDA patients compared to healthy controls. However, neither the healthy nor the mutant gene showed any discernible changes in IDA patients.

Keywords: IDA- Hemoglobin- AQP1- rs10244884 (T>C)

المستخلص

اغلب الاشخاص في العالم يعانون من فقر الدم الناجم عن نقص الحديد، حيث انه مرض متعدد العوامل مع معدل امراضيه عالي حيث وجد ان تعدد الاشكال يلعب دورا اساسيا في توازن الحديد في جسم الانسان .

هدفت الدراسه الى التحقق من العلاقه بين تعدد الاشكال الجيني لانزيم البروتين AQP1 rs10244884 محمل بالصفات الجينية وخطر فقر الدم بسبب نقص الحديد. حددت مجموعه من 100 شخص لهذه الدراسه 50 مريض يعانون من فقر الدم بسبب نقص الحديد و50 من المتطوعين الاصحاءو جمعت البيانات للمرضى (العمر، الجنس، تركيز خضاب الدم، متوسط حجم الكرية الدم متوسط حجم خضاب الدم، متوسط حجم تركيز خضاب الدم) من الملفات الطبية للمرضى بواسطه الاستبيان كما جمع «5 مل من الدم تم من جميع المشتركين ووضع (2)مل في تيوب EDTA المضاده للتخثر وخزنه تحت درجه -20» سيليزيه كي يستعمل فيما بعد لاستخلاص DNA .

استخلص الحمض النووي من الدم لتحليل تعدد الاشكال الجيني للجين AQP1 rs10244884 بواسطة تفاعل البلمرة لاجزاء متضخمة فرقت بواسطة 2% من الجل المصبوغ ببرمايد الاثيديوم واظهرت النتائج بنظام الهلام الكهربائي التي انتجت جزء واحد عند 192 تمثل C السائده (CC) و التي تحللت بواسطة الحزمة الاحصائية الاصدارة 7 والتي بينت وجود اختلافات ذات دلاله معنوية في مرضى ($P\leq0.00$) و تم الكشف عن الجين انزيم البروتين AQP1 rs10244884 بواسطة تفاعلات البلمره المتسلسلة النمط الوراثي (TT) من تعدد الاشكال الجيني لانزيم البروتيني AQP1 مرتفع بنسبه كبيره في مرضى فقر الدم بسبب نقص الحديد اظهرت النتائج انخفاض في تركيز HB,MCHC,MCH,MCV، مقارنة مع الاشخاص الطبيعين .أما بالنسبه للجين الهجين والجين السائد وكذلك بالنسبه لمرضى نقص الحديد فلا يوجد اي تفاعل بين الجينات المذكورة والباراميترات للصورة الكاملة للدم .واخيرا فان تعدد الاشكال الجيني AQP1rs10244884 يوضح لنا ارتباط شديد بفقر الدم في المرضى في العراق و خاصة بمدينة بغداد كذلك لوحظ وجود ارتباط بين تعدد الاشكال الجيني AQP1 rs10244884 و Hb,MCHC,MCH,MCV، في حالة فقرالدم الناجم عن نقص الحديد مقارنة مع الاشخاص الاصحاء.

الكلمات المفتاحية: فقر الدم الناتج عن نقص الحديد، تعددالاشكال الجينية و بروتين AQP1 rs10244884

Introduction

Iron deficiency (ID) is one of the most frequent dietary deficits in industrialized and poor countries. It is the leading cause of anemia in women and children under five (Hershko, 2018).

Anemia is the most common hematologic disorder, iron deficiency being the leading cause worldwide (Elstrott, *et al*., 2020). Often, anemia is the presenting sign of a more serious underlying condition that, if left untreated, can generate consequent morbidity (Portugal-Nunes. *et al*., 2020)

Iron deficiency anemia (IDA) occurs when the body does not get enough iron, either because of poor iron absorption from meals or excessive blood loss (Burz, *et al*., 2018). IDA can be caused by diseases, genetics, lack of vitamins, and internal or external injuries that cause blood loss (Cappellini, *et al*., 2020). Approximately 38% of pregnant women, 29% of non-pregnant women, and 29% of all women of reproductive age were anemic in 2011, according to the World Health Organization (WHO), and 50% of these women have iron deficiency anemia (IDA) (WHO, 2021).

Due to increased hepcidin levels, individuals with iron-resistant iron deficiency anemia (IRIDA) (1 out of 1 M) are unable to absorb intestinal iron (Pei, *et al*., 2014). Hepcidin controls iron homeostasis by modulating macrophage iron absorption and release (Camaschella, *et al*., 2019).

Materials and Methods

Patients and Control

The equation for Daniels's sample size formula was used to calculate sample size includes (100) individuals divided into two groups. The first group comprises the (50) patients diagnosed earlier as IDA. While the second group consists of (50) seemingly healthy controls.

Collecting samples:

From each individual in group one and two 3ml of blood drawn and stored in EDTA-anticoagulant-treated containers before analysis.

Inclusion criteria

1. Persons with IDA.
2. Age from 20 to 50 years.
3. Pregnancy

Exclusion criteria:

Microcytic anemia patients who have another known hereditary cause.

Samples Collection:

Characteristics of Red Blood Cells Each participant had 5 ml of blood drawn, and the sample was divided in half for serum and whole blood analysis. A contemporary hematology analyzer (CBC) was used to determine hemoglobin level, hematocrit%, red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Genotyping

DNA was isolated from whole blood was used the salting method (Iranpur-Mobarakeh and Esmailizadeh, 2010). The association between rs10244884 and IDA susceptibility was studied using the PCR-RFLP technique.

Methodology of PCR test

DNA extraction

RBC Lysis

1. An anticoagulant-treat collecting tube was used to collect fresh human blood.
2. A total of 300 µl of fresh blood was added to a 1.5 milliliter micro-centrifuge tube to fill it halfway (not provided). If the sample is bigger than 300 µl, fill a sterilized 15 milliliters centrifuge tube with it (up to 1 ml).
3. Left the sample combination to remain at 25°C for ten minutes.
4. It is centrifuged at 3000-xg for five minutes, and all the supernatants are removed.
5. Then 100 µl of RBC Lysis Buffer was added to the pellet and the cells were re-suspended by pipetting.

Cell Lysis

1. Then 200 µl of FABG Buffer was added to the sample mixture, and mixed well by vortex.
2. Permitted the sample combination to rest at room temperature for ten minutes, or until it became clear. During incubation, the test tube inverted every 3min.
3. In a 70°C water bath, preheated the needed Elution Buffer (for Step 5 DNA Elution).

DNA Binding

1. The samples vortexed for 10 seconds after added 200µl of ethanol (96- 100%). If a precipitate has developed, the sample was pipetted to mix it.
2. The FABG Column Installed to the Collection Tube. Carefully the sample was transferred to the FABG Column. For one minute, centrifuge at 18,000 x g. the FABG Column replaced in a new Collection tube after discarding the old one.

Column washing

1. The FABG Column was filled with 400 µl of W1 buffer and centrifuged for 30 seconds at 18,000 x g. The FABG Column returned to the collection tube after discarding the flow-through.
2. The FABG Column was filled with 600µl of wash buffer and centrifuged for 30 seconds at 18,000 xg. The FABG Column returned to the collection tube after discarding the flow-through.
3. Dried the column tube by centrifuging for a further 3 minutes at 18,000 x g.

Elution

1. The FABG Column was transported to a 1.5mL micro-centrifuge tube after drying.
2. 100µl of Pre-heated Elution. Buffer or TE added to the membrane center. Of FABG Column.
3. The FAGB Column placed in an incubator at 37°C for 10 minutes.

4. The DNA was eluted by centrifugation at maximum speed for 1 minute at 18,000x g.
5. The DNA was kept at 4 °C or -20 °C.

DNA concentration and purity

The concentration and purity of the isolated DNA were analyzed by a Nano drop spectrophotometer. DNA concentration measurements were recorded as µg/ml, while DNA purity was taken from the ratio obtained from the A260/280 absorbance formula. The 260/280 and 260/230 ratios were calculated to determine both the quantity and quality of the DNA. Whenever the 260/280 ratio was less than 1.7 and/or the 260/230 ratio was less than 1.7, the sample was re-extracted. (Ngole, *et al*., 2022) . Table (1) showed the set of primers used in this investigation as a forward and reversed for Aquaporin 1 gene rs10244884.

Table [1]: The set of primers used

Gene	Type primer	Sequence
Aquaporin 1 gene rs10244884	Forward Reverse	F: 5'- ATAGGTGCCACCCATGCTCC - : 5'- GCCTCTGCTCTGCTGACTCG-3' 3'

Agarose Gel Electrophoresis

Agarose gel electrophoresis was the most effective way and standard method to separate, identify, purify DNA fragments (DNA fragments of varying sizes ranging from 100 bp to 25 kb) and it is simple, rapid to perform. The location of DNA within the gel can be determined directly using staining. Bands containing as little as 1-10 mg of DNA can be detected by direct

examination of the gel in ultraviolet light. Electrophoresis is the movement of a charged molecules, chiefly proteins and nucleic acids under the influence of an electric field, Loading dyes used in gel electrophoresis serve three major purposes:

1- They add density to the sample, allowing it to sink into the gel.
2- The dyes provide color and simplify the loading process.
3- The dyes move at standard rates through the gel, allowed for the estimation of the distance that DNA fragments had migrated. The rate of migration of a DNA molecule through a gel was determined by the following:
   - size of DNA molecule.
   - agarose concentration.
   - DNA conformation.
   - voltage applied.
   - presence or use of staining.
   - type of agarose and electrophoresis buffer.

Gel Electrophoresis for analyze DNA quality

1. Preparation of Tris-borate-EDTA buffer (TBE): A weight of 27g of Tris base with 14 g boric acid, and 1.86 g of EDTA (pH 8) dissolved in 500 ml distilled water.
2. The gel was prepared at a concentration of 1% for DNA extraction (2% for PCR production) by dissolving 0.3 of agarose in a 30 ml buffer solution TBE 0.5X and then heated the mixture for 1 min in the microwave.

3. The homogeneous solution of agarose was left until its temperature reaches 55°C, then 3µl of ethidium bromide (10 mg/µl) was added to it and mixed with the mixture by turning the beaker.
4. The homogeneous mixture of agarose was poured into the gel tray and left to polymerize for 30 minutes.
5. After hardening, the agarose was transferred to an electrophoresis device and immersed in a TBE running buffer at a concentration of 0.5X. 6. Ten µl of DNA extraction (five µl of PCR production) was combined with 2 µl of loading-dye and carefully loaded by a mechanical pipette into the wells of the gel.
6. The electrophoresis was set to 100 volts and 70 A for 20 minutes for DNA extraction (50 minutes for PCR production) and the devise was carried out.
7. After completing the electrophoresis, the gel was imaged and the image was analyzed in order to determine the molecular weights of the DNA segments.
8. The gel was then photographed and analyzed used the CS analyzer® software to determine the extracted DNA molecular weight.
9. The gel was exposed to the UV light and the DNA bands was visible. The PCR product (192bp) were digested with stu1 restriction enzyme to detect T > C polymorphism as shown in Figure (1).

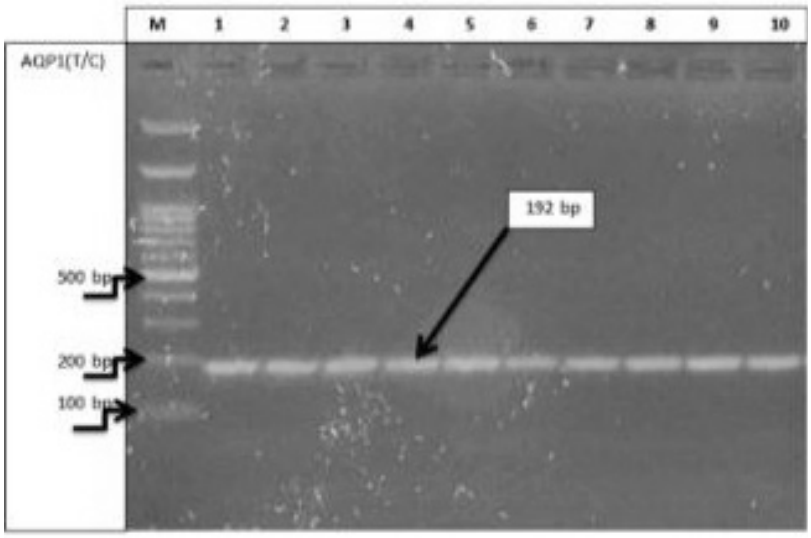

Figure (1): Electrophoresis for Genomic DNA. Extracted genomic DNA agarose gel electrophoresis, the electrophoresis was conducted on 2% agarose, 100 volts for 20 min.

Restriction enzyme add

10 µl of PCR product was taken and added to the PCR tube, 2.5 µl of the restriction enzymes and 2.5 µl of CutSmart® Buffer, and 10 µl of nuclease free water added to the same PCR tube to complete the total volume of the mixture, 25µl. The mixture was incubated at a temperature of 37°C for two hours in incubator, and after two hours the activity of the restriction enzyme was proven at a temperature of 65oC for 20 minutes.

The Polymerase Chain Reaction (PCR) steps

1- Initialization step

The reaction was heated to about 94-96°C (this study used 95.0°C). It required for DNA polymerases activation.

2- Denaturation step

This step was included heat the reaction to about 94-98°C (this study used 95.0 °C) for melting the DNA template by distraction of hydrogen bonds. The single stranded DNA molecules are yielded in this step.

3- Annealing step

The temperature of reaction was lowered to 50-65 °C (this study used 60.0°C). Allowing for the primers to annealing with single stranded of DNA template. The Taq polymerase attaches to the primer- template hybrid and begins the formation of DNA.

4- Extension / Elongation step

The optimum activity of Taq polymerase occurs at 72-80 °C and (this study used 72.0 °C) was used with this enzyme.

DNA polymerase synthesizes a new DNA strand that complementary to the DNA template strand by adding dNTPs in 5 to 3 direction. The extension time depends on both the length of the DNA target and the type of DNA polymerase.

PCR Components of Amplification

The PCR G0 Taq® G2 Green Master- Mix Kit was used for amplification of ARG1gene. The PCR G0 Taq® G2 Green Master - Mix Kit was a premixed ready-to- use solution containing G0 Taq® G2 DNA Polymerase, MgCl2, dNTPs, and reaction buffers at optimal concentrations for efficient. Amplification of a wide range of DNA templates by PCR. G0 Taq® G2 Green Master Mix contains two dye (blue and yellow) that allow monitoring of

progress during electrophoresis. The reactions assembled with G0 Taq® G2 Green Master Mix have sufficient density for direct loading onto agarose gels. G0 Taq® G2 DNA Polymerase exhibits 5 ' 3 ' exonuclease activity.

Thermo-cycler Program of Amplification

The PCR Thermo-cycler program that gave the results of amplification of AQP1 gene was shown in Table (2). Thermo-cycler program

Table (2): PCR amplification of AQP1rs10244884 gene

Name of cycle	Temp °C	Time	No. of cycle
Initial denaturation	95	5 min	1 cycle
Denaturation	95	1min	35 cycle
Annealing	60	1min	35 cycle
Extension	72	1min	35 cycle
Final extension	72	5min	1 cycle

Agarose Gel Electrophoresis for PCR Products

After execution PCR, 5 µL of each sample were loaded on 2 % agarose gel and stained with Ethidium bromide. The electrophoresis was performed at (55V for 45 minutes) to evaluate the PCR product.

Optimization of PCR Conditions

Started the optimization of PCR reaction for rs10244884 genotyping with the thermo- cycling condition listed in the (Table 3).

Table (3): Optimized reaction mixture for PCR

Composition	Concentration	Volume
Master mix	2.5 X	8
Mgcl2	25 MM	0.5
Forward primer	10 PM	1
Revers primer	10-20 ng/µl	1
DNA sample		2
Nucleases free water		7.5
Total volume		20

PCR-RFLP

Restrictive Digestive Digestion for the Gene AQP1 rs10244884 PCR-RFLP represented in Table (1) .

PCR-RFLP was a two-part method as indicated by its name. The first part consists of a traditional PCR with primers surrounding the possibly mutated area. The PCR product is then subjected to restriction enzymes which have the ability to cut DNA at specific sequences, also called restriction sites, thus creating DNA fragments. The restriction enzymes can either be used as positive or negative markers meaning that if the DNA was mutated the enzyme can either gain a restriction site, creating more fragments and of different sizes than with normal DNA, or lose a site that normally exists in un mutated DNA.

The latter will then result in less fragments and of different sizes than with normal DNA. After restriction, the fragments were analyzed by gel electrophoresis, which creates visible bands, and by assessing; how far the bands have travelled through the gel in comparison to a DNA ladder with known band sizes the restriction fragments' sizes can be estimated. The number of fragments combined with their sizes allows the original sequence to be identified as mutated.

The following ingredients are combined to produce the restriction reaction:

1- An amount of (5 µl) of PCR product.
2- The restriction enzyme (Taq 1) 0.25 µl.
3- Restriction buffer 1.5 µl (each restriction enzyme has its own restriction buffer, which is given by the manufacturer).
4- Bovine serum albumin (BSA) 0.15 g/L add 0.1 µl.
5- Using molecular grade water, the reaction mixture was brought to an end at 15 µl add 20 µl of mineral oil.
6- The reaction mixture was incubated for 24 hours at 65°C in a water bath.
7- The RFLP reaction product was resolved on 2% Agarose gel electrophoresis as illustrated in Figure (2) as figure of RFLP.

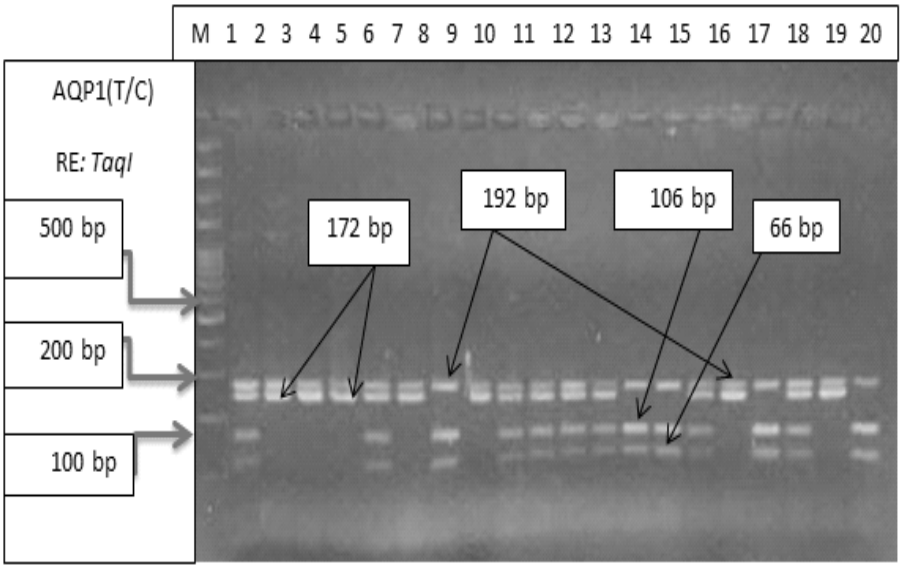

Figure. (2): Genotyping of AQP1 by RFLP. PCR products were digested by stu1. Single band 192 bp represented homozygous C and single band at 172 bp represented homozygous T. Two bands represented heterozygous TC.

Statistical analysis

The statistical analysis of this prospective study performed with the statistical package for social sciences (SPSS) 20.0 and Graph pad prism Version 7. Numerical data were tested for normality testing using Shapiro-Wilk test found that the data were abnormally distributed. The data described as mean and standard deviation and independent sample t test used for comparison between two groups. Categorical data were described as count and percentage. Chi-square test or Fisher exact test used to estimate the association between variables. Odds ratio and its 95%(Chaparro and Suchdev, 2019)

Results

This study aims to look for a link between IDA susceptibility and the AQP1 gene polymorphism at RS10244884. The study had 192 individuals in total. The PCR-RFLP method was used for genotyping. The PCR result, which was 192 base pairs long and had T > C polymorphism, was digested with the stu1 restriction enzyme (Figure 3, a and b).

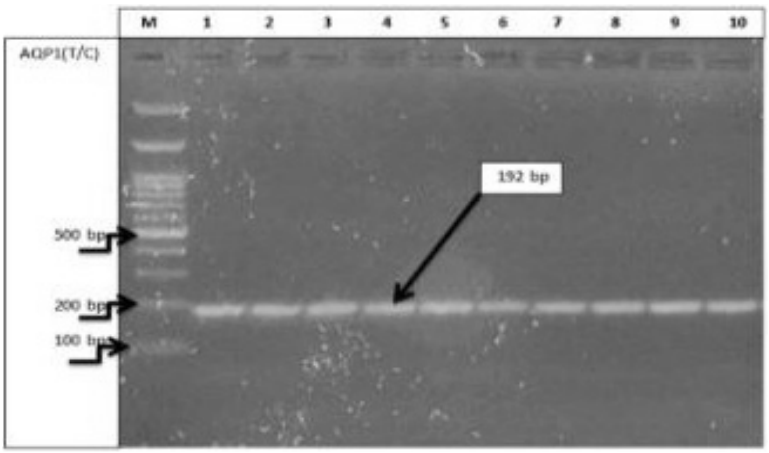

Figure 3 (a): AQP1 genotyping by PCR (PCR product = 129 bp).

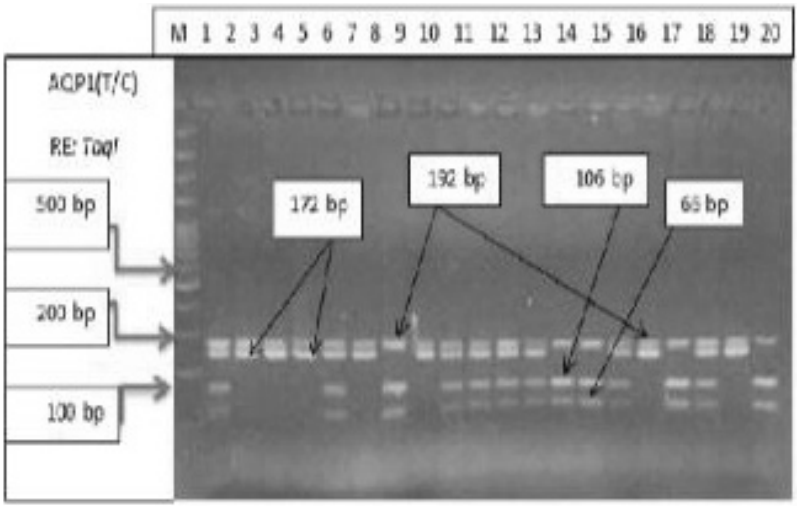

Figure 3 (b): RFLP genotyping of AQP1. stu1 digested the PCR products. A single 192-bp band indicated homozygous C, whereas a single 172-bp band represented homozygous T. Two bands denoted heterozygous TC.

Participants' baseline and clinical features

Table 1 compares the baseline and clinical characteristics of research participants. There were significant variations in all of the baseline characteristics between the patients and controls. Cases were older than controls, with a mean age of 31.2 years against 33.76 years. There were significant variations in all of the baseline characteristics between the patients and controls. The mean ages of cases and controls were 31.02 and 33.76, respectively, indicating that cases were older than controls. Hb levels in patients were 11.11 g/dl vs. 13.89 g/dl (P< 0.001).

Furthermore, MCV was lower in cases than in controls, 72.39 vs. 81.36 (P< 0.001). Low levels of MCH and MCHC were also seen in the control group (P 0.001), but RBC levels were lower in the case (P< 0.001). Hb levels in patients were 11.11 g/dl vs. 13.89 g/dl (P<0.001). In addition, MCV was lower

in cases than in controls, 72.39 vs. 81.36 (P< 0.001). The levels of MCH and MCHC were also low in controls (P< 0.001), but the levels of RBC were lower in the case (P< 0.001). Table (3) show the distribution of mean and SD of hematologic parameters among the study groups (IDA patients and controls).

Table (3): 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	

Association of Aquaporin1 rs10244884 with IDA

Both patients and controls had rs10244884 genotype frequencies in HWE. In codominant, additive, dominant, and recessive models, they looked at the association between rs10244884 and IDA pathogenicity. In the codominant model, rs10244884 was found to have a significant association with IDA (P 0.05, OR: 2.1, and 95% CI: 1.2-6.3). Similarly, the Additive model revealed a significant association (P 0.05, OR: 1.3, and 95% CI: 1.1- 01.72). However, P-values (P > 0.05) were discovered in Dominant and Recessive models. Among the four analyzed genetic models, rs10244884 was shown to

have the strongest association with IDA in the co-dominance and additive models (P-values of 0.012 and 0.045, respectively). Table (4) show that the Different genetic models for the association between AQP1 rs10244884(T > C) and IDA.

Table (4): Different genetic models for the association between AQP1 rs10244884 (T > C) and IDA

	Study groups		P value	Odds ratio (CI95%)
rs10244884	Control	Patients		
Codominant				
CC Homozygous	8 (16.0)	17 (34.0)	0.012*	2.1 (1.2-6.3)
TC Heterozygous	28 (56.0)	23 (46.0)		
TT Homozygous	14 (28.0)	10 (20.0)		
Additive				
C allele	44 (44.0)	57 (57.0)	0.045*	1.3 (1.01-1.72)
T allele	56 (56.0)	43 (43.0)		
Dominant				
TT	14 (28.0)	10 (20.0)	0.483NS	0.642 (0.24-1.5)
TC+CC	36 (72.0)	40 (80.0)		
Recessive				
CC	8 (16.0)	17 (34.0)	0.063 NS	2.7 (1-7)
TC+TT	42 (84.0)	33 (66.0)		

Association analysis between hematological traits and AQP1 rs10244884 polymorphism

Table (4) illustrate a significant relationship between Hb level and mutant C allele (10.11) vs. T allele (11.12). Similarly, the CC genotype has a lower Hb level (10.26), the CT genotype (10.88), and TT genotype (11.4). While, Table (5) show the findings of an association study between hematological traits and the AQP1 rs10244884 polymorphism.

Table (5): The findings of an association study between hematological traits and the AQP1 rs10244884 polymorphism

	rs10244884				
	Genotype			Allele	
	CC	CT	TT	C	T
Hb	10.26±0.60	10.88±0.86	11.40±0.35	10.11±0.74	11.12±0.72
p value	0.008*			0.015*	
RBC	4.08±0.42	4.12±0.45	4.35±0.42	4.10±0.43	4.23±0.45
p value	0.075 NS			0.147 NS	
MCV	72.45±7.56	73.13±6.77	70.59±8.10	72.72±7.23	71.95±7.48
p value	0.435 NS			0.603 NS	
MCH	22.73±1.69	23.44±2.97	22.79±1.61	23.02±2.31	23.14±2.45
p value	0.347 NS			0.800 NS	
MCHC	28.36±1.49	30.36±2.59	28.90±3.39	29.17±2.23	29.68±3.05
p value	0.002*			0.357 NS	

Discussion

Patients of adult age in Iraq have an increased chance of acquiring IDA. Genome-wide association studies (GWAS) conducted on people in Europe, India, Asia, Chianti, Italy, and Baltimore, Maryland, found that TMPRSS6 SNPs are strongly linked to anemia and poor iron stores (Ngole, *et al*., 2022). The researchers in this study set out to learn how the rs10244884 mutation in the AQP1 gene affected adult IDA patients in Iraq. We found that AQP1 rs10244884 was significantly associated with IDA in our study population. The PCR analysis of the AQP1 gene revealed a single band at 192bp, indicative of a highly represented gene (C homozygous CC). The TMPRSS6 rs10244884 polymorphism's mutant (TT) genotype was more common in IDA patients. The genotypes of the AQP1 C/T polymorphism were significantly associated with the incidence of IDA (P0.038). Moreover, our findings revealed that the means of Hb, MCV, MCH, and MCHC were significantly lower in IDA patients

than in the control group (P ≤0.00). An imbalance in iron hemostasis (poor hem reduces Hb synthesis) caused by TMPRSS6 polymorphisms underlies the low Hb concentration, MCV, MCH, and MCHC values. Values for Hb concentration, MCV, and RDW in IDA patients revealed here are consistent with those established by (Sun, *et al*., 2021) (8.5 g/dl, 69 fl, and 18%). AQP1 rs10244884 this study was proved that SNP plays a crucial role in IDA pathogenicity, and genetics play a major role in IDA pathogenesis.(Sung. 2014) found that patients with IDA had Hb concentration and MCV values of (8.5 g/dl, 69 fl, and 18%), which aligns with the current findings.

Conclusions

There was a strong correlation between the AQP1Rs10244884 polymorphism genotype and the likelihood of developing IDA. Interactions between AQP1 C/T genotypes and the means of Hb, MCV, MCHC, and MCH were seen in IDA patients compared to healthy controls.

References

- Chaparro CM, Suchdev PS, (2019), Anemia Epidemiology, Pathophysiology, and Etiology in Low- and Middle-income Countries. Ann. N Y Acad. Sci. 1450,15–31.
- Ngole, M., Race, V., Mbayabo, G., Lumbala, P., Songo, C., Lukusa, P. T., ... & Lumaka, A., (2022), DNA Testing for Sickle Cell Anemia in Africa: Implementation Choices for the Democratic Republic of Congo. Journal of Clinical Laboratory Analysis, 36(5), e24398.
- Sun, T., Chen, Y., Wen, Y., Zhu, Z., & Li, M., Prempli, (2021), A Machine Learning Model for Predicting the Effects of Missense Mutations on Protein-ligand Interactions. Communications biology, 4(1), 1-11.
- Sung, N. P. , Ming, C. M., Huey, L. Y., Hung, C. Fu, Ching, Y. K., Kun, M. R., Ming, C. W. and Chien, T. L., (2014), TMPRSS6rs855791 Polymorphism Influences the Susceptibility to Iron Deficiency Anemia in Women at Reproductive Age, International Journal of Medical Sciences; 11(6), 614-619.
- Hershko, C., (2018), Assessment of Iron Deficiency. Hematologica, 103 (12), 1939–1942.
- Elstrott, B., Khan, L., Olson, S., Raghunathan, V., DeLoughery, T., &Shatzel, J. J., (2020), The Role of Iron Repletion in Adult Iron Deficiency Anemia and Other Diseases. European journal of hematology, 104(3), 153-161.
- Portugal-Nunes, C., Castanho, T. C., Amorim, L., Moreira, P. S., Mariz, J., Marques, F., ... & Palha, J. A., (2020), Iron Status is Associated with Mood, Cognition, and Functional Ability in Older Adults: A Cross-sectional Study. Nutrients, 12(11), 3594.
- Burz, C., Cismaru, A., Pop, V., Bojan, A., (2018), Iron-Deficiency Anemia, Iron Deficiency Anemia: In tech. Open.
- Cappellini, M.D., Musallam, K.M., Taher, A.T., (2020), Iron Deficiency Anemia. J. Intern. Med., 287 (2), 153–170.
- World Health Organization, (2020), Global Anemia Reduction Efforts Among Women of Reproductive Age: Impact, Achievement of Targets and the Way Forward for Optimizing Efforts. Geneva: World Health Organization.
- Pei, S. N., Ma, M. C., You, H. L., Fu, H. C., Kuo, C. Y., Rau, K. M., Wang, M. C., Lee, C.T., (2014), TMPRSS6 rs855791 Polymorphism Influences the Susceptibility to Iron Deficiency Anemia in Women at Reproductive Age. Int. J. Med. Sci. 11 (6), 614.
- Camaschella, C., Pagani, A., Nai, A., Silvestri, L.,(2019), Hepcidin and Anemia: A Tight Relationship. Front. Physiol. 10, 1–7.
- Iranpur-Mobarakeh, V., Esmailizadeh, A., (2010) , Rapid Extraction of High Quality DNA from Whole Blood Stored at 4oC for Long Period Protocol Online PID:4175.