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Association between P582S *HIF-1A* gene polymorphism and hematological parameters among women: A cross-sectional study

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ABSTRACT

Women are prone to low red blood indices due to increased physiological requirements and frequent blood loss in menstrual periods. Hypoxia-inducible factors (HIFs) act as master regulators of oxygen and iron balance. In this study, the association between P582S *HIF-1A* polymorphism and red blood indices among women was examined. A total of 310 participants were recruited in the study. PCR followed by RFLP technology was used to genotype *HIF-1A* polymorphism. The mean age of participants was 27.0 years, and the mean BMI was 26.4±7.73 kg/m^2. Most of the participants did not exercise (89.1%), and about 21.1% were current tobacco smokers. Frequency of 582S (T) mutant allele was 17.5% while the frequency of 582P (C) wild-type allele was 82.5%. No association was found between P582S *HIF-1A* and hemoglobin level (p=0.37), red blood cell count (p=0.33), hematocrit (p=0.96), mean body size (p=0.20), mean corpuscular volume (p=0.34), mean corpuscular hemoglobin concentration (p=0.22), red blood cell distribution width (p=0.77), ferritin (p=0.19), and erythropoietin (p=0.15). In addition, no significant differences were found in distribution of P582S genotypes according to age of participants, body mass index, smoking status, and exercise habits (p>0.05). In conclusion, P582S *HIF-1A* polymorphism may not be associated with red blood indices among women. More studies in other populations are needed to confirm this finding.

Keywords: *HIF-1A*, P582S, rs11549465, red blood, hemoglobin, ferritin

INTRODUCTION

Women are exposed to regular blood loss during menstruation [1]. On average, females who menstruate lose about 40-80 ml of blood per period. In about 10% of females, menstrual bleeding accounts for more than 1.4 mg iron loss per day [2]. Women with menorrhagia usually experience fatigue, distress, depression and negative social interactions [3, 4] and are prone to anemia and tissue hypoxia [1]. Among the proposed causes of menorrhagia are infection and hormonal changes [5, 6]. Understanding the factors that affect the amount of blood loss and hematological homeostasis in menstruating women can help improve women's health during their reproductive age [7, 8].

Hypoxia-inducible factor (HIF-1) is a dimeric protein complex that functions as a master oxygen regulator in conditions with low oxygen concentrations [9, 10]. HIF-1 consists of *HIF-1A* subunit, which is regulated by oxygen tension and the constitutively expressed HIF-1B subunit. Hydroxylation of *HIF-1A* subunit by prolyl hydroxylase in the normal oxygen state signals its degradation [11]. This mechanism plays an important role in regulating HIF abundance and oxygen homeostasis [12]. However, when oxygen levels decrease, *HIF-1A* becomes stable and translocate from the cytoplasm to the nucleus where it binds to HIF-1B to form HIF complex [11]. HIF-1 complex binds to hypoxia response elements (HRE) of HIF-1 target genes to induce gene expression. Iron-carrying transferrin, transferrin receptors, and erythropoietin are among the targets of HIF-1 [13, 14]. HIF-1 also reduces the expression of hepcidin, a protein that inhibits iron transport into cells by activating ferroprotein degradation [15]. Studies have shown that prolyl hydroxylase inhibitors (HIF-1 stabilizers) can be used as a treatment for anemia in patients with chronic kidney disease [16, 17]. Thus, HIF-1 is among the factors that might be involved in hematological homeostasis in the body [18].

P582S *HIF-1A* (rs11549465; C1772T) is a single nucleotide polymorphism that replaced the C at position 1772 with a T, as a result of which proline at position 582 is replaced by a serine [19]. A previous study demonstrated that the P582S polymorphism is associated with red blood cell indices among regular male blood donors by protecting the donors from iron deprivation [20]. Other studies showed an association between the P582S polymorphism and the higher endurance capacity of elite athletes [21] and increased muscle activity in humans [22].

The beneficial impact of P582S polymorphism on hematological homeostasis is attributed to the better iron levels and oxygen environment in individuals with the mutant allele of this polymorphism. In the current study, we hypothesized that the P582S polymorphism may protect females from the negative impact of menstrual cycle on red blood indices. Therefore, the aim of the current investigation was to examine the relationships between the P582S polymorphism and blood indices among women.

MATERIALS AND METHODS

Subjects

Adult females aged between 18 and 40 years were recruited from King Abdullah University Hospital to participate in the study. Subjects signed a consent form declaring their agreement to participate in the study in accordance with the policy of the Institutional Review Board of Jordan University of Science and Technology. Exclusion criteria include having acute or chronic blood disorders such as thalassemia and sickle cell anemia, pregnancy or lactation, age of >40 years, and use of iron supplements four months before the start of the study [23]. The sample size was calculated using G-power 3.1. software (Universitat Kiel, Germany). Based on an effect size of 0.15, alpha of 0.05 and a power of 0.80, a sample size of 270 is required. A total of 502 subjects were invited to participate in the study: 159 subjects were refused participation and 33 were excluded based on selection criteria. A total of 310 participants were finally included in the study. The study was conducted during the year 2020 including the recruitment of subjects.

Sample Demographics

Subjects were asked to fill out a questionnaire that collected information about weight, height, age, smoking habits, exercise habits, chronic diseases, and supplement use.

Blood Sampling

Blood samples were drawn into ethylene-diamine-tetra acetic acid (EDTA) and plain tubes. A portion of EDTA blood samples was used for complete blood count analysis (Blood Count Analyzers, Abbott Diagnostics, USA). The remaining EDTA blood samples were stored at -30 °C for molecular analysis. Plain tubes were centrifuged at 500xg for five min and serum was transferred to sterile tubes and stored at -80 °C for erythropoietin measurements. Ferritin levels were determined using an immunoassay analyzer (Elecsys 1020, Roche Diagnostics, USA) in the Medical Laboratories of King Abdullah University Hospital.

DNA Extraction

DNA was extracted from whole blood taken from EDTA tubes using a genomic DNA isolation kit obtained from Zymo Research (catalogue number: D3024, Irvine, CA, USA). The quality of the extracted DNA was assayed using a Nano-Drop spectrophotometer (Thermo Scientific, USA). DNA samples were stored at -20 °C until used for further analysis.

Genotyping of P582S *HIF-1A* Polymorphism

DNA fragment of the P582S *HIF-1A* polymorphism was amplified by PCR using a master mix purchased from Promega (USA). The primer sequences were forward 5-GAC TTT GAG TTT CAC TTG TTT-3 and reverse 5-ACT TGC GCT TTC AGG GCT TGC GGA ACT GCT T-3 [22]. PCR conditions were denaturation at 94 °C for five min, 34 cycles of denaturation at 94 °C for 60 sec, annealing at 55 °C for 60 sec, extension at 72 °C for one min, and a final extension step of 10 min at 72°C. This yields a 197 bp fragment. The amplified fragment was analyzed using RFLP technique and Tsp451 restriction enzyme (catalog number: ER1511, Thermo Scientific, USA) as previously described [24]. RFLP products were separated by electrophoresis at 120 V for 1 h using 3% agarose gel. DNA bands were visualized using ethidium bromide and UV light.

Table 1. Demographic characteristics of participants

Variable	n (%)
Mean age	27.03±6.46
Age group	
18-30	208 (67.1)
>30-40	102 (32.9)
Body mass index (BMI)	
<18.5	14 (4.5)
18.5-24.9	155 (50.0)
25-29.9	75 (24.2)
>30	66 (21.3)
Mean BMI	26.40±7.73
Smoking	
Yes	66 (21.2)
No	244 (78.8)
Exercise	
Yes	34 (10.9)
No	276 (89.1)

Table 2. Distribution of P582S polymorphism in examined population

P582S*		n (%)
Genotypes	CC	214 (69.0)
	CT	83 (26.8)
	TT	13 (4.2)
Alleles	C	511 (82.4)
	T	109 (17.6)

Note. *Hardy Weinberg equilibrium: Chi-squared value=1.795 & p=0.180

Measurement of Serum Erythropoietin Level

Serum erythropoietin was measured using an ELISA kit obtained from Fine Test (catalogue number: EH0357, Wuhan Fine Biotech Co, Wuhan, China) according to the manual provided by the manufacturer. Changes in optical density at 450 nm were measured using an ELx800 plate reader (BioTek Instruments, Winooski, VT, USA) [25].

Statistical Analysis

The relationships between P582S *HIF-1A* polymorphism and hemoglobin levels were analyzed using SNPstat statistical program. Serum levels of different parameters were compared between the different genotypic groups using the ANOVA test. Categorical variables were compared using the Chi-square test. The distribution of the different P582S *HIF-1A* genotypes was examined for their concordance with the Hardy-Weinberg equilibrium. A p less than 0.05 was used to indicate a significant difference.

RESULTS

310 women (18-40 years old) were recruited to participate in the study (**Table 1**). According to the sample, the mean age was 27.03 years, and the mean BMI was 26.4±7.73 kg/m^2. Most of the participants did not exercise (89.1%), and about 21.1% were current tobacco smokers.

The distributions of the different genotypes and alleles of P582S SNP among the sample are shown in **Table 2**. The frequency of the wild type C allele encoding proline was 82.4%, while the frequency of the T allele encoding serine was 17.6%. The majority of participants carried the CC genotype (69.0%), followed by CT genotype (26.8%) and the TT genotype (4.2%).

Table 3. P582S frequencies among different population

Population	Percentage (%)	Reference
Jordan	17.5	Current study
Italy	15.4	[20]
Iran	10.6	[38]
USA	12.9	[39]
Korea	10.5	[40]
Turkey	14.7	[41]
China	11.0	[42]
Russia	7.5	[43]
Mexico	10.9	[44]
Japan	6.7	[46]
Poland	7.0	[53]

Table 4. Distribution of different genotypes of P582S polymorphism according to different demographic variables

Variable	CC	CT	TT	p
Age group				
18-30	141 (67.8)	58 (27.9)	9 (4.3)	0.795
>30-40	73 (71.6)	25 (24.5)	4 (3.9)	
Body mass index				
<18.5	9 (64.3)	5 (35.7)	0 (0.0)	0.913
18.5-24.9	106 (68.4)	41 (26.5)	8 (5.2)	
25-29.9	51 (68.0)	22 (29.3)	2 (2.7)	
>30	48 (72.7)	15 (22.7)	3 (4.5)	
Smoking				
Yes	43 (65.2)	21 (31.8)	2 (3.0)	0.563
No	171 (70.1)	62 (25.4)	11 (4.5)	
Exercise				
Yes	20 (58.8)	12 (35.3)	2 (5.9)	0.443
No	194 (70.3)	71 (25.7)	11 (4.0)	

Table 5. Association of Hb levels with P582S polymorphism

Genotype	Controls: n (%) Hb>12.0 g/dL	Cases: n (%) Hb<12.0 g/dL	p
C/C	169 (67.9)	45 (73.8)	0.37
C/T	68 (27.3)	15 (24.6)	
T/T	12 (4.8)	1 (1.6)	
Allele C	406 (81.5)	105 (86.1)	0.24
Allele T	92 (18.5)	17 (13.9)	

Table 6. Correlation of P582S with hematological parameters

Parameter*	Mean±SD			p
	C/C	C/T	T/T	
WBCs (cell×10^3/µL)	7.39±2.00	7.44±2.90	8.11±3.00	0.56
RBCs (cell×10^6/µL)	4.75±0.38	4.70±0.43	4.60±0.36	0.33
Hct (%)	39.52±4.77	39.38±3.40	39.56±3.32	0.96
MCV (fL)	82.77±8.25	84.07±7.47	85.98±6.20	0.20
MCH (pg)	27.53±2.97	27.88±2.73	28.54±2.48	0.34
MCHC (g/dL)	32.97±1.95	36.74±32.62	33.18±1.38	0.22
RDW (%)	14.09±1.49	13.97±1.53	14.18±1.36	0.77
Platelets (×10^3/µL)	302.40±72.7	295.9±72.80	280.3±84.5	0.49
Lymphocytes (%)	34.03±9.70	32.67±9.59	30.65±9.91	0.30
Monocytes (%)	6.54±1.79	7.14±4.44	6.29±1.64	0.20
Neutrophils (%)	56.99±10.24	58.03±10.91	60.0±10.96	0.48
Eosinophils (%)	1.81±1.48	1.63±1.32	2.49±3.50	0.18
Basophils (%)	0.59±0.33	0.55±0.31	0.56±0.33	0.65
Erythropoietin (pg/ml)	149.0±129.6	172.3±133.5	107.2±57.40	0.15
Ferritin (pg/mL)	20.68±18.38	20.73±18.18	18.03±17.78	0.89

Note. *WBCs: White blood cells; RBCs: Red blood cells; Hct: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; & RDW: Red cell distribution width

Table 3 shows the distribution of P582S polymorphism in different world populations. P582S polymorphism is relatively common in most populations with frequencies ranging from 6.7% to 17.5%.

Table 4 shows the distribution of different P582S genotypes according to demographic variables (**Table 4**). No significant differences were found in the distribution of P582S genotypes according to age of participants, body mass index (BMI), smoking status, and exercise habits (p>0.05).

To examine the association between P582S polymorphism and hemoglobin among participants (**Table 5**), the sample was divided into normal hemoglobin level (Hb≥12 g/dL) and low hemoglobin levels (Hb<12 g/dL). No association was found between P582S genotypes and hemoglobin levels (p=0.37). In addition, no association was found between the distribution of P582S alleles and hemoglobin levels (p=0.24).

Relationships between different blood parameters and the P582S are shown in **Table 6**. There are no effects of the P582S SNP genotypes on the different blood parameters (p>0.05). These includes red blood cell count, hematocrits, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT), erythropoietin, ferritin, and count of the different types of white blood cells.

DISCUSSION

In the current study, the association of P582S *HIF-1A* polymorphism with blood parameters among women was examined. The results showed no association between P582S *HIF-1A* polymorphism and red blood indices among women.

HIF-1A encodes *HIF-1A* subunit that is part of the hypoxia-inducible factor (HIF-1), which plays an essential role in regulating oxygen utilization under hypoxic conditions [9]. HIF-1 is known to be involved in the regulation of hematopoiesis via interactions with erythropoietin, transferrin and hepcidin [13, 26]. Therefore, we hypothesized in the current study that such polymorphism might also affect red blood indices among women who are susceptible to blood loss due to menstruation. The results showed no association between red blood indices and P582S *HIF-1A* polymorphism among women. The results also showed no association between the low hemoglobin phenotype and all genotypes and alleles of P582S *HIF-1A* polymorphism. Furthermore, there are no effects of P582S *HIF-1A* genotypes on serum erythropoietin and ferritin levels. These findings indicate that P582S *HIF-1A* polymorphism might not be important for homeostasis in healthy menstruating women.

A previous study showed that P582S *HIF-1A* polymorphism was associated with better red blood indices and iron homeostasis after blood loss among male blood donors but not among female blood donors [20]. Male donors with the homozygous wild type allele showed significantly better blood indices of hemoglobin, hematocrit, ferritin, and mean corpuscular hemoglobin than male donors with the mutant allele [20]. Although the number of women included in [20] was very small (n=12) and blood donation is not comparable to menstrual blood loss, the present study findings, and those of [20] suggest that the impact of P582S *HIF-1A* polymorphism on blood indices might be sex-specific, being associated with blood indices in males. Thus, testing for P582S *HIF-1A* polymorphism to predict the blood indices outcomes after

blood donations/blood loss could be useful in males but not in females. It noteworthy that the effect of P582S *HIF-1A* polymorphism for oxygen utilization has been shown to be also beneficial in other situations [27]. For example, it has been shown that weightlifters, long-distance runners, and high-performance athletes are more likely to have the mutated allele for the P582S polymorphism [21]. In addition, P582S polymorphism was found to be associated with endurance training responses in women [28] and subjects' responses to hypoxia training [29].

The clinical significance of P582S polymorphism extends far beyond the efficient use of oxygen. It was found that P582S polymorphism is associated with an improved survival rate of cancer cells and an improvement in their proliferation and expansion [30]. P582S polymorphism has been shown to be associated with breast cancer [31], prostate cancer [32], gastrointestinal tract cancer [33], coronary artery disease [34], and diabetes complications [35-37].

P582S polymorphism is common, and its frequency has been reported in several populations. In the current study conducted in Jordan, the frequency of the mutant T allele was 17.5%. This frequency is similar to that of the Turks, Italians, Iranians, Chinese, Koreans, and the Americans [20, 38-42]. A relatively low frequency of the mutant allele was reported in Japanese, Polish, and Russian populations [43-46].

In the current study, there were no differences in the distribution of P582S polymorphism when the sample was stratified by age, BMI, smoking and exercise. In agreement with the present findings, no association between P582S polymorphism and parameters such as age and BMI was reported in previous studies conducted in Hungary, China, and Mexico [47-49]. However, a study in Caucasians showed a slight and significant enrichment of PP genotype in male athletes compared to controls [21]. In the present investigation, about 11% of the study sample reported exercise training. In a systematic review, exercise training was shown to improve bone mineral density, muscle strength, balance, function, and quality of life in postmenopausal women with osteoporosis via mechanisms that involved HIFs [50, 51]. Therefore, the association between P582S polymorphism and exercise training needs to be explored in a future study with a better representation of exercise training among the sample.

Study Limitations, Future Directions, and Clinical Implications

A limitation of the current study is that the subjects were healthy females. It is strongly recommended that females with anemia and other ages be included in future investigations. In addition, the study is cross-sectional in design, and therefore, it is recommended that findings be confirmed using a cohort or randomized clinical study design. Moreover, the study did not collect data regarding menses and the time of sampling relative to menstrual cycle. Furthermore, sexual and reproductive hormones cause differences in iron absorption, storage, and retention. These are further influenced by age, lifestyle, menstruation, pregnancy, lactation, and iron intake. A more comprehensive study that considers such factors could give us a better understanding of the relationship between P582S *HIF-1A* polymorphism and hematological indices among females. The current findings should be confirmed in other populations because the population's genetic background may have an influence on the results of association studies [52, 53].

CONCLUSION

In conclusion, P582S *HIF-1A* polymorphism may not be associated with red blood indices among women. More studies in other populations are needed to confirm this finding.

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Declaration of interest: No conflict of interest is declared by authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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