http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.146.150

Research Article
Frequency of the HAMP (c.-582 A>G) Polymorphism in Iron Deficiency in Saudi Arabia

[1,2]Osama Al-Amer and [3]Khalaf F. Alsharif

[1]Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk, Saudi Arabia
[2]Genome and Biotechnology Unit, Faculty of Sciences, University of Tabuk, Tabuk, Kingdom of Saudi Arabia
[3]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, Kingdom of Saudi Arabia

Abstract

Background and Objective: Hepcidin, a key element in iron hemostasis, is a small antimicrobial peptide encoded by the HAMP gene on 19q13. Several studies have revealed that the expression of hepcidin is influenced by single nucleotide polymorphisms located in the promoter region of HAMP. Therefore, this research aimed to study the frequency distribution of HAMP promoter genetic variants and their associations with serum iron, serum transferrin and serum ferritin levels in Saudi Arabian women (aged 15-25). **Materials and Methods:** The study was conducted on 108 female subjects, among whom 50 had normal levels of iron and 58 were iron deficient. All participants were enrolled at the University of Tabuk. The HAMP promoter rs10421768 A>G gene polymorphism (c.-582 A>G) was detected by using an allele-specific or amplification-refractory mutation PCR system. The AS-PCR primers were designed by using Primer3 software. **Results:** The frequencies of HAMP promoter rs10421768 genotypes AA, AG and GG were 3.45, 96.55 and 0% in the iron-deficient women and 12, 88 and 0% in the healthy women, respectively. The distributions of the HAMP promoter c.-582 A>G genotypes observed between the iron-deficient and normal women were not significantly different ($p = 0.239$). A significant difference in the HAMP genotype (c.-582 A>G) between the iron-deficient women and healthy women was associated with reduced serum iron ($p = 0.049$). **Conclusion:** The results indicated that the HAMP genotype (c.-582 A>G) was associated with reduced serum iron in women in northern Saudi Arabia. However, no significant difference was found between healthy women and iron-deficient women.

Key words: Iron-deficiency, anemia, HAMP, SNPs, c.-582 A>G, hepcidin, rs10421768

Citation: Al-Amer, O. and K.F. Alsharif, 2021. Frequency of the HAMP (c.-582 A>G) polymorphism in iron deficiency in Saudi Arabia. Pak. J. Biol. Sci., 24: 146-150.

Corresponding Author: Osama Al-Amer, Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk, Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hepcidin is a hepatic peptide hormone that plays a major role in the regulation of iron homeostasis through facilitating the entry of iron into the circulation. In humans, hepcidin is encoded by the HAMP gene. Many studies have reported that the HAMP gene is mainly expressed in the liver and is associated with iron overload[1,2]. Olthof *et al.*[3] reported that the estimation of iron overload was based on Liver Iron Concentration (LIC) and Serum Ferritin (SF) levels. Several diseases are linked to iron metabolism, mainly through genetic alterations of hepcidin that alter the uptake of iron[4-6]. Several Single Nucleotide Polymorphisms (SNPs) reported in the HAMP promoter could act as modulators of iron overload. The c.-582 A>G genetic variant was found to be associated with beta-thalassemia patients with higher serum ferritin levels and a higher liver iron concentration[7]. Marco Andreani *et al.*[7] investigated the iron status in patients with a Wild Type (WT) profile and compared their data to the iron status of patients with the p.H63D HFE and/or the c.-582 A>G HAMP-P variants[7]. Moreover, it was reported by Parajes *et al.*[8] that patients carrying the HAMP promoter c.-582A G gene variant have decreased expression of the HAMP gene. Similarly, Island *et al.*[9] investigated the HAMP promoter c.-582 A>G gene variant and showed that it impaired the response to BMPs and IL-6.

This study aimed to determine the frequency of the (c.-582 A>G) polymorphism in the HAMP promoter in the Saudi female population and to assess its association with serum ferritin and serum iron.

MATERIALS AND METHODS

Study area: This study was conducted at the University of Tabuk, Kingdom of Saudi Arabia and Taif University, Kingdom of Saudi Arabia. The research was conducted between January 1-August 20, 2020.

Sample population: A total of 108 female students were included in the present study. There were 50 healthy controls and 58 iron-deficient women. All participants were enrolled at the University of Tabuk. All procedures were conducted according to the ethical standards of the Research Ethics committees of Tabuk University.

Biochemical determinations: Serum iron and serum transferrin were assayed using a modular machine (Hitachi, UK). A Complete Blood Count (CBC) was assayed using a Beckman Coulter LH750 (Beckman Coulter Inc., Miami, FL, USA). With these methods, ferritin <15 ng mL^{-1} and hemoglobin >12 g dL^{-1} were considered as iron deficiency. Besides, ferritin <15 ng mL^{-1} and hemoglobin <12 g dL^{-1} were considered as iron deficiency anemia.

Variant genotype study: DNA was extracted from peripheral blood according to standard procedures using a QIAmp DNA blood kit (Qiagen, Valencia, CA, USA). The ratios of A260/A280 and A260/A230 by Nanodrop were used to determine the purity of the DNA. PCR was used to detect the rs10421768 polymorphism in the HAMP promoter according to the manufacturer's instructions. The Fig. 1 shows the AS-PCR of

Table 1: Primers used to study c.-582 A>G variant genotype

HAMP promoter rs10421768 A/G polymorphism c.-582 A>G variant genotype			
AS-PCR for HAMP rs10421768 (G allele); a mutant allele			
HAMPF1	TCTGACACTGGGAAAACACCG	180 bp	59°C
HAMPR1	CCGCTCTCCTCCACTCACTTC		
AS-PCR for HAMP rs10421768 (A allele); a wild-type allele			
HAMPF2	CTCAAGCGACCCTCCTGC	219 bp	59°C
HAMPR2	GTGTGCCCGATCCGCACGT		

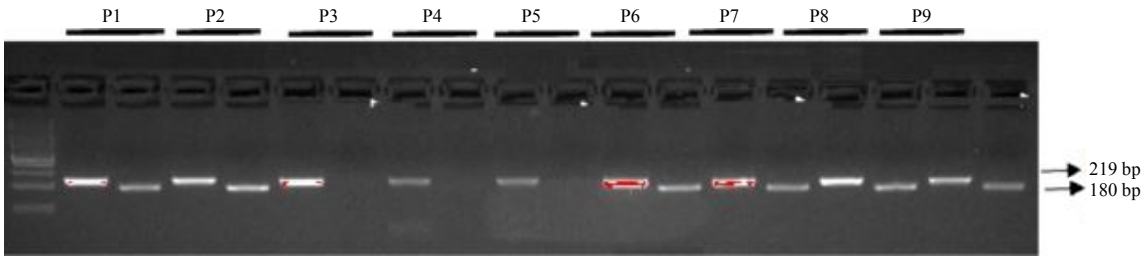

Fig. 1: Allele-specific PCR the HAMP promoter (c.-582 A>G)

the HAMP promoter with the rs10421768 (c.-582 A>G) polymorphism. Primers HAMPF1 and HAMPR1 were used to amplify the GG genotype of the HAMP promoter rs10421768 polymorphism, a mutant allele (G allele) and primers HAMPF2 and HAMPR2 were used to amplify the AA genotype, the wild-type allele (A allele) as following in Table 1.

Statistical analysis: Statistical analysis of the genetic variant of the rs10421768 polymorphism in the HAMP promoter was determined using SPSS version 16.0 software (SPSS, Chicago, USA). P-values were considered significant when p was less than 0.05. Odds ratios (ORs) and 95% confidence intervals were analyzed by the chi-square test.

RESULTS

Frequencies of HAMP c.-582 A>G genotypes in iron-deficient women and healthy women: This study was conducted on 108 female subjects, among which 50 were normal and 58 were iron deficient. In the iron-deficient women, the AA, AG and GG genotype frequencies were 3.45, 96.55 and 0%, respectively, whereas, in the healthy controls, the AA, AG and GG genotype frequencies were 12, 88 and 0%, respectively (Table 2). The distributions of HAMP promoter c.-582 A>G genotypes observed between the iron-deficient women and the normal women were not significantly different (p = 0.239).

Clinicopathological Characteristics of Iron-deficient Women: The demographic features of the subjects are depicted in Table 3. Out of the 108 consecutive women, 85 (78.70%) were carrying normal levels of hemoglobin, whereas 23 (21.30%) had lower levels of hemoglobin. This result indicated that there were no significant differences in the genotype distribution of the HAMP promoter between healthy women and hemoglobin-deficient women. Out of 108 consecutive women, 38 (35.19%) had normal levels of iron, whereas 70 (64.81%) had lower levels of iron. This result indicated that there was a significant difference in the genotype distribution of the HAMP promoter between healthy women and iron-deficient women (p = 0.049). Out of 108 consecutive women, 50 (46.29%) had a normal level of serum ferritin, whereas 58 (53.71%) had lower levels of serum ferritin. This result indicated that there were no significant differences in the genotype distributions between healthy women and ferritin-deficient women.

Out of 108 subjects, 85 (78.70%) were carrying normal levels of RBC, whereas 23 (21.30%) had lower levels of RBC. This result indicated that there were no significant differences in genotype distributions of the HAMP promoter between healthy women and RBC-deficient women. Of the 108 consecutive women, 106 (98.14%) had normal levels of platelets, whereas 02 (1.86%) had lower levels of platelets. This result indicated that there were no significant differences in the genotype distribution of the HAMP promoter between

Table 2: Frequency of the *c.-582 A>G* polymorphism in HAMP promoter

	N	AA (%)	AG (%)	GG (%)	Chi-square	DF	p-value
Normal	50	6 (12)	44 (88)	0 (0)	2.86	2	0.239
Iron deficient	58	2 (3.45)	56 (96.55)	0 (0)			

A: Adenine, G: Guanine, N: Number, %: Percentage, DF: Degrees of freedom

Table 3: Frequency of the c.-582 A>G polymorphism in HAMP promoter with respect to clinical parameters

Clinical parameters	N (%)	AA (%)	AG (%)	GG (%)	Chi-square	DF	p-value
Hemoglobin							
Normal	85 (78.70)	5 (5.88)	80 (94.12)	0 (0)	1.35	2	0.509
Abnormal	23 (21.30)	3 (0.13)	20 (87.00)	0 (0)			
Iron							
Normal	38 (35.19)	6 (15.78)	32 (84.21)	0 (0)	6.01	2	0.049
Abnormal	70 (64.81)	2 (02.85)	68 (97.14)	0 (0)			
Ferritin							
Normal	50 (46.29)	6 (12.00)	44 (88.00)	0 (0)	2.86	2	0.239
Abnormal	58 (53.71)	2 (03.45)	56 (96.55)	0 (0)			
RBC							
Normal	85 (78.70)	6 (7.06)	79 (92.94)	0 (0)	0.07	2	0.966
Abnormal	23 (21.30)	2 (8.70)	21 (91.30)	0 (0)			
Platelets							
Normal	106 (98.14)	7 (6.60)	99 (93.40)	0 (0)	5.39	2	0.068
Abnormal	02 (01.86)	1 (0.50)	01 (00.50)	0 (0)			
WBC							
Normal	80 (00.74)	6 (7.50)	74 (92.50)	0 (0)	0	2	1.000
Abnormal	28 (00.26)	2 (7.14)	26 (92.86)	0 (0)			

A: Adenine, G: Guanine, N: Number, %: Percentage, DF: Degrees of freedom

healthy women and platelet-deficient women. Of 108 consecutive women, 80 (74%) were carrying normal levels of WBC, whereas 28 (26%) had lower levels of WBC. This result indicated that there were no significant differences in genotype distributions of the HAMP promoter between healthy women and WBC-deficient women.

DISCUSSION

This study demonstrated that the AA, AG and GG genotype frequencies in iron-deficient women were 3.54, 96.55 and 0%, respectively, whereas, in healthy controls, the AA, AG and GG genotype frequencies were 12, 88 and 0%, respectively. The data showed that there is an increase in the heterozygosity of the HAMP promoter polymorphism (96.55%) in women with iron deficiency compared with health women (88%) but it was not significant.

This research assessed the association of the (c.-582 A>G) polymorphism in the HAMP promoter with the levels of serum iron and ferritin. This study showed that there was no significant association between the HAMP promoter c.-582 A>G genotype and the level of serum ferritin. Similar results were reported to demonstrate that no association was found between serum ferritin levels and the c.-582 A>G variant[8,10].

The HAMP gene controls the expression of the hepcidin protein, which is the main component of iron homeostasis regulation[11,12]. The deficiency of hepcidin is associated with many diseases, especially with anemia and also chronic diseases caused by iron overload[13]. It was reported in many studies that the c.-582 A>G genotype in the HAMP promoter could be correlated with serum iron and ferritin levels. In, addition, it has been reported in several studies that the presence of "G" at -582 in the HAMP promoter is associated with a reduction of serum ferritin levels. In thalassemia patients, the "G" variant has also been found to be associated with abnormal iron and ferritin levels[7]. To date, there is no publication demonstrating a correlation of the c.-582 A>G genotype in the HAMP promoter with a reduction of serum iron and ferritin levels in a Saudi population. Therefore, this study aimed to determine the frequency of the (c.-582 A>G) polymorphism in the HAMP promoter in the Saudi female population and to assess its associations with serum ferritin and serum iron.

On the other hand, Petrak *et al.*[14] studied iron overload in liver-derived human hepatoma HepG2 cells that were exposed to a high concentration of iron for 3 days. Interestingly, the findings showed that there was a significant association between the HAMP (c.-582 A>G) genotype and a reduction in the level of serum iron. This finding is supported by Marco Andreani *et al.*[7], who reported an association between the c.-582 A>G polymorphism and iron metabolism. However, some studies showed no significant relationship between the c.-582 A>G polymorphism and serum iron[8].

To our knowledge, this is the first study examined the association of HAMP (c.-582 A>G) polymorphism with iron deficiency in Saudi Arabia. Limitations of this study could be highlighted, such as small-sized samples of the participants. However, this finding can enhance our understanding the role of SNPs in different types of diseases.

CONCLUSION

In this study, we found that there were no significant differences in the genotype distributions of the HAMP promoter c.-582 A>G genotype between healthy women and iron-deficient women. However, we found that the HAMP genotype (c.-582 A>G) was associated with reduced serum iron.

SIGNIFICANCE STATEMENT

This study discovers the association of HAMP genotype (c.-582 A>G) with reduced serum iron that can be beneficial for understanding the role of (c.-582 A>G) in iron deficiency anemia. This study will help the researcher to uncover the critical areas of reducing serum iron in Saudi Arabia that many researchers were not able to explore. Thus a new theory on the HAMP genotype (c.-582 A>G) was associated with reduced serum iron may be arrived at.

ACKNOWLEDGMENT

Authors would like to thanks the University of Tabuk and Taif University.

REFERENCES

1. Park, C.H., E.V. Valore, A.J. Waring and T. Ganz, 2001. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J. Biol. Chem., 276: 7806-7810.
2. Hunter, H.N., D.B. Fulton, T. Ganz and H.J. Vogel, 2002. The solution structure of human hepcidin, a peptide hormone with antimicrobial activity that is involved in iron uptake and hereditary hemochromatosis. J. Biol. Chem., 277: 37597-37603.

3. Olthof, A., P. Sijens, H. Kreeftenberg, P. Kappert, R. Irwan, E. van der Jagt, M. Oudkerk, 2007. Correlation between serum ferritin levels and liver iron concentration determined by MR imaging: Impact of hematologic disease and inflammation. Magn. Reson. Imaging., 25: 228-231.
4. Ganz, T., 2003. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood, 102: 783-788.
5. Ashby, D.R., D.P. Gale, M. Busbridge, K.G. Murphy and N.D. Duncan *et al*., 2009. Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. Kidney Int., 75: 976-981.
6. Nemeth, E., 2010. Hepcidin in β-thalassemia. Ann. N. Y. Acad. Sci., 1202: 31-35.
7. Andreani, M., F.C. Radio, M. Testi, C. De Bernardo and M. Troiano *et al*., 2009. Association of hepcidin promoter c.-582 A>G variant and iron overload in thalassemia major. Haematologica, 94: 1293-1296.
8. Parajes, S., A. Gonzalez-Quintela, J. Campos, C. Quinteiro, F. Dominguez and L. Loidi, 2010. Genetic study of the hepcidin gene (HAMP) promoter and functional analysis of the c.-582A > G variant. BMC Genet., Vol. 11. 10.1186/1471-2156-11-110.
9. Island, M.L., A.M. Jouanolle, A. Mosser, Y. Deugnier, V. David, P. Brissot and O. Loreal, 2009. A new mutation in the hepcidin promoter impairs its BMP response and contributes to a severe phenotype in HFE related hemochromatosis. Haematologica, 94: 720-724.
10. Bruno, F., S. Bonalumi, C. Camaschella, M. Ferrari and L. Cremonesi, 2009. The -582A>G variant of the HAMP promoter is not associated with high serum ferritin levels in normal subjects. Haematologica, 95: 849-850.
11. Nemeth, E. and T. Ganz, 2006. Regulation of iron metabolism by hepcidin. Annu. Rev. Nutr., 26: 323-342.
12. Kwapisz, J., A. Slomka and E. Zekanowska, 2009. Hepcidin and its role in iron homeostasis. J. Int. Fed. Clin. Chem., 20: 124-128.
13. Ganz, T. and E. Nemeth, 2010. Hepcidin and disorders of iron metabolism. Annu. Rev. Med., 62: 347-360.
14. Petrak, J., D. Myslivcova, P. Man, R. Cmejla, J. Cmejlova and D. Vyoral, 2006. Proteomic analysis of iron overload in human hepatoma cells. Am. J. Physiol. Gastrointest Liver Physiol., 290: G1059-G1066.