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Hepcidin Polymorphism in Association with Plasma Hepcidin Level as Potential Risk Factors for Acne Severity and Post Acne Scarring

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ABSTRACT

Background: Acne vulgaris (AV) is an inflammatory disease of pilosebaceous follicles. It has multifactorial causes and is manifested as blackheads, papules, pustules, nodules, as well as cysts. The significantly greater serum hepcidin values among acne cases that do not develop post-acne scarring support its antifibrotic activities that were clarified by its capability of impeding transforming growth factor β1(TGF β1) induced Smad3 phosphorylation.

Objective: The aim of the current work was to assess hepcidin gene polymorphism and plasma hepcidin level in acne vulgaris cases of varying severity with and without post-acne scaring.

Patients and Methods: This case-control study included a total of 30 cases with AV with no post-acne scars, 30 cases with AV and post acne scar and 30 subjects of age and gender matched healthy controls, attending at Outpatient Clinic of Dermatology, Department of Andrology and STD, Mansoura University Hospitals, Delta, Egypt.

Results: The distribution of acne severity in acne patients (group A) was graded according to GAGS grading system. Mild acne severity was the commonest form of acne (56.7%), followed by moderate acne severity (30%), severe acne (6.7%) and very severe acne (6.7%). After applying Goodman grading system on the group of patients with post- acne scars (group B), moderate affection was the most common form (43.4%).

Conclusion: This study concluded that no significant association was found in hepcidin level between both case groups versus control group and scarred versus non scarred case groups. No significant association was found regarding HAMP genotypes and alleles with acne occurrence nor scar formation.

Keywords: Acne vulgaris, Comedones, Plasma Hepcidin.

INTRODUCTION

AV affects pilosebaceous unit and is most frequently observed during adolescence with different severity between people [1]. Despite acne vulgaris is one of the most common dermatologic complaints, it negatively affects psychosocial functioning with higher rates of depression, anxiety, social isolation [2]. The lesions of acne vulgaris are categorized into noninflammatory (open and closed comedones) as well as inflammatory (papules, pustules, nodules, and cysts) [3].

There are a lot of factors that are responsible for acne vulgaris, such as genetic factors, diet, hormone, stress, and environment [4]. Genetics are supposed to be the main cause in 80% of patients. The role of dietary factors and smoking is not clear [5]. Another common factor is excess growth of bacterium Cutibacterium acnes present on skin [6]. Likewise, the association between genetic factors and acne has been found in various populations, the majority of genes linked to acne are either major players in the innate immune system or are linked to steroid hormones metabolism [7].

Hepcidin is secreted predominantly by the liver which acts as a key regulator of iron metabolism [8]. Beside its major role in iron homeostasis, hepcidin is considered as an inflammatory marker [9]. Hepcidin, is classified as a type II acute phase protein which increases during various infections and inflammatory disorders [10]. Hepcidin has antifibrotic activities repressing hepatic fibrosis by suppressing transforming growth factor β1 (TGFβ1)- induced Smad3 phosphorylation [11]. Hepcidin wass reported to be upregulated and activated in fibrotic disorders that modulates phenotype and function of fibroblast, through the induction of myofibroblast trans-differentiation and promotion of matrix deposition [12].

El-Taweel *et al.* [13] reported a significantly reduced serum hepcidin values. so, hepcidin can have a likely role in active acne and post-acne scars.

This work was aimed to assess hepcidin gene polymorphism and plasma hepcidin level in cases with AV of varying severity with and without post-acne scarring to explain its role in pathogenesis of active acne and post-acne scars.

SUBJECTS AND METHODS

This case-control study included a total of 60 patients with AV of varying severity with or without post acne scar and 30 subjects of age and gender matched healthy controls, attending at Outpatient Clinic of Dermatology, Department of Andrology and STD, Mansoura University Hospitals, Delta, Egypt.

The included subjects were divided into three groups, 30 each; ***group A*** included patients with AV with no post acne scars, ***group B*** included patients with acne and post acne scars and ***group C*** was the control group that included age and gender matched healthy controls.

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Inclusion criteria: Patients aged 13 to 35 years and did not receive any systemic medical therapy for acne in the past 3 months.

Exclusion criteria: Patients with history of acute or chronic hepatic diseases, history of kidney diseases, history of malignancy, and history of any other cutaneous or fibrotic disease. Pregnant and lactating Females were also excluded.

Each patient was subjected for detailed history taking, general and dermatological examination to rule out any systemic or other skin disorders, acne lesion examination to detect the type of the lesions and its distribution. Determination of acne severity was done using global acne grading system (GAGS), which divides the face, chest, and upper back into 6 areas. GAGS Location factors are; forehead factor is 2, right cheek factor is 2, left cheek factor is 2, nose factor is 1, chin factor is 1 and chest and upper back factor are 3.

Each lesion was given a value (Grade) according to its severity where no lesion was 0, comedones were 1, papules were 2, pustules were 3 and nodules were 4. The local score for each area is the product of the most severe lesion, multiplied by the area factor [Local score = location factor × Grade (0-4)]. The global score is the sum of all local score [Score of 0 =No acne, 1-18 mild; 19-30 moderate; 31-38 severe] [14]. In the case of post-acne scars, Goodman and Baron scaring grading system was used

The Qualitative Scarring Grading System subdivides acne scars into grades 1–4 depending upon scar severity (macular, mild, moderate, and severe, respectively) [15].

Assessment of serum hepcidin by ELISA, Genomic DNA extraction and hepcidin genotyping:

A blood sample (3ml) was withdrawn from all subjects into EDTA containing tubes. Each sample was divided. Plasma was collected from one division for hepcidin protein assay using ELIZA while DNA was extracted from the second division of collected blood samples using commercially available spin column DNA extraction kits. Hepcidin gene was assessed through PCR reaction using commercially available kits and gene specific primers followed by RFLP detection by Mspl enzyme.

A) Assessment of serum hepcidin levels using ELISA:

Serum hepcidin concentrations were measured with a commercially available ELISA kits (Catalog No. E-EL-H6013), according to manufacturer instruction.

B) Gene Polymorphism Determination

One ml of collected blood was used for DNA extraction into tubes which contained EDTA and kept at -20 C. The blood was utilized for determination of Hepcidin G to A Gly71Asp gene polymorphisms by PCR and restriction enzymes followed by agarose gel electrophoresis.

C) Genotyping of Hepcidin G to A Gly71Asp gene polymorphism

Hepcidin G to A (Gly71Asp) substitution at nucleotide 212 in exon 3 was analyzed by conventional method of PCR amplification using commercially available kits and gene specific primers followed by RFLP enzyme detection.

Ethical consideration:

This study was ethically approved by Institutional Review Board (IRB) of the Mansoura College of Medicine. Written informed consent of all the participants was obtained. Confidentiality and privacy were upheld. The study protocol conformed to the Helsinki Declaration, the ethical norm of the World Medical Association for human testing.

Statistical Analysis

The SPSS application, version 18 (SPSS Inc., PASW statistics for Windows version 18), was used to analyse the data. SPSS Inc., Chicago. Numbers and percentages were used to express qualitative data. Means and standard deviations were used to depict quantitative data. The qualitative data between groups were compared using Chi-Square and Monte Carlo testing. For non-normally distributed data, the Kruskal Wallis and Mann Whitney U tests were employed to compare two examined groups and more than two studied groups, respectively. A one-way ANOVA test that used the Post Hoc Tukey test to identify pairwise comparisons between more than two independent groups. P value less than 0.05 was regarded as significant.

RESULTS

This study included ninety persons who were subdivided into: Group A: 30 AV cases with no post-acne scars. Group B: 30 cases with acne and post acne scars. Group C (control group): 30 healthy persons who matched the patients in age and sex.

No significant differences were found between studied groups regarding gender, age, and marital status. No significant differences were found between studied groups regarding BMI. The BMI range in group ***A*** was 25 to 40 kg/m² with a mean of 31.73 ± 4.01 kg/m²; in group ***B*** was 26 to 38 kg/m² with a mean of (30.88 ± 3.61 kg/m²) and in the control group was 24 to 38 kg/m² with a mean of (31.46 ± 3.10 kg/m²). Regarding smoking, no significant differences were found between patient and control groups. **(Table 1).**

Table (1): Demographics, BMI, obesity, and smoking status of the studied groups (group with acne lesions (Group A), group with post acne scars (Group B) and the control group).

	Group A		Group B		Control group		Test of significance
	No.	**%**	**No.**	**%**	**No.**	**%**	
Gender							
Male	8	26.7	9	30.0	16	53.3	χ^2 = **5.46**
Female	22	73.3	21	70.0	14	46.7	**P = 0.065**
Age (years)							
Min. – Max.	14-33		15-35		15-35		**H = 1.28**
Mean ± SD.	23.5±5.77		24.63±5.05		25.80±5.81		**P =0.281**
Marital status							
Single	18	60.0	12	40.0	14	46.7	χ^2 = **2.49**
Married	12	40.0	18	60.0	16	53.3	**P = 0.288**
Obesity							
Non-obese (<30)	12	40	14	46.7	9	30.0	χ^2 = **1.78**
Obese (≥30)	18	60	16	53.3	21	70.0	**P = 0.411**
BMI (kg/m^2)							
Minimum – Maximum	25.0 – 40.0		26 – 38		24 – 38		**F = 0.439** **P = 0.646**
Mean ± SD.	31.73 ± 4.01		30.88 ± 3.61		31.46 ± 3.10		
Smoking							
Non-Smokers	23	76.7	21	70.0	20	66.7	**χ2 =0.757**
Smokers	7	23.3	9	30.0	10	33.3	**P = 0.685**

χ^2: Chi square test
H: H for Kruskal Wallis test, Pairwise comparison between each two groups was performed with Post Hoc Test
IQR: Inter quartile range, F: F for ANOVA test, SD: Standard deviation
SD: Standard deviation,*: Statisticall y significant at $p \leq 0.05$

Table (2) demonstrates distribution of acne severity in the acne patients group (group A) according to GAGS grading system for, mild acne severity was the commonest form (56.7%), followed by moderate acne severity (30%), severe acne (6.7%) and very severe acne (6.7%). **After applying** Goodman grading system on patients with post-acne scars (group B), moderate affection was the most common form (43.4%), followed by mild affection (33.3%) and severe affection (23.3%).

Table (2): Severity of AV based on GAGS grading system among Group A and Severity of AV based on Goodman grading system among group B.

GAGS Grading	**Group A**		**Goodman Grading**	**Group B**	
	No.	**%**		**No.**	**%**
Mild	17	56.7	**Mild**	**10**	**33.3**
Moderate	9	30.0	**Moderate**	**13**	**43.4**
Severe	2	6.7	**Severe**	**7**	**23.3**
Very severe	2	6.7			

Table (3) and figure (1) show that the levels of serum Hepcidin in both group A (3.38±0.83 ng/ml) and group B (3.47±0.85 ng/ml) are non-significantly greater than controls (3.24±0.79 ng/ml) and also no statistically significant difference between patients with acne and those with post acne scars as regard level of serum Hepcidin.

Table (3): Levels of serum hepcidin in the subjects of studied groups

Serum hepcidin (ng/ml)	**Group A**	**Group B**	**Control group**	**Test of significance**
Mean ± SD	3.38±0.83	3.47±0.85	3.24±0.79	**H** = 0.224
Significance	P_1=0.804, P_2=0.680, P_3=0.509			P =0.800

SD: Standard deviation
p1: between Groups A and B
p2: between Group A and Controls
p3: between Group B and Controls
(*): Statistically significant at $p \leq 0.05$

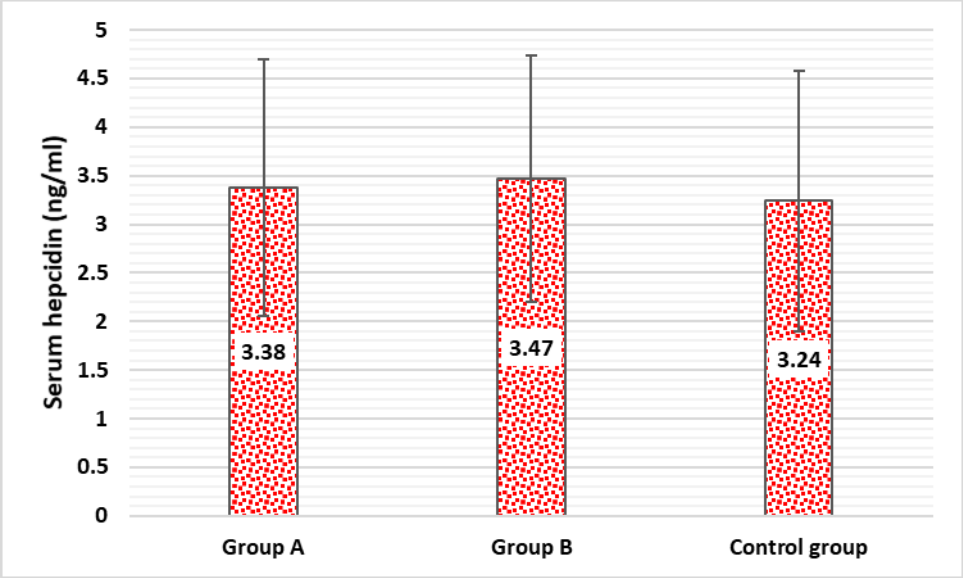

Figure (1): Mean serum hepcidin values in the studied groups

Table (4) shows serum Hepcidin values in cases with mild, moderate and severe acne are 3.39, 3.59 and 1.59 ng/ml, respectively among Group A. The serum Hepcidin levels among the various grades of acne patients do not show statistically significant difference except between very severe and severe grades. The Level of serum Hepcidin in cases with mild, moderate and severe post-acne scars are 3.27, 3.47 and 3.76 ng/ml, respectively in group B. There was no statistically significant increase in Hepcidin value with increased severity of post-acne scars.

Table (4): Levels of serum hepcidin levels in patient with different grades of acne (group A) and in patient with different grades of post-acne scars (group B)

	Mild Acne (n = 17)	**Moderate acne (n = 9)**	**Severe acne (n = 2)**	**Very severe (n=2)**	**Test of Significance**
Serum hepcidin (ng/ml) in Group A	3.39±0.82	3.59±0.88	1.59±0.38	4.26±0.15	P_1=0.711, P_2=0.073, P_3=0.370 P_4=0.057 P_5=0.506 P_6=0.047*
	Mild post acne scars (n=10)	**Moderate post acne scar (n=13)**	**Sever post acne scars (n=7)**		
Serum hepcidin (ng/ml) in Group B	3.27±0.80	3.47±0.85	3.76±0.93		P_1'=0.723, P_2'=0.453, P_3'=0.636

p1: between mild and moderate acne patients
p2: between mild and severe acne patients
p3: between mild and very severe acne patients
p4: between moderate and severe acne patients
p5: between moderate and very severe acne patients
p6: between severe and very severe acne patients
p_1': between patients with mild and moderate post-acne scars
p_2': between patients with mild and severe post-acne scars
p_3': between patients with moderate and severe post-acne scars

According to **Table (5)**, there is no discernible difference in the genotype distribution of Hepcidin genotype among the three groups under investigation.

Table (5): Frequency of genotype polymorphism in the 3 groups

	Group A		**Group B**		**Control group**		**Significance**		
	No.	**%**	**No.**	**%**	**No.**	**%**	**p_1**	**p_2**	**p_3**
AA	2	6.7	4	13.3	5	16.7	**^{MC}p= 0.714**	**^{MC}p= 0.221**	**^{MC}p= 0.235**
GA	15	50.0	19	63.3	18	60.0			
GG	13	43.3	7	23.3	7	23.3			
$^{HW}\chi^2$ (p)	**0.724 (0.395)**		**2.34 (0.125)**		**1.27 (0.261)**				

MC: Monte Carlo
HWχ2: Chi square for goodness of fit for Hardy-Weinberg equilibrium (If P < 0.05 - not consistent with HWE.)
p1: between patients with Acne and patients with Post acne scars
p2: between patients with Acne and Control
p3: between patients with Post acne scars and Control

In the different grades of patients with acne lesions, no statistically significant difference in the genotype distribution was found among Group A and also in the different grades of post-acne scar patients, no statistically significant **difference** in the genotype distribution was found according to **Table (6).**

Table (6): Frequency of hepcidin genotype polymorphism in patient with different grades of acne(group A) and in patient with different grades with post-acne scar (group B)

		Mild acne (n=17)	**Moderate acne (n=9)**	**Severe acne (n=2)**	**Very severe acne (n=2)**	**Significances**
Group A	**AA**	1(5.9%)	1(11.1%)	0	0	**$^{MC}p_1$=0.899**
	GA	7(41.2%)	6(66.7%)	1(50.0%)	1(50.0%)	**$^{MC}p_2$=0.675**
	GG	9(52.9%)	2(22.2%)	1(50.0%)	1(50.0%)	**$^{MC}p_3$=0.504**
	$^{HW}\chi^2$ (p)	**0.056 (0.812)**	**1.10 (0.294)**	**0.222 (0.637)**	**0.222 (0.637)**	
		Mild post acne scars (n=11)	**Moderate post acne scars (n=13)**	**Severe post acne scars (n=7)**		
Group B	**AA**	2 (18.2%)	0 (0.0%)	2 (28.6%)		**$^{MC}p_1$'=0.155**
	GA	8 (72.7%)	9 (69.2%)	3 (42.9%)		**$^{MC}p_2$'=0.390**
	GG	1 (9.1%)	4 (30.8%)	2 (28.6%)		**$^{MC}p_3$'=0.409**
	$^{HW}\chi^2$ (p)	**2.39 (0.122)**	**3.64 (0.05)**	**0.143 (0.705)**		

MC: Monte Carlo.

$^{HW}\chi^2$: Chi square for goodness of fit for Hardy-Weinberg equilibrium (If P < 0.05 - not consistent with HWE.)
p_1: p value for comparing between AA frequency among different severities of Acne patients
p_2: p value for comparing between GA frequency among different severities of Acne patients
p_3: p value for comparing between GG frequency among different severities of Acne patients
p_1': p value for comparing between AA frequency among different severities of post-acne scar patients
p_2': p value for comparing between GA frequency among different severities of post-acne scar patients
p_3': p value for comparing between GG frequency among different severities of post-acne scar patients

No statistically significant difference was reported between Group A and Group B's various gene polymorphisms in the serum Hepcidin levels **(Table 7).**

Table (7): Relation between serum Hepcidin levels and gene polymorphism in group (A) and group (B).

	Frequency (%)	Serum Hepcidin level (ng/ml)	Test of significance
Group A			
AA	6.7	4.459±0.237	P1 = 0.241
GA	50.0	3.65±0.81	P2 = 0.275
GG	43.3	2.91±0.43	P3 = 0.086
Group B			
AA	13.3	3.82±0.84	P1 = 0.560
GA	63.3	3.35±0.82	P2 = 0.501
GG	23.3	3.59±0.88	P3 = 0.766

P1: Significance change in Hepcidin level between AA and GA polymorphism
P2: Significance change in Hepcidin level between AA and GG polymorphism
P3: Significance change in Hepcidin level between GA and GG polymorphism

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DISCUSSION

A very common skin condition is AV. Around 85% of adolescents have AV, which can cause symptoms throughout adulthood [16]. When the pore of the pilosebaceous unit becomes obstructed or irritated, AV lesions form [17]. The quantity of inflammatory pustules, papules, and nodules as well as non-inflammatory comedones determines the severity of AV. More severe AV may also include cysts, scars, erythema, and hyperpigmentation [18]. All varieties of AV, including papules, pustules, comedones, and nodulocystic acne, are capable of causing scarring, which can manifest itself over the course of acne healing. High psychological distress and social hardship can result from scarring [19].

Hepcidin has antifibrotic activities repressing hepatic fibrosis by suppressing TGFβ1- induced Smad3 phosphorylation. Its levels have inverse correlation with exacerbation of fibrosis [11]. The HAMP gene encodes hepcidin that regulates ferroprotein in enterocytes [20].

Ali and Samweil [21] concluded that hepcidin concentration predicts occurrence and severity of post-acne scars. Likewise, it is indicator for the initiation of aggressive acne therapy like systemic retinoid.

This study was aimed to assess hepcidin gene polymorphism and plasma hepcidin level in patients with AV of varying severity with and without post-acne scaring.

Our results demonstrated that the range of patients age with acne lesions was 14.0 to 33.0 years with a mean of (23.5±5.77), in patients with post acne scars was15.0 to 35.0 years with a mean of (24.63±5.05) and in control group was 15.0 to 35.0 years with a mean of (25.80±5.81).The mean age in our study was in agreement with **El-Taweel *et al.*** [22] who found that the mean age of the acne patients was(19.57 ± 2.81 years).

Reinholz *et al.* [23] revealed that the mean age of acne patients with post acne scar was (28.6 ± 9.2 years). Likewise**, Chuah and Goh** [24] and **Agrawal & Khunger** [25] found that the mean age of acne scar cases was (25.6 ± 5.2 years).

In our work, patients with acne lesions (Group A) included eight males (26.7%) and twenty two females (73.3%), the group of patients with post-acne scars (Group B) included 16 male patients (53.3%) and 14 female patients (46.7%) while the healthy controls had 16 male subjects (53.3 %) and 14 female subjects (46.7%). There were no statistically significant differences between the patient groups and control healthy group regarding gender and age and marital status between the studied groups (matched groups). This came in agreement with **El-Taweel et al.** [22] who found that There were 44 (73.3%) female and 16 male (26.7%) patients. Female predominance may be due to hormonal changes during menstruation or higher level of stress among females [18]**.** Conversely**, Kaushik et al.** [26] reported that male cases were 2-times the female cases.

In our study**,** the severity of acne in acne cases (group A) were classified according to GAG system, into mild acne severity was the most common (56.7%), followed by moderate AV (30%), severe AV (6.7%) and very severe AV (6.7%). According to **GAGS, Fouda *et al.*** [27] reported that acne cases were classified into moderate acne severity (53.3 %the most common), followed by mild acne severity (41.7%less common), severe acne (5%the least common), very severe acne (0%no cases). The severity of acne vulgaris varied from 10 to 36 with a median score was 20.0. This was not consistent with **Alsalem *et al.*** [28] the GAGS degree distribution between studied patients is severe acne (35% the most common), followed by moderate acne severity (32.5%less common), then mild acne (27.5%less common) and very sever (the least 5%).

In this study**,** after applying **Goodman grading system** on cases with post- acne scars (group B), moderate affection was the most common form (43.4%), followed by mild affection (33.3%) and severe affection (23.3%). In the same line**, Chuah & Goh** [24] observed that moderate affection (53%the most common), followed by mild affection (34%less common) and sever affection (13%least common).

In this study**,** the mean levels of serum hepcidin in both group A (3.38±0.83 ng/ml) and group B (3.47±0.85 ng/ml) are slightly greater than that in controls (3.24±0.79 ng/ml),However, without statistically significant differences. Also, no statistically significant difference exited between patients with AV and patients with post acne scars as regard level of serum Hepcidin. In contrast, **El-Taweel *et al.*** [22] revealed that patients with post acne scarring had significantly lower serum values of hepcidin (P-value<0.001) this is consistent with **Ali and Samweil** [21].

The significantly elevated hepcidin values among AV cases that do not develop post-acne scarring support the antifibrotic activities of hepcidin. Hepcidin from hepatocyte or exogenous hepcidin can improve hepatic fibrosis via inhibition of hepatic stellate cells [11].

In the current study, no significant association was found regarding hepcidin level according to age, onset, gender, duration, marital status and family history in acne group. The mean level of serum hepcidin in cases with mild, moderate, and severe AV are 3.39±0.82, 3.59±0.88 and 1.59±0.38 ng/ml, respectively. The serum hepcidin levels among patient with various grades of acne do not show statistically significant differences except between patient with very severe and severe grades. The mean level of serum hepcidin in cases with mild, moderate, and severe post-acne scars are 3.27±0.80, 3.47±0.85 and 3.76±0.93 ng/ml, respectively. There is no statistically significant increase in hepcidin value with increased severity of post-acne scars.

As regards the hepcidin value and acne scar severity, **Ali & Samweil** [21] reported non-significant difference between hepcidin value in controls versus mild or moderate acne scars however there was significant difference between hepcidin value in controls versus severe acne scars ($p < 0.05$).

Beside its major role in iron homeostasis, hepcidin is considered as an inflammatory marker. During inflammation, activated macrophages release different proinflammatory cytokines, including IL-6, which causes hepcidin overexpression [9].

In our work**,** there is no discernible difference in HAMP genotype distribution of the hepcidin genotype between the three groups under investigation. In the different grades of acne in group A, there is no significant differences in the genotype distribution of HAMP polymorphism. In patient with different grades of post-acne scar, there was no significant difference in genotype distribution between different grades. There is no significant difference between Group A and B's various gene polymorphisms as regard the serum hepcidin levels.

To the best of our knowledge, this is the 1st work to assess hepcidin gene polymorphism in patients with acne vulgaris of varying severity with and without post-acne scaring.

Although multiple genetic loci that control acne have been revealed recently [29, 30], studies explaining genetic factors linked to acne scarring, its type or extent are scarce. Few earlier studies have tried to evaluate the specific immunologic factors linked to scars [31].

CONCLUSIONS

This study concluded that no significant association was found regarding hepcidin level between both cases groups versus control group and scarred versus non scarred cases groups. No significant association was found regarding HAMP genotypes and alleles with acne occurrence nor scar formation.

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Competing interests: Nil.

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