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Effect of Serum Homocysteine in Pathogenesis and Activity of Vitiligo.

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ABSTRACT

Vitiligo is a common, acquired, multifactorial **and** polygenic depigmenting disorder caused by the destruction of melanocytes. The exact etiopathogenesis is unknown. There has been conflicting reports on the association between the disease and the serum levels of homocysteine (Hcy) in vitiligo and its severity. Hcy may mediate melanocyte destruction via increased oxidative damage, interleukin 6 production and nuclear factor κB (NFκB) activation. Sera from patients and controls were assayed by using using a TOSOH AIA-1900 system analyzer. The mean serum level of Hcy in patients group was significantly higher than that in healthy controls. Disease activity was assessed as stable, regressive and active and there were a significant differences between three types of activities. There were elevated levels of serum Hcy in extensive vitiligo compared to healthy controls. Elevation in serum levels of Hcy were observed in patients with active as well as stable disease, but not in patients with regressive disease.

The aim of the study was to determine the level of Hcy in the sera of patients with vitiligo and healthy individuals to compare between them and to assess the role of Hcy in the development and progression of vitiligo.

Keywords: Activity, Homocysteine and Vitiligo.

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INTRODUCTION

Vitiligo is an acquired noncontagious, idiopathic, persistent, and a worldwide common depigmentation disorder in which progressive loss of functional melanocytes results in a patchy depigmentation of the skin, hair and oral mucosa, affecting people of all ages and both sexes in the same extent. It is not life-threatening but it is life-altering. Patients lose their skin color over time, most commonly in a patchy and progressive way[1,2].

Vitiligo is classified as follows: 1. Non-segmental vitiligo (NSV); acrofacial, mucosal (more than one mucosal site), generalized, universal, mixed (associated with segmental vitiligo [SV]), and rare variants, 2. Segmental; uni-, bi-, or plurisegmental, and 3. Undetermined/unclassified Vitiligo; focal or mucosal (one site in isolation) according to Bordeaux Vitiligo Global Issues Consensus Conference (VGICC) classification and consensus nomenclature [3]. Alterations in humoral and cellular immunity with abnormal cytokine profiles, autoimmunity and genetic factors can play a role in the pathogenesis of vitiligo[4,5]. Intrinsic abnormality of melanocytes, increased catecholamine release, free radicals, cytomegalovirus infection, and stress can be considered as other causes of pathogenesis [6-8]. The effect Hcy in vitiligo was studied in several studies, but the results are contradictory.Increased serum level of Hcy has been reported in patients with vitiligo[9-13].

Hyperhomocysteinemia possibly contributes to vitiligo pathogenesis through several mechanisms; Hcy auto oxidation leads to the production of toxic reactive oxygen species, which adds to the oxidative stress already present on the melanocytes of vitiligo patients [14]. Oxidative modification and alteration of self-antigens trigger autoimmunity and contribute to immune system dysregulation, with abnormal activation and processing of cell-death signals[15]. In addition, Hcy has an inhibitory action on both histidase and tyrosinase activity of the skin by binding to copper at its active site, resulting in reversible hypopigmentation[16].

Moreover, a common genetic background for both vitiligo and hyperhomocysteinemia has been detected (11q23 is a common susceptibility locus for both**)** [17,18].

Finally, the ability of Hcy to enter the protein biosynthetic pathways (due to structural similarity to cysteine) interferes with protein biosynthesis, causes protein damage and cell death, and elicits immune response against altered proteins, which is likely to contribute to the pathogenesis of autoimmune diseases[19].

PATIENTS AND METHODS

The subjects were divided into two groups: 1. Patient**s** Group = Subjects with vitiligo, 2. Control**s** Group = Subjects without vitiligo. A number of 44 (43.20% females, 56.80% males) patients with vitiligo with a mean age ± SD of (27.82±4.94) years old, and 44(40.9% females, 59.10%males) age and sex matched controls with mean age ± SD of (27.56±4.55) years old were selected. Both patients and controls were enrolled in the study at the same time interval. Disease activity was assessed as stable, progressive or regressive: If any change in vitiligo lesions was not observed by the patients within 3 month before the study, the patients were assessed to had stable vitiligo. When the enlargement of already present lesions and ⁄or the appearance of new lesions within the 3 months prior to the study are observed by the patients, the patients were assessed to had progressive vitiligo. When improvement of vitiligo lesions whether spontaneous or after ultraviolet therapy during the 3 months prior to the study, the patients were assessed to had regressive vitiligo. All blood samples were recruited from the out patients dermatology clinic in Marjan medical city, Iraq, Babylon province, Hilla during the period from November 2015 till March 2016.

Blood samples were drawn at the laboratory of the clinic and the studied biomarker was performed at the laboratories of Biochemistry Department at the college of medicine, university of Babylon.

Exclusion criteria for all subjects were age over 40 and under 15 years old, body mass index over 30, smoker, coffee drinker, patient who clinically and laboratory diagnosed with hypertension, pernicious anemia, diabetes mellitus (D.M), alopecia areata, thyroid dysfunction, pregnancy and lactation which affect the level of Hcy, intake of some medicine or drugs such as vitamins, oral contraceptives and anticoagulants, carbamazepine, phenytoin, nitrous oxide or 6-azauridine triacetate which may elevate levels of Hcy due to their effect on the metabolic pathway disease, chronic hepatic disease, chronic kidney disease who are on

drug therapy involving S- adenosyl methionine (SAM) which may show falsely elevated level of Hcy and finally animal proteins consumption 24 hours before drawing blood samples. The extent of vitiligo was measured by Wallace rule of nines to determine the total body surface area (TBSA) of the vitiligo lesion[20].

The following investigations were done for all subjects before determination of Hcy level: complete blood picture, serum alanine aminotransferase and aspartate aminotransferase, serum creatinine, fasting blood sugar and serum T3, T4 and TSH,only patients with normal results were included in this study.

After explaining the procedure and obtaining consent from every patient and control, an eight hours fasting 5mL blood sample was drawn and centrifuged at 3000 Xg after clotting for 10 minutes. Serum was obtained and stored at (-80 C^0) until the time of analysis [21].

Determination of serum homocysteine level

Serum Hcy was measured by using the ST AIA-PACK Hcy kit which is designed for the quantitative measurement of Hcy in human serum using a TOSOH AIA-1900 system analyzer.The principal of it is a competitive immune-enzymatic assay. Sample Hcy competes with enzyme-labeled Hcy for a limited number of binding sites on Hcy-specific antidody immobilized on magnetic beads. After incubation, the beads are washed to remove the unbound enzyme-labeled Hcy and then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4-MUP). Oxidized Hcy is reduced by Tris,2-carboxyethylphosphine (TCEP) to the free form and converted to S-Adenosyl homocysteine (SAH) by the SAH hydrolase and excess adenosine prior to the immunoassay. SAH present in the pre-treated sample competes with immobilized SAH on magnetic beads for binding sites on the enzyme- labeled anti-SAH mouse monoclonal antibody. The magnetic beads are washed to remove unbound anti-SAH mouse monoclonal antibody and are then incubated with a fluorogenic substrate, 4-MUP. The rate of fluorescence produced by the enzyme reaction indicates the amount of enzyme-labeled anti-SAH mouse monoclonal antibody. The amount of antibody that binds to the beads is inversely proportional to Hcy concentration in the test sample [22].

Statistical analysis

Data were analyzed using the SPSS [Statistical Package of Social Sciences for windows (version 21)] (SPSS Inc., Chicago, IL, U.S.A.) computer statistics program. Data were expressed as mean ± SD. Differences between the different variables were calculated using the Student's t-test. Annova test was used for comparing between the three means of (stable, progressive and regressive) activities of disease. The level of significance was defined as $P \leq 0.05$.

RESULTS

Twelve (27.3%) patients had vulgaris and segmental, 8 (18.1%) patients had acrofacial and 12(27.3%) patients had focal distributions. Regarding the activity of the vitiligo, the disease was progressive in 14 (31.9%) patients, regressive in 6 (27.3%) and stable in 18 (40.9%). There was a positive family history of vitiligo in first-degree relatives in nine patients (20.5%). Sixteen patients (36.4%) were receiving no treatment and 28 (63.6%) were receiving ultraviolet therapy, as it shown in (table 1). Mean duration of vitiligo was (10.73±6.48) years, mean extent of disease was (47.16±18.08) % of BSA, as it shown in (table 2). Mean serum Hcy level in the patient group was significantly higher than in the control group (14.31±6.68µmol/L vs. 7.57±2.14µmol/L respectively; $P < 0.001$, as it shown in (table 3). Mean Hcy level in patients with progressive disease was significantly higher than in the control group (23.21± 2.65µmol/L vs. 7.57± 2.14µmol/L; $P < 0.001$). In patients with stable disease the mean Hcy level was also significantly higher than in the control group (11.56± 2.20µmol/L vs. 7.57± 2.14µmol/L; $P < 0.01$), while in patients with regressive disease the mean Hcy level was almost the same as that of the controls (8.05± 1.74 µmol/L vs. 7.57± 2.14µmol/L; $P > 0.05$), as it shown in (table 4).

There was a significant relationship between Hcy level and sex of the controls; the level was significantly higher in male than female controls (9.11± 1.26µmol/L vs. 5.35 ± 0.68µmol/L; $P<0.001$). Meanwhile the level of Hcy was higher in male than female patients (15.94± 6.92µmol/L vs. 12.16± 5.84µmol/L respectively; $P > 0.05$), but it was not statistically significant as it shown in (table 5) and (table 6).

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Table1: Distribution of patients with vitiligo by type, activity, treatment and family history of disease.

Variable	Frequency (%)
Type of Vitiligo	
Vulgaris	12 (27.3%)
Segmental	12 (27.3%)
Acrofacial	8 (18.1%)
Focal	12 (27.3%)
Activity of Vitiligo	
Progressive	14 (31.8%)
Stable	18 (40.9%)
Regressive	12 (27.3%)
Patient on Treatment	
Yes	28 (63.6%)
No	16 (36.4%)
Family History of Vitiligo	
Yes	9 (20.5%)
No	35 (79.5%)

Table 2: Distribution of patients with vitiligo by duration (years) and extent of disease (% of BSA).

Parameter	group	N	Mean	S.D	t-test	p value
Homocysteine	Patients	44	14.31	6.68	6.366	<0.001*
	Control	44	7.57	2.14		

Table 3: Mean differences of homocysteine between patients with vitiligo and controls.

Variable	Mean± SD
Duration of disease	10.73±6.48
Extent of disease	47.16±18.08

*p value ≤ 0.05 is significant

Table 4: Mean differences of homocysteine by activity of vitiligo

Parameter	Activity of Vitiligo	N	Mean	S.D	ANOVA	P value
Homocysteine	Progressive	14	23.21	2.65	169.565	<0.001*
	Stable	18	11.56	2.20		
	Regressive	12	8.05	1.74		
	Total	44	14.31	6.68		

*p value ≤ 0.05 is significant

Table 5: Correlation between homocysteine by Sex among Controls

Parameter	sex	N	Mean	S.D	t-test	p value
Homocysteine	Male	26	9.11	1.26	12.714	<0.001*
	Female	18	5.35	0.68		

*p value ≤ 0.05 is significant

Table 6: Correlation between homocysteine by Sex among patients

Parameter	Sex	N	Mean	S.D	t-test	p value
Homocysteine	Male	25	15.94	6.92	1.916	0.062
	Female	19	12.16	5.84		

*p value ≤ 0.05 is significant

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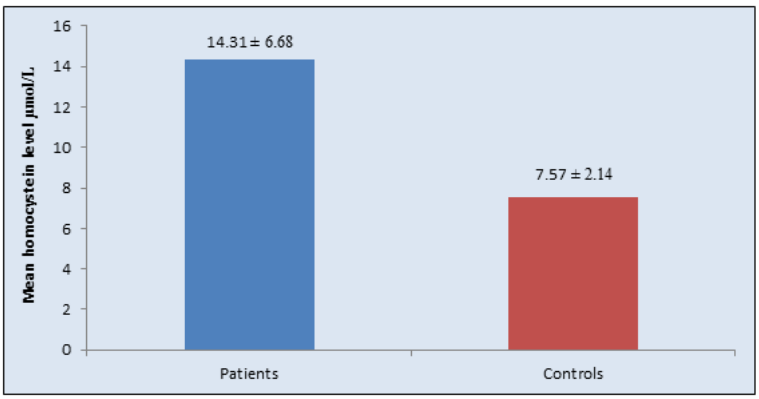

Figure 1: Comparison of mean serum level of homocysteine in patients with vitiligo and controls

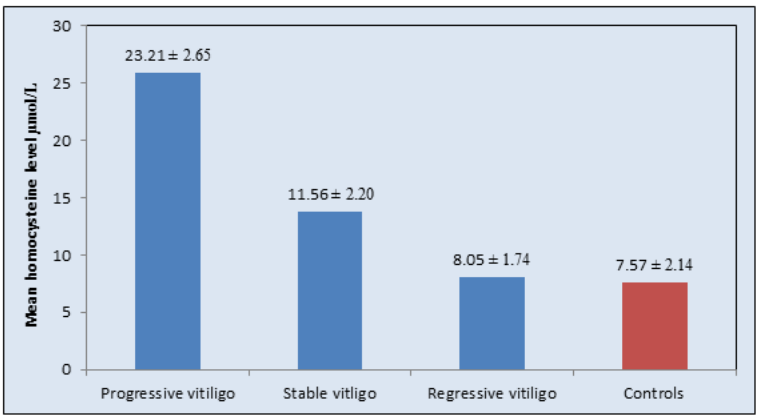

Figure 2: Comparison between mean serum levels of homocysteine and the activity of the disease in patients with vitiligo.

DISCUSSION

Hcy is a sulfhydryl amino acid product of normal protein metabolism [23]. Its balance is dependent on its production, its irreversible catabolism to other compounds and its remethylation to methionine. The latter process uses folate as the methyl donor and involves vitamin B12-related enzymes [24]. Consequently, low plasma levels of vitamin B12 and folic acid have been implicated in elevated concentrations of Hcy[25], hence the presumed contribution to the pathogenesis of vitiligo. Hcy has an inhibitory effect on the histidase and tyrosinase activity of the skin and increases reactive oxygen species (ROS) formation and lipid peroxidation, as well as decreases antioxidant defenses[1]. Increased reactive oxygen species which impair cell function and bind covalently to the catalytic center of tyrosinase, may impair or inactivate the enzyme and may reduce melanogenesis[26].

In our study, the serum Hcy level was significantly highly elevated in patients with vitiligo than in controls (14.31± 6.68µmol/L vs. 7.57± 2.14µmol/L respectively; $P < 0.001$) as it shown in (Fig.1). These findings are logical as Hcy metabolism depends on both folic acid and vitamin B12,[27] both of which are lowered in patients with vitiligo[28,29]. Hcy may mediate hypomelanogenesis or melanocyte destruction in vitiligo through inhibition of key melanogenic enzymes and increased reactive oxygen radicals, which may have toxic effects on melanocytes. It may also play a role in the development of autoimmunity through altered protein structure [30]. Our results were compatible with studies which reported high Hcy levels in vitiligo[9-11,31].

Increased serum level of Hcy may be associated with our patients' disease. However, genetic factors can be effective on increased serum Hcy levels both in patients and controls groups. Polymorphism owing to genetic variations modifying activity of implicated enzymes such as methylenetetrahydropholate reductase (MTHFR) increases mean Hcy levels by about 25%. It is high in whites, whereas it is very low in black populations [32]. However, we do not know the effect of MTHFR on levels of Hcy because of the fact that we

did not analyze MTHFR polymorphism in this study. This was the one of the limitations of our study. The results of Hcy should be correlated with MTHFR polymorphism. Hcy was related to the gender of patients, activity of disease, and dietary habits according to Singh *et al* [9]. .In contrast to the present study, other studies reported that no significant difference in the levels of Hcy were found between vitiligo patients and the controls groups[33-38].

The controversy in the results among different studies may result from ethnic differences among the different populations, difference in the mean body surface area affected by the disease, and differences in age, sex distribution and stability of the disease.

In this study also there was elevation of Hcy levels observed in patients with active as well as stable disease, but not in patients with regressive disease, pointing to a possible relationship to vitiligo as it shown in (Fig.2). These findings coincide with Shaker *et al* who reported that serum Hcy levels were significantly higher in patients with progressive vitiligo than healthy sex- and age-matched controls. Silverberg *et al* have also been proposed Hcy to be a biomarker for the disease[10,32]. Hcy was also related to gender and disease activity; it was higher in males than in females as well as in patients with active vitiligo versus those with a stable and regressive disease [9]. Similar results were also obtained in other studies [11,12,38].

CONCLUSION

In conclusion, Hcy may play a role in the pathogenesis of vitiligo and we demonstrated an association between homocysteine and vitiligo.It was also demonstrated that serum Hcy levels significantly elevated in extensive stages of the disease. Hcy may be a precipitating factor in the pathogenesis of vitiligo in predisposed individuals.We recommend the routine estimation of Hcy level in patients.The limitation of our study was its small sample size and therefore it might not be representative of all Iraqi vitiligo patients because of the diversity of them in factors like ethnical, ecological and genetic factors.

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