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Study of Serum Levels Homocysteine in Vitiligo Patients and Controls

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ABSTRACT
Vitiligo is an acquired depigmentary condition caused by selective destruction of melanocytes in the basal layer of epidermis. It may involve the hair follicle melanocytes also. To estimate the serum levels homocysteine in patients with vitiligo and control group. 35 patients with vitiligo and 35 age and sex matched controls presented to Dermatology OPD in a tertiary Hospital between January to December 2021 were included in the study. Venous samples were obtained from the study subjects and the levels of homocysteine, vitamin B12 and folic acid were measured by enzyme chemiluminiscent essay method and the data were analyzed using SPSS software version 2020. Serum homocysteine was related to the sex of the patients and type of vitiligo. No significant difference were found in the levels of serum homocysteine between vitiligo patients and controls ($p>0.05$). No significant relation was found between serum levels of homocysteine and age of the patients, duration of the disease and vitiligo activity ($p>0.05$). It seems that the presence of a relationship between vitiligo and the serum homocysteine remains controversial and needs to be vigorously investigated.

INTRODUCTION

Around 0.5-1% of the world's population is affected by vitiligo[1]. Highest incidence was recorded in India, followed by Mexico and Japan[2]. Majority of the affected population belong to less than 30 years of age and greater number of cases have been reported in females, which may be due to social stigma and seeking early medical attention[3].

Principal clinical manifestation of vitiligo is the appearance of milky white macules with fairly homogenous depigmentation and well defined borders involving skin and mucous membranes[4]. It can affect any part of the body but sites most commonly affected are extensor body surface areas like pretibial regions, sides of ankles, knees, elbows and skin overlying digits, periorificial areas such as the periocular, circumoral and anogenital area (glans penis, prepuce and vulva) and also the flexor aspect of wrists, axillae, groins, lower back and loin, palms, soles and scalp[5]. On the basis of polymorphic distribution, extension and number of white patches, vitiligo is broadly classified into generalized (vulgaris, acrofacial, mixed) and localized (focal, segmental, mucosal) types[6].

The exact cause of vitiligo is unknown but there are different theories including autoimmune, genetic, toxic metabolites or oxidative stress, neural causes and the lack of melanocyte growth factors[7]. Vitiligo is associated with various other autoimmune diseases. Abnormal thyroid functions are reported in around 10.74% and it seems to have the strongest association with vitiligo[8]. Family members of children with vitiligo have a higher incidence of vitiligo and other autoimmune diseases[9]. Although not a life threatening disease, poor body image due to cosmetic disfigurement leads to low selfesteem and psychological trauma and therefore poor quality of life[10].

Increased levels of homocysteine causes activation of various cytokines and lipid peroxidation and increased reactive oxygen species that may have toxic effects on melanocytes[11]. In addition, homocysteine acts through inhibition of tyrosinase enzyme[12]. Serum homocysteine has been suggested as bio-marker of vitiligo severity[13] Several studies reported that vitiligo is associated with elevated homocysteine levels and homocysteine may play a role in the destruction of melanocytes. Hence this study was conducted to estimate the serum levels homocysteine in patients with vitiligo and control group.

MATERIALS AND METHODS

All clinically diagnosed cases of vitiligo aged above 18 years attending the Dermatology Out Patient Department (OPD), who gave in a tertiary Hospital consent were included. The study was conducted between January to December 2021. Ethical clearance was obtained from Institutional Ethical Committee (IEC).

Method of data collection:

- **Sample size:** 35 patients with vitiligo and 35 age and sex matched controls were included in the study

- **Inclusion criteria:** All clinically diagnosed cases of vitiligo aged above 18 years

Exclusion criteria:

- Age younger than 18 years
- Patients who have undergone gastrointestinal surgery
- Diseases known to affect the homocysteine levels including genetic disorders of aminoacid metabolism, hypertension, diabetes mellitus, thyroid dysfunction, cardiovascular disease and renal failure
- Cigarette smoking, alcohol intake and hormonal therapy

The disease activity was assessed as follows:

- Stable disease no change in the vitiligo lesions during the 2 months prior to the study as observed by the patient
- Active disease-enlargement of already present lesions and the appearance of new lesions within 2 months prior to the study as observed by the patient

Investigations: After explaining the procedure and obtaining written informed consent from every patient and control, A 5 mL of venous blood were drawn from each participant. The serum levels of homocysteine, vitamin B12 and folic acid were measured using the electro chemiluminescence immunoassay method (RocheE411, Germany). The normal range of serum levels homocysteine was taken as 3.7-13.9 micro mol/L. The cost of the investigations were borne by the researcher. Other investigations like complete hemogram with peripheral smear, Random blood sugar, Liver and Renal function tests, Thyroid function test, Urine Routine examination were done wherever necessary. Any abnormalities in the above-mentioned tests were recorded (Table 1-6).

Statistical analysis: The study was a hospital-based case control study. Study subjects were entered in excel spread sheet and analyzed using statistical

Table 1: Serum levels of homocysteine in study subjects

Serum marker	Group	N	Mean	SD	Student's t-test	p-value	Inference
Homocysteine (micromol/L^{-1})	Cases	35	28.82	15.12	-0.4	0.66	
	Controls	35	30.40	17.46		(>0.05)	Not significant

Table 2: Serum levels of homocysteine in different types of vitiligo

	Vitiligo type	N	Mean	SD	F-test	p-value	Inference
Homocysteine (micromol/L^{-1})	Acral	3	15.6233	12.89642	3.203	0.026	
	Acrofacial	11	22.3336	10.99124		(<0.05)	Significant
	Focal	6	35.1367	18.78750			
	Mucosal	5	23.7260	9.65610			
	Vitiligo vulgaris	10	38.6840	14.02814			
	Total	35	28.8237	15.12400			

Table 3: Serum levels of homocysteine in patients with stable and active vitiligo

Serum markers	Disease activity	Mean	SD	Student's t-test	p-value	Inference
Homocysteine (micromol/L^{-1})	Stable	24.74	16.38	-1.011	0.320	
	Active	30.46	14.62		(p>0.05)	Not significant

Table 4: Serum levels of homocysteine in patients with unilateral and bilateral vitiligo

Serum markers	Laterality	Mean	SD	Student's t-test	P value	Inference
Homocysteine (micromol/L^{-1})	Unilateral	24.71	9.06	-0.573	0.571	
	Bilateral	29.35	15.76		(p>0.05)	Not significant

Table 5: Serum levels of homocysteine in patients with vegetarian and mixed diet

Serum markers	Diet	N	Mean	SD	Students 't' test	p-value	Inference
Homocysteine (micromol/L^{-1})	Vegetarian	18	24.10	12.90	-1.979	0.056	
	Mixed	17	33.82	16.06		(>0.05)	Not significant

package for the social sciences software version(SPSS) 20.0. Mean and standard deviation (SD) were calculated for continuous parameters. Unpaired student's t test was used to compare quantitative variables and chi square test was used to compare qualitative variables. $p<0.05$ was considered statistically significant.

RESULTS

The mean age among cases was 38.11±13.14 years, mean age among controls was 37.86±12.82 years. So the maximum number of cases and controls were clustered within the age group of 20-40 years. Male to female ratio among cases and controls was 1.5:1 and 1.9:1 respectively. Males outnumbered females among both cases and controls. Mean duration of the disease in study was 48.80±91.94 months with minimum and maximum duration of disease being 1 month and 360 month respectively.

Maximum number of patients presented within 6-12 months of onset of their disease. Most common type seen was acrofacial vitiligo (n = 11) followed by vitiligo vulgaris (n = 11) and the least common was acral vitiligo (n = 3). In this study, family history of vitiligo was present in n = 7 (20%) of vitiligo patients. The disease was stable in n = 10 (28.6%) and active in n = 25 (71.4%) of vitiligo patients. Vitiligo was found to be unilateral in n = 4 (11.4%) and bilateral in n = 31 (88.6%) of patients. Serum homocysteine levels were significantly higher in patients with vitiligo vulgaris (38.68±14.02: $p<0.05$) as compared to other clinical types.

Serum homocysteine level was increased in 80% of patients versus 82.85% of controls and normal in 20% of patients versus 17.14% of controls. There was no statistically significant difference in serum levels of homocysteine between patients and controls. Serum homocysteine levels were significantly higher in patients with vitiligo vulgaris (38.68±14.02: $p<0.05$) as compared to other clinical types. There was no statistical significant difference in serum levels of homocysteine in patients with stable and active vitiligo.

There was no statistical significant difference in serum levels of homocysteine in patients with unilateral and bilateral vitiligo. There was no statistically significant difference in the serum levels of homocysteine in patients with vegetarian and mixed diet.

DISCUSSIONS

The present study is a case control study involving 35 cases with vitiligo and 35 age and sex matched controls. The age range was 18-65 years. The mean age along with SD in cases and controls was 38.11±13.14 years and 37.86±12.82 years respectively with no statistical significant difference between case and controls ($p = 0.93$). Balci *et al*.[14] in a study noted that Mean age among cases and controls were 37.94±16.27 and 39.32±13.15 years respectively ($p = 0.692$).

Yasar *et al*.[11] reported mean age group among cases and controls to be 27.77±13.44 years and 25.42±4.48 years respectively and the age range was 10-56 years in cases and 20-41 years in controls ($p = >0.05$). Vitiligo is reported more frequently in females than males, which may be the result of increased reporting rates in females due to

Table 6: Comparison of serum homocysteine levels in case and controls among various studies

	Homocysteine (micromol/L^{-1})		
Various studies	cases	controls	p-value
Present study	28.82±15.12	30.40±17.46	0.66
Singh *et al*.[20]	28.8±7.7	23.1±1.9	0.000
Agarwal *et al*.[17]	15.39±7.2	11.88±4.81	0.02
Sabry *et al*.[15]	17.77±7.7	11.81±3.41	0.006
Karadag *et al*.[21]	11.4 , 4.6-55.4	9.4, 5.5-17.0	<0.01

Data presented as median, interquartile range

greater social consequences in females affected by vitiligo. El-Dawela *et al*.[13] noted mean duration of disease as 6.4±6.05 years ranged between 1-20 years. Balci *et al*.[14] noted mean duration of disease as 9.28±9.32 years in their study.

In contrast to the present study, Sabry *et al*.[15], Ghiasi *et al*.[16] and Agarwal *et al*.[17] reported vitiligo vulgaris as the most common type of vitiligo followed by acrofacial vitiligo in their study. In the present study, family history of vitiligo was present in 20% of the patients. Studies by Agarwal *et al*.[17] and Sabry *et al*.[15] showed positive family history in 16% and 11.4% of vitiligo patients respectively. Sabry *et al*.[15] in their study observed stable disease in 45.7% and active disease in 54.3% of patients. The difference in the disease activities could be because of the difference in observations of progression of vitiligo lesions by the patients. Sabry *et al*.[15] reported vitiligo was found unilaterally in 20% of patients and bilaterally in 80% of patients.

In this study there was no significant difference in serum levels of homocysteine, between vitiligo patients and healthy controls. Consistent with our results, Ghiasi *et al*.[16], Balci *et al*.[14] and Kim *et al*.[19] reported no significant difference in the serum levels of homocysteine, between vitiligo patients and controls. Similarly, Yasar *et al*.[11] also found no significant difference in the serum homocysteine in vitiligo patients compared to healthy individuals. In contrast to the present study, Singh *et al*.[20] reported that in comparison with healthy individuals, patients with vitiligo had significantly higher levels of homocysteine. Similarly, Agarwal *et al*.[17] and Sabry *et al*.[15] also reported that mean serum homocysteine level in vitiligo patients were significantly higher than that of controls. Karadag *et al*.[21] also showed higher homocysteine in the serum of patients with vitiligo compared to healthy subjects, However, El-Dawela *et al*.[13] indicated that while serum homocysteine levels were higher in vitiligo patients when compared to healthy individuals. However the difference in patient selection, particularly in terms of the severity, type and duration of vitiligo, as well as their ethnicity may account for the discrepancies.

In present study, the mean homocysteine level was significantly higher in male than female patients between male and female vitiligo patients. The results obtained were in accordance with study conducted by Singh *et al*.[20] who reported significantly higher serum homocysteine levels in male than female patients. Similarly, El- Dawela *et al*.[13] and Sabry *et al*.[15] noted significantly higher serum homocysteine levels among male patients than female patients. The sex disparity may be attributed to hormonal status, greater muscle mass in men, sex related lifestyle differences and the effect of female sex steroid hormones on homocysteine metabolism. However in contrast to our results Agarwal *et al*.[17] and Ghiasi *et al*.[16] reported no significant difference in serum levels of homocysteine between male and female vitiligo patients. In the present study, No significant relation was found between serum homocysteine in male and female controls.

In the present study, no statistically significant relation was found between serum levels of homocysteine and patients on vegetarian and mixed diet. The results obtained were in accordance with the study conducted by Agarwal *et al*.[17] ($p = 0.377$). In contrast to our study, singh *et al*.[22] noted that serum homocysteine level was significantly elevated in vegetarian as compared with nonvegetarian within the patient group (32.7±5.7 vs. 21.6±5.2 micromol lL^{-1} $p = 0.001$).

CONCLUSION

In the present study, no significant difference was found in serum levels of homocysteine between vitiligo patients and controls. Serum homocysteine was related to the sex of the patients and type of vitiligo. Our study despite the small sample size gives some indications for the use of homocysteine as a marker for severity of vitiligo. More large studies are needed to evaluate homocysteine as a risk factor in extensive vitiligo.

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