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ORIGINAL ARTICLE

Homocysteine Level in Children with Systemic Lupus Erythematosus

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a chronic acquired autoimmune multisystemic disease. Homocysteine (HCys) plasma levels increase frequently in children with (SLE). Hyperhomocysteinemia (HHCys) represents a risk factor for many morbidities in SLE patients, such as the formation of atherosclerotic plaques and atherothrombotic events. Also, HHCys was found to be associated with dyslipidemia and vitamin D deficiency in these patients. This study aimed at measurement of the level of HCys in children with SLE.

Methods: A retrospective case-control study was carried out in Pediatric Department, Zagazig University Hospital during the period from December 2016 to January 2018. 35 patients diagnosed as SLE, and 35 healthy controls were included in the study. All participants were subjected to; Full clinical history taking, clinical examination and investigations as complete blood count (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), antinuclear antibodies (ANA), renal and liver functions, lipid profile, Vitamin D level and serum homocysteine level.

Results: Serum homocysteine level was significantly higher in children with SLE than control group ($P<0.05$). Dyslipidemia was found to be frequent in children with SLE and related to elevated homocysteine level, which both represented cardiovascular risk factors in these patients. Also, children with SLE showed deficiency in Vitamin D level.

Conclusions: The present study proved that elevated plasma homocysteine level was frequent in children with SLE, which may help in predicting cardiovascular risk in these patients.

Keywords: Systemic lupus erythematosus (SLE); Homocysteine (HCys); Vitamin D; Children; Dyslipidemia.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune multisystemic disease with heterogenous clinical presentation in children and adolescents [1]. SLE is one of the most common autoimmune diseases with different pathogenesis [2]. Pediatric SLE represents a unique example of the genetic role in lupus pathogenesis [3]. Other mechanisms involve inadequate clearance of apoptotic debris, aberrant presentation of self-nucleic antigens and inadequate activation of T and B cells. Hormonal, and environmental factors also play a role [4].SLE can present by different clinical pictures include fever, malaise, fatigue, arthralgia, temporary loss of cognitive functions, malar rash, discoid Rash, photosensitivity, and oral ulcers [5]. Systemic manifestations include arthritis, serositis, kidney and neurological. Laboratory criteria as anti-nuclear antibodies, anti-DNA antibodies and hematological abnormalities as anemia, leucopenia, and thrombocytopenia. For diagnosis any 4 or more out of the 11 criteria should be present [6]. Homocysteine (HCys) is a non-essential amino acid obtained by a biochemical synthesis from the demethylation of methionine (Met), then homocysteine itself can be either retransformed to methionine or degraded to cysteine. The resynthesis of methionine requires a methylation reaction enhanced by methionine synthase, which requires vitamin B12 as a cofactor and 5-methyltetrahydrofolate as a methyl donor [7].HCys physiological levels are considered normal between 5 and 15 µmol/l. Hyperhomocysteinemia (HHCys) is common in children with SLE, and considered as a risk factor for several pathologies, as the formation of atherosclerotic plaques and atherothrombotic events [8].The main cause of HHCys in children is the genetic defect in the transcription of enzymes responsible for the HCys metabolism. In addition

to genetic causes, other factors include nutritional deficiencies of some cofactors involved in HCys metabolism such as folic acid, vitamin B6, vitamin B12, and betaine may be responsible for the development of HHCys [9].

This study aimed at measurement the level of homocysteine in children with SLE and its relation to dyslipidemia and vit D deficiency in these patients and predicting cardiovascular risk in SLE patients.

METHODS

Study Design:

A retrospective case-control study was carried out on 35 patients, diagnosed as SLE and admitted to Pediatric Department, Zagazig University Hospital, and 35 healthy controls during the period from December 2016 to January 2018. Children between **6** to 18 years old were included in the study they were followed by the nephrology unit after being referred to internal medicine department.

Inclusion Criteria:

All patients with SLE who were diagnosed, treated, and followed in nephrology unit Pediatric department, Zagazig University Hospital were included in the study, duration of treatment was at least 6 months. Both sexes were included.

Exclusion Criteria:

New cases and patients who had been followed less than 6 months were excluded from the study.

Ethical Approvals:

A written informed concent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Procedures:

All participants were subjected to full clinical history taking as clinical history as regards presenting symptoms, age of onset of disease, duration of disease, drugs used to control the disease, and frequency of disease activity. Clinical examination regarding for vital signs, measurements, local examination (cardiac, chest, abdominal, neurological), and investigations as complete blood count (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), antinuclear antibodies (ANA), renal, liver function, lipid profile, Vit D level and serum homocysteine level.

Method of measuring Homocysteine level in the serum:

Up to 100 mL of serum sample, calibrators and quality control were added 20mL of internal standard solution (75mmol/Lhomocystine-d8, 25mmol/L homocystine-d4 final concentration in the sample after reduction). The mixture was treated with 20mL of 500 mmol/L.

Dichlorodiphenyltrichloroethane (DTT) and left to react for 15 min at room temperature to accomplish the complete reduction of disulfides. Protein precipitation was carried out by the addition of200mL of methanol. After 5 min of centrifugation at10 000g, 200mL of the clear supernatant was transferred into a vial and injected into the high-performance liquid chromatography (HPLC) system. Using enzyme linked immunosorbent assay (ELISA) kits, Shanghai YL Biotech Co.Ltd.

Method of measuring lipid profile:

4-5 ml of blood after 9-12 hours of fasting were obtained by vein puncture into plain tube. Samples were allowed to clot at room temperature for 15 minutes and then the serum was separated from the clotted blood by centrifugation at 4000 rpm for 10 minutes, then transferred into clean and sterile plain tubes and then lipid profile were analyzed on the day it was drawn. A stock solution of cholesterol (200 mg/dL) was used. The spectrophotometric method performed to measure absorbance of standards. Using Spinreact kits, Spain.

Method of measuring Vitamin D level:

Blood plasma samples were extracted with acetonitrile at ratio of (1;2) or (1;3) and plasma was added to acetonitrile drop-wise this preparation was centrifuged for 10 minutes and the supernatant was placed in amber glass vial. Radioimmunoassay method (RIA) was used to measure 25(OH)D, or high-performance liquid chromatography (HPLC) was applied to 25 (OH)D assay. This assay included a lipid extraction of the serum followed by preparative chromatography and the 25(OH)D fraction was applied to HPLC and the UV absorption of 25(OH)D was used to measure its concentration. Using enzyme linked immunosorbent assay (ELISA) kits, [DRG international, Inc, New Jersy, United States].

STATISTICAL ANALYSIS

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

Quantitative variables were described using their means and standard deviations. Categorical variables were described using their absolute frequencies and were compared using Chi square test and Fisher exact test when appropriate.

Kolmogorov-Smirnov (distribution-type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric tests.

To compare continuous variables between two groups, independent sample t test (used with normally distributed data) was used to compare means of two groups. Pearson correlation coefficient was used to assess strength and direction of a linear relationship between two variables. The level of statistical significance was set at 5% (P<0.05). Highly significant difference was present if p≤0.001.

RESULTS

Regarding the demographic characteristics, 80% of SLE patients were females, mean age was 14.26 ± 2.36. Age of onset of SLE mean 9.09 ± 2.22 years in SLE group as shown in (Table 1).

Regarding the presenting symptoms of SLE, 60% of patients presented with joint swelling, and 8.6% had joint pain. About 91%, 83%, 69% and 54% had edema, malar rash, photosensitivity, and fever respectively. Only 10% presented with CNS manifestation as demonstrated in (Figure 1).

Regarding the activity of SLE disease in relation to season, more than half of the studied patients had disease activity in winter as demonstrated in (Figure 2).Regarding the lipid profile level, there was a statistically significant difference between the studied groups, as triglycerides, total, low density lipoprotein cholesterol (LDL), and very low-density lipoprotein cholesterol (VLDL) cholesterol were higher and high-density lipoprotein cholesterol (HDL) was lower in SLE groups (p<0.05) (S) as shown in (Table 2).

Regarding homocysteine and vitamin D levels, there was a statistically significant difference between the studied groups regarding to both homocysteine level and vitamin D level. Homocysteine level was higher and Vit D level was lower in SLE group than control group p<0.05 is statistically significant as shown in (Table 3).

Regarding the Correlation between homocysteine level and lipid profile in SLE group, there was a significant strong positive correlation between homocysteine level and triglycerides, total, LDL, and VLDL cholesterol. On the other hand, there was a significant negative correlation between homocysteine level and HDL cholesterol as shown in (Table 4).

Table (1): Distribution of the studied patients according to demographic characteristics and anthropometric measures

	N=35	%
Gender:		
Male	7	20
Female	28	80
	Mean ± SD	**Range**
Age (years)	14.26 ± 2.36	6 - 18
Age of onset (years)	9.09 ± 2.22	4 - 13
Weight (kg)	54.23 ± 10.3	18 - 79
Length (cm)	150 ± 13.54	93 - 165
BMI	23.83 ± 3.43	18 - 34

BMI: body mass index

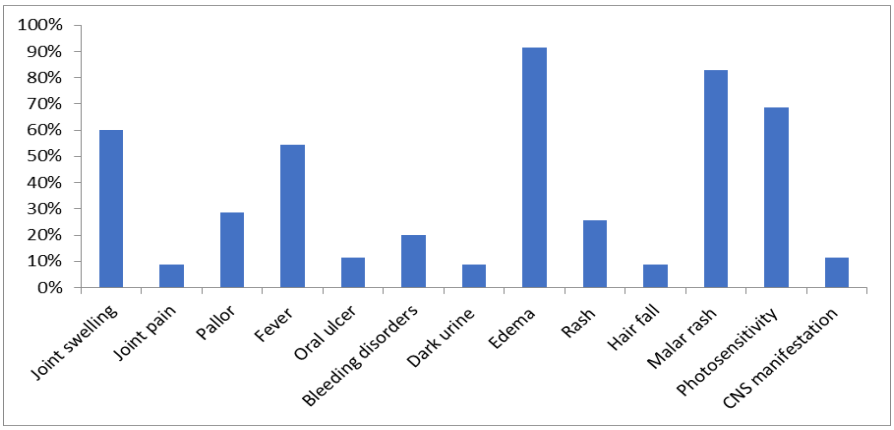

Figure (1): Simple bar chart showing distribution of the studied patients according to presenting symptoms.

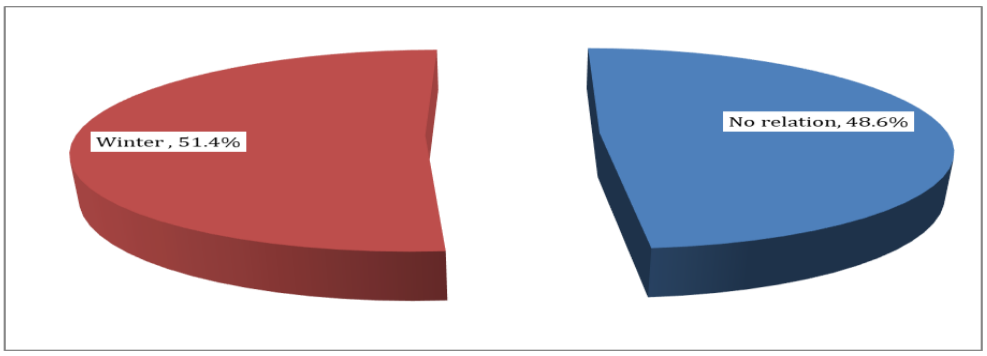

Figure (2): Pie chart showing distribution of the studied patients according to activity of SLE disease in relation to seasons.

Table (2): Comparison between the studied groups regarding lipid profile:

Lipid profile	Groups		Test	
	SLE group	Control group	t	p
	Mean ± SD	Mean ± SD		
Triglycerides	161.09 ± 54.38	79.33 ± 12.01	8.428	<0.001**
Total cholesterol	206.26 ± 38.31	150 ± 17.78	7.088	<0.001**
HDL cholesterol	53.46 ± 23.64	68.6 ± 11.21	-3.069	0.004*
LDL cholesterol	154.23 ± 45.88	88.13 ± 5.08	8.404	<0.001**
VLDL cholesterol	51.4 ± 26.16	13.53 ± 2.48	8.764	<0.001**

p<0.05 is statistically significant. t independent sample t test SD: standard deviation. SLE: Systemic lupus erythematosus. HDL: high density lipoprotein cholesterol. LDL: low density lipoprotein cholesterol. VLDL: very low-density lipoprotein cholesterol.

Table (3): Comparison between the studied groups regarding homocysteine level and Vit D level.

	Groups		Test	
	SLE group	Control group	t	p
	Mean ± SD	Mean ± SD		
Homocysteine level (µmol/L)	21.94 ± 9.46	7.53 ± 2.26	8.5	<0.001**
25 (OH) D (nmol/L)	36.63 ± 8.11	42.13 ± 3.54	-3.341	0.002*

p<0.05 is statistically significant. t independent sample t test

Table (4): Correlation between homocysteine level and lipid profile in SLE group.

Parameters	Homocysteine level (µmol/L)	
	r	P
Triglycerides	0.808	<0.001**
Total cholesterol	0.616	<0.001**
HDL cholesterol	-0.82	<0.001**
LDL cholesterol	0.744	<0.001**
VLDL cholesterol	0.825	<0.001**

p<0.05 is statistically significant r Pearson correlation coefficient

DISCUSSION

Homocysteine (HCys) is a non-essential amino acid that comes from the biosynthesis and metabolism of methionine (Met). Within the Met metabolic pathway, HCys is irreversibly degraded to cysteine (Cys) via the trans-sulfuration pathway or re-methylated back to Met. HCys is highly important for the cell's homeostasis, as its physiological activity is important to Met, which plays an essential role for the cell's viability [10].

There was a significant relation between SLE and sex, as 80% of patients were females. Also, there was a significant relation between SLE and age, as most cases were between 6-18 years old with mean age was 14.26 ± 2.36. Age of onset of SLE ranged from 4 to 13 years old with mean age was 9.09 years in SLE group. This is consistent with a study by Beth et al [11], who reported that approximately 20% of all patients having SLE are diagnosed in childhood. The onset of SLE is rare before 5 years of age; most pediatric patients are diagnosed in adolescence. SLE commonly occurred in female.

There was a statistically significant difference between the studied groups regarding lipid profile,

as triglycerides, total, LDL, and VLDL cholesterol were higher, and HDL was lower in SLE group. This is consistent with a study by Dakua et al., [12] which detected that high cholesterol, high triglyceride, high LDL and low HDL levels were observed in lupus patients.

There was a statistically significant difference between the studied groups regarding both homocysteine level and vitamin D level. Homocysteine level was higher and Vit D level was lower in SLE group. This correlated with a study by Jelic et al., [13], which stated that patients with SLE are at high risk of developing 25 (OH) D deficiency and correlated with a study by Timlin et al., [14], who reported that patients with lupus had elevated homocysteine levels.There was a significant strong positive correlation between homocysteine level and both all of triglycerides, total, LDL, and VLDL cholesterol. On the other hand, there was a significant negative correlation between homocysteine level and HDL cholesterol in SLE group. This correlated with a study by Ardoin et al., [15], which stated that homocysteine (Hcy) was significantly and independently accompanied by the presence of dyslipidemia, and higher LDL levels in the juvenile SLE group. Also, a study by Martins et al., [16] reported that all participants with SLE with moderately elevated homocysteine level had dyslipidemia that supported our study. Our study detected that 60% of patients with SLE presented with joint swelling, 8.6% had joint pain. About 91%, 83%, 69% and 54% had edema, malar rash, photosensitivity, and fever respectively. Only 10% presented with CNS manifestation. This correlated with a study done by Liu et al., [17], which stated that kidney damage is one of the commonest health problems caused by lupus. In children who have lupus, 8 out of 10 had renal symptoms as edema. Our study detected that more than half of the studied patients had disease activity in winter this is not matched with a study done by Fang et al., [18], which stated that activity of lupus exaggerated during the spring and summer. This may be related to viral infection in winter and low socioeconomic standard with patients in our study. Limitations of the study: They include the small number of patients and the short duration of the study. Also, the single center hospital-based study that does not reflect the national situation at the community level represented a limitation for the study.

Recommendations:

Further studies are needed with larger number of patients and longer duration to study the effect of elevated serum homocysteine level in patients with SLE and to evaluate the effect of supplementation with folic acid, Vit D and Vit B complex in lowering its level and protect patients with SLE from complications as atherosclerosis.

CONCLUSIONS

This study confirmed that pediatric patients with SLE have had increased serum homocysteine level which may increase risk of morbidity and mortality in these patients later in life.

Conflicts of Interest: Nothing to declare.

Financial Disclosures: Nothing to declare.

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