CLINICAL IMPACT OF HOMOCYSTEINE AND FOLIC ACID ON VASO-OCCLUSIVE CRISIS IN SICKLE CELL DISEASE

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ABSTRACT

Vaso-occlusion is a determinant for most signs and symptoms of sickle-cell anemia (SCA).Elevated concentration of Homocysteine contribute to thrombosis , a frequent event in sickle cell anemia . Folic acid deficiencies lead to dangerous increase in plasma Homocysteine.The aim of study is to test whether children with sickle cell anemia have elevated concentration of serum homocysteine with diminished level of folate, and to determine whether hyperomocysteinaemia has a correlation with the frequency of Vasoocclusive crisis. A case- control study was carried over a period of one year from Jan.- Dec. 2014 inclusive, 50 patients were collected from the Sickle cell centre in Abo Elresh Hospital together with healthy 30 cases, age and sex matched ,were taken from Menoufia Hospital.Venous blood sample were aspirated from both groups to estimate serum Homocysteine and folic acid, Statistical analysis was done, using the student T-test (P. value < 0.05 is considered as statistically significant) . Pearson correlation analysis was performed. The mean and standrd deviation of age of the patients and controls was (6.20± 2.55) and (6.03 ± 2.64) respectively. 66% of patients were males . Sickle cell – Thalassemia constituted 64% of patients. Homocysteine level was significantly higher in the patients group compared with control group with a mean and standard deviation of (44.68 ± 9.096) and (18.81 ± 3.76)µmol/L respectively and p value < 0.01 . Folic acid level was lower, (12.02 ± 2.76) and (14.68 ± 2.99) ng/ml respectively, the results were statistically significant, P. value 0.02. Significant inverse correlation was found between Homocysteine folic acid with correlation coefficient -0.337 and p value 0.017. A strong positive correlation between Homocysteine level and the frequency of Vaso-occlusive crisis was found ($\times^2$ 4.836 and p value 0.04). We conclude that patients with sickle cell disease have high serum level of Homocysteine with low level of folic acid. This Hyperomocysteinaemia is positively correlated with the frequency of Vaso-occlusive crisis.

Keywords: Sickle cell disease , ,Homocysteine , Folic acid , Vaso-occlusive crisis.

INTRODUCTION

Sickle cell anemia (SCA) is a genetic disorder caused by homozygosity for a single β-globin gene mutation (β6GAG→GTG), in which glutamic acid has been substituted for valine at the sixth codon of β-globin chain.

Despite this fact, the clinical course of patients suffering from SCA is extremely variable, the severity of manifestations ranging from asymptomatic to a very severe course (**Steinberg MH, Adewoye AH**, **2006**; **Adams GT, 2003)**. The phenotypic variability maybe explained by some genetic factors, those related to globin genes have been well recognized[2]. There is evidence that SCA and other chronic hemolytic anemia are characterized by a hypercoagulable state with increased of thrombin and fibrin generation as well as platelet activation with an augmented risk for thromboembolic **complications (Ataga KI, 2003)**.

Homocysteine is a sulfur amino acid and a normal intermediate in methionine metabolism. In many individuals with inborn errors of homocysteine metabolism,

kidney or liver disease, nutrient deficiencies, homocysteine levels can rise beyond normal levels and lead to adverse health outcomes. Elevated plasma tHcy is an independent risk factor for cardiovascular-related as well as non-cardiovascular-related mortality**(Vollset SE, 2001; Bostom AG, 1999).**

So the aim of this study to test whether children with sickle cell disease have elevated concentration of serum Homocysteine with diminished levels of folate, to determine the correlation between hyperomocysteinaemiaand Vaso-occlusive .

PATIENTS AND METHOD:

A case-control study was carried over a period of one year from first of Jan. 2104 to the end of Dec. 2014 , 50 cases of patients (sickle cell anemia and Sickle cell – Thalassemia) were collected from the Haematology centre in Abo Elresh Hospital together with 30 healthy cases, age and sex matched ,were taken from Menoufia Hospital.

Hostory of renal, hepatic or cardiac disease was considered as exclusion criteria. All the patients were subjected to the following after signing an informed consent by one of the parents or the patient himself: Full history taking laying stress on age , gender, residence ,frequency of occurrence of Vaso-occlusive crisis, severity of pain and site, whether they took folic acid. The severity of Vaso-occlusive crisis was determined according to the pain scale (1-10) plus whether the patient use hospital, emergency or unscheduled ambulatory care for pain in the previous day. Physical examination was performed. A 3cc of venous blood sample were aspirated from both groups and centrifuged ,after centrifugation the serum were taken to the Medical Research Unit, in the Genetic Engineering Institute / University of Sadat city , Menoufia, to estimate serum homocysteine and folic acid level.

Using High Performance Liquid Chromatography (HPLC), Shimadzu (Kyoto, Japan) which consisted of a system controller model SCL-10 AVP, a degasser model DGU-12A, two liquid delivery pumps model LC-8AVP, UV-Visible detector model SPD-10AVP, and injector model SIL-10A, equipped with 20 µl sample loop.

Biochemical Assessment

Total homocysteine was determined after reverse phase HPLC by using isocratic elution and fluorimetric detection. Plasma folate concentrations were determined by a microbial assay with the use of a 96-well plate and manganese supplementation, as described previously**(Tamura, 1990).**

Statistical analysis

Data were analyzed using SPSS Win statistical package version 18. Numerical data were expressed as mean, standard deviation and range. Qualitative data were expressed as frequency and percentage. Pearson correlation analysis was done between serum Homocysteine level and Folic acid, as well as between Homocysteine level and the frequency of Vaso-occlusive crises, r. value range from -1.0 to 1.0 inclusive and reflects the extent of a linear relationship between two data sets. P.value was estimated, a value < 0.05 indicates statistical significance.

RESULTS

In this study a total number of 50 cases of patients and 30 cases of the control were studied. The mean and standard deviation of age of the patients and controls was (6.20± 2.55) and (6.03 ± 2.64). Applying the ttest, no statistical difference was found between the age of the two groups with p . value = 0.781 i.e. > 0.05

Of the patients group 17 (34%) cases were female and 33 (66 %) cases were male, male: female ratio equal to 1.0:1.9 In the control group, 17 cases (60%) were male and 13 cases (40%) were female.

The patients group includes 18 cases (36%) with Sickle cell anemia and 32 cases (64%) with Sickle- Thalassemia.

Sickle cell disease (SS) patients included 12 cases with frequent vaso-occlusive crisis while sickle-Thalassemia patients (S/B) included 11 cases only. $\times^2$ test revealed a significant association

between sickle genotype and frequency of vaso-occlusive crisis ($\times^2$=4.836 and p value 0.04).

Homocysteine level was higher in the patients group compared with control group with mean and standard deviation was (44.68 ± 9.096) for the patients group while in the control group standard deviation of (18.81 ± 3.76) as it is shown in (Table- 1), applying the student T-test the result is statistically significant with a P.value < 0.01.

Folic acid level was lower in the patients group compared with control group with mean and standard deviation was (12.02 ± 2.76) while in the control group (14.68 ± 2.99) ng/ml, applying the student T-test the result is statistically significant with a P.value 0.002.

Pearson correlation shows a negative significant correlation between Homocysteine level and folic acid level (r. value =- 0.1, p. value 0.04) as it is shown in (Figure1).

For the patients group, a positive strong correlation was found between Homocysteine level and the frequency of Vasoocclusive crises (r.value = 0.9 , p. value < 0.05) as it is shown in (Figure 2)

DISCUSSION

In the present study, patients with homozygous sickle cell have a higher frequency of vaso-occlusive crises than patients with sickle cell–β+-thalassemia genotype .This observation was consistent with the results of other previous studies**(Platt, 1994; Nagel, 1985)**.

In this study, Homocysteine level was higher while Folic acid level is lower in the patients than the control , thereis a negative significant correlation between Homocysteine level and folic acid level.

Table (1): Clinical and laboratory measurements of Study group and homocysteine correlations in SCD patients

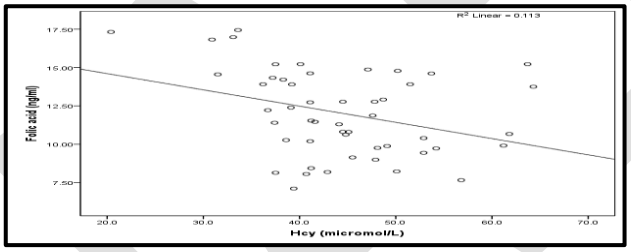
Parameter	**Clinical & laboratory Parameters Of Study Group**			**Homocysteine in SCD patients**	
	SCD(N=50) Mean ± SD	**Controls(N=30) Mean ± SD**	***p* value**	**Correlation Coefficient**	**P value**
Gender	Male: 33 (66%) Female: 17 (34%)	Male: 17 (60%) Female: 13 (40%)	0.797		
Age (years)	6.20± 2.55	6.03 ± 2.64	0.781	0.039	0.787
Weight (kg)	19.28 ± 8.01	21.73 ± 7.41	0.169	0.083	0.567
Height (centimeters)	103.90 ± 15.98	111.67 ± 16.99	0.079	0.043	0.768
Heart rate/minute	88.42 ± 13.92	90.10 ± 11.26	0. 577	0.017	0.907
Systolic BP	101.74 ± 9.50	104.83 ± 8.95	0. 154	0.109	0.109
Diastolic BP	66.90 ± 6.98	66.17 ± 5.97	0. 633	0.235	0.100
Haemoglobin (g/L)	7.97 ± 1.66	10.947 ± 1.1383	< 0.01	0.036	0.804
MCV	82.08 ± 11.04	75.28 ± 4.74	0.004	-0.015	0.918
WBCs (thousands /cm)	11.528 ± 5.18	8.64 ± 3.43	0.009	-0.217	0.130
Platelets (thousands /cm)	307.24 ± 191.35	295.63 ± 76.37	0. 752	-0.212	0.139
HbA (%)	13.89 ± 13.33	95.79 ± 1.366	< 0.01	0.085	0.556
HbA2 (%)	3.47 ± 1.59	3.473 ± 0.80	0. 992	0.126	0.382
Homocysteine(µmol/L)	44.68 ± 9.096	18.81 ± 3.76	< 0.01		
Folic acid (ng/ml)	12.02 ± 2.76	14.68 ± 2.99	0.002	-0.337	0.017

Table (2): ×2 test between avso-occlusive crisis and sickle genotype in SCD patients

	Vaso-Occlusive Crisis		
	Positive	Negative	**Total**
SS	**12**	**6**	**18**
S/B	**11**	**21**	**32**
Total	**23**	**27**	**50**
Chi-squared:		4.836	
DF:		1	
Significance level:		0. 040	

In comparison with studies done elsewhere , Homocysteine level was also higher in the patients than the control, Ohio**(Balassa,2002**),Cincinnati**(Balassa,2002**), Birmingham(**Lowenthal, 2000)**), folate is significantly higher in patients than the control.

Concerning correlation, Homocysteine is inversely correlated to folate **(Houston,1997).**



Figure(1): homocysteine & folic acid correlations

In this study significant positive correlation was found between Homocysteine level and the frequency of crisis which indicate that Hyperomocysteinaemia contributes for initiation of Vaso –occlusive crisis through occlusion of small blood vessels. This hyperomocysteinaemia may be attributed to pyridoxine deficiency, as it is known that Homocysteine is an intermediate compound of methionine degradation ,is normally remethylated to methionine , This methionine-sparing reaction is catalyzed by the enzyme methionine synthase, which requires a metabolite of folic acid (5-methyltetrahydrofolate) as a methyl donor and a metabolite of vitamin B12 (methylcobalamin) as a cofactor**(Iraj, 2008)**, in addition Homocysteine is trans –sulferated to cystathionine ,this pathway require Vitamin B6 **(Guilland, 2003)**,deficiencies of this vitamin will contribute to high serum Homocysteine level through disturbance in the metabolic pathway of Homocysteine**(Iraj, 2008).**

CONCLUSION

Patients with sickle cell anemia and Sickle- Thalassemia have high serum level of Homocysteine with low level of folic acid compared with the control group. There is strong positive correlation betweenHomocysteinelevel and the frequency of vasoocclusive crisis.

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