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Evaluation of liver enzymes activity and some trace elements in sickle cell anemia patients in Basrah Governorate – Iraq.

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Abstract.

Sickle cell anemia is one of genetic diseases caused by hemoglobin biochemical disorder in especially in polypeptidic chain of this protein. The study was conducted from October 2019 to December 2020 and included two sets of samples Patient and Healthy .The current study was curried out to evaluate some enzymes and trace elements in patients with sickle cell anemia. The levels of copper (Cu) were estimated spectrophotometrically by reaction of copper with 4 - (3,5 - dibromo - 2)pyridylazo) - N - ethyl sulphopropylaniline to produce a chelating complex. The determination was carried out at wavelength equal to 520 nm. Finally, absorbances were recorded and they are proportional to concentration of copper in the sample. Also, the levels of zinc in serum of blood belonging to sickle cell anemia (SCA) were investigated and estimated by reaction of zinc with chromogen existing in the characteristic kit to produce coloured complex. The absorbances were recorded spectrophotometrically which are proportional to concentration of zinc in blood sample. The levels of biochemical variables were estimated using ARCHITECT c4000 systems provided by ABBOTT Company, which are designed with advanced technology, accuracy in clinical results and ease of handling. The device uses a sample size at a rate of 6µL in all measurements of chemical variables. The total values of the current results were made and expressed by calculation of the mean \pm standard deviation. Analysis of all results data were carried out by using a statistical procedure according to analysis of variance univariate . Statistical programmer of social science (SPSS, v.23) was depended for calculation of significant differences for the different groups of patients infected by SCA disease and healthy persons . The correlation regression coefficient (r) was used and depended to distinguish between the means of groups belonging to all patients and healthy individuals (control group) .The p-value was agreed for less than 0.05 to the limit of lowest significance . A highly significant difference was recorded belong to levels of GPT, ALP and GOT in patients compared with healthy individuals (control group) according to age and sex factor. Also, the study indicated presence of great significant difference in concentration of Zn and Cu in patients' group when compared with healthy group also according to age and sex factor. We conclude from that a high moral rise in AST and ALT concentrations in sickle cell patients depending on age and sex factor. Also there is a high moral decrease in the effectiveness of the ALP enzyme in sickle cell patients by age factor in the first period only and there is a significant increase in the concentration of copper in sickle cell patients for both males and females and also a very significant increase in the concentration of copper in the first and second ages. It was also observed that a marked decrease in the concentration of zinc in sickle cell patients in both males and females as well as a very significant decrease in the second, third and fourth ages. As a result, sickle cell disease is associated with oxidative stress resulting in increased free radicals, lower antioxidant levels and higher liver enzyme levels.

Keywords: Sickle cell; sickle cell disease (SCD); anaemia; Sickle cell anemia; complications; homozygous sickle cell disease; hemoglobinopathy; haemoglobin; liver; copper; zinc; Antioxidants; oxidative stress.

1. Introduction

Hemoglobin is an essential blood protein which is responsible for biochemical action of red blood cells and the abundance of this protein in blood with normal levels leads to natural work of the living cell while the existence of biological or biochemical disorders in hemoglobin will lead to high disorder in blood serum[1,2].Sickle cell anemia is one of various diseases which referred to genetic disorders which happen due to presence of genetic mutation leading to elimination of glutamic acid from location in polypeptidic chain of normal hemoglobin then valine occupies the same location instead of glutamic acid and this statement leads to produce abnormal hemoglobin. Therefore, red blood cells have sickle form and this biochemical process leads to decrease oxygen amount in these cells [3,4]. This biochemical process leads to produce an abnormal HbA in the living cell then the biological and chemical activity of red blood cells will decrease. Homozygous B⁵ (Hbss) disease is considered as one common sickle cell anemia .In the cases, the cell is unable to form normal hemoglobin because of oxidative damage in the chemical antioxidant system present in the RBC so, more subjects suffer from this status in the worldwide leading to complication problems in biochemical system of various organs in circulatory system [5,6].

Clinically the sickle cell anemia (SCA) is diagnosed by a vaso – occlusion hemolytic anemia and highly providing to infect by this disease because of a hemoglobinopathy concerning occurrence and abundance of blood hemoglobin. So, this complex disease has become a real public problem hurts different peoples in various countries therefore the protection from this disease demands more biochemical agents in order to strength the immune system which is responsible for biochemical synthesis of blood proteins such as hemoglobin, albumin and globulin. Also concentrations of biochemical parameters in living cell such as antioxidants enzymes, lipid profiles , vitamins such as E, A and C, trace elements, malondialdehyde, uric acid and

glutathione must be at normal levels [7,8]. The early diagnosis and therapy can decrease infections with the SCA therefore more countries carried out the serious health information concerning dangers which belong to this genetic disease. These procedures are used to evaluate and investigate the safety of blood protein presented by hemoglobin (Hb) by following the normal sequence of amino acids in this protein's polypeptidic chain. However, when there is a chemical disorder in the location of amino acids, this statement results in significant variables, weakness, instability, and hemoglobin chemical action [9,10]. As a result, the current study was designed to assess the levels of liver enzymes and trace elements in patients with sickle cell anemia in Basrah Governorate, Iraq.

Forty five serum samples were collected from Al Mawani teaching hospital in Basrah governorate-Iraq from different patients with sickle cell anemia. These patient were divided into (20 males and 25 females) with ages ranged between (1-80 years). Fifty individual were participate in the present work as a control group, this group was classified to 22 males and 28 females according to their gender. Their ages were within the range (1-80 years). Clinically the current research was designed according to sex and age factors for both patients and healthy mankinds.

2. Materials and Methods

2.1 Collection of blood samples

Five ml of venous blood were collected from the study individuals (patients and healthy). The sample of blood was collected and centrifuged for 12 minutes at rotation speed was 4000 rpm, then the sera were kept at -20° C until use while residue blood was placed in heparin – containing tubes. Samples of blood belonging to patients and healthy persons were underwent centrifugation process other time with speed was 3000 rpm for 10 minutes in order to get plasma while the enduring red blood cells were washed gently by using 9% W / V of NaCl solution. Then erythrocytes were lasted with deionized water with ration is (1:1 v/v) [11].

2.2 Investigation and estimation of GPT, GOT and ALP enzymes in sera sample

Glutamate- pyruvate transaminase (GPT), Glutamate – oxaloacetate transaminase (GOT) and Alkaline phosphatase (ALP) enzymes concentrations were measured sera the study groups [12,13,14].

2.3 Evaluation copper and zinc concentrations in sera samples of the study groups

The levels of copper (Cu) were estimated spectrophotometrically by reaction of copper with $4 - (3,5 - \text{dibromo} - 2 - \text{pyridylazo}) - \text{N} - \text{ethyl sulphopropylaniline to produce a chelating complex. The determination was carried out at wavelength equal to 520 nm. Finally, absorbances were recorded and they are proportional to concentration of copper in the sample [15]. Also, the levels of zinc in serum of blood belonging to sickle cell anemia (SCA) were investigated and estimated by reaction of zinc with chromogen existing in the characteristic kit to produce coloured complex.$

The absorbances were recorded spectrophotometrically which are proportional to concentration of zinc in blood sample [16].

3. Statistical Analysis

The mean standard deviation was used to calculate the total values of the current results. All results data were analyzed using a statistical procedure based on a univariate analysis of variance. The statistical program for social science (SPSS,v.23) was used to calculate significant differences between different groups of SCA disease patients and healthy people. To distinguish between the means of groups belonging to all patients and healthy individuals (control group), the correlation regression coefficient (r) was used and depended on. To the limit of lowest significance, the p-value was agreed to be less than 0.05.

4.Results and Discussion

The age periods were divided into four groups are the first is(1-15 years), the second is (16-25 years), the third is (26-45 years) and the forth is (46-80 years). Table(1) indicates the oxidative stress belonging to GOT,GPT and ALP enzymes in patients infected by sickle cell anemia according to age factor. The concentrations which were recorded for enzymes in the blood represented by GOT, GPT and ALP were with values equal to 40.766, 31.840 and 94.040 U/L respectively for the first age period in sickle cell anemia patients while the same enzymes showed different concentrations with values equal to 35.902, 17.910 and 57.000 U/L at the second age period. At the third period of age , the concentrations of the three enzymatic antioxidants gave various values equal to 48.588, 39.120 and 61.695 U/L whereas the same enzymes showed the concentrations represented by 26.224 ,25.355 and 57.000 U/L respectively in the fourth age period.

Table 1: Concentrations of GOT , CPT , ALP enzymes in blood serum of
patients with SCA disease according to age factor.

Age (Years)	Groups	GOT conc.(U/L)	GPT conc.(U/L)	ALP conc. (U/L)
1-15	Control (No. =8)	27.000±1.88982	10.840±1.14700	193.000±12.65758
	patients(No.=25)	40.766±3.26281 ^{**}	31.375±4.45188 ^{**}	94.040±6.37874 ^{***}
16-25	Control (No. =6)	24.855±2.15028	14.571±4.28095	59.808±5.57525
	patients(No.=7)	35.902±4.69630 ^{**}	17.910 ± 3.61572^{NS}	57.000 ± 14.45024^{NS}
26-45	Control(No.=22)	26.224 ± 2.92024	22.600±8.19241	72.900±9.83356

	patients(No.=10)	48.588±7.22845 ^{**}	39.120±5.31350 ^{**}	61.695±5.06359 *
46-80	Control(No.=14)	25.666±8.77598	18.333±0.88192	60.962±5.26115
	patients(No.=3)	26.224±2.92024 ^{NS}	$25.355 {\pm} 3.05704^{**}$	57.000 ± 4.72582^{NS}

Concentration values were expressed as mean \pm SD levels of significance between SCA patients and control group.

***P< 0.0001, ** P < 0.01, * P < 0.05, N.S: Non significance.

The greatest level of GOT enzyme was recorded at the third age stage but the lowest one of the same enzymes was measured at the fourth age stage. Concerning GPT enzyme, the highest concentration was found at the third age period but the lowest level was recorded to be at the second age stage whereas the highest and lowest concentrations belonging to ALP enzyme were measured at the first and fourth age stage respectively.

According to sex factor of SCA patients, it was noticed presence of variant differences in the concentrations belonging to GOT, GPT and ALP enzymes when compared with healthy individuals (control group) as in table (2).

 Table 2: Concentrations of GOT, GPT and ALP enzymes in blood serum of patients infected by SCA disease according to sex factor.

Sex	Groups	GOT conc.(U/L)	GPT conc. (U/L)	ALP conc.(U/L)
Male	Control (No.=22)	28.601±2.59997	11.750±1.30561	83.867±10.27201
	Patient (No.=20)	42.526±4.32174 ^{**}	39.375±5.21619***	83.300 ± 7.80860^{NS}
Femal	Control (No.=28)	21.257±1.60581	15.560±3.62449	81.019±11.19366
e	Patient (No.=25)	39.313±3.34856***	$25.279 \pm 2.34099^{**}$	79.360±6.99330 ^{NS}

Concentration values were expressed as mean \pm SD levels of significance between SCA patients and control group.

***P< 0.0001, ** P < 0.01, * P < 0.05, N.S: Non significance.

The mean concentrations of GOT enzyme were recorded to be with values equal to 42.526 and 39.313 U/L in males and females respectively but the mean levels belonging to GPT enzyme were estimated with valves equal to 39.375 and 25.279 U/L for males and females respectively in SCA patients. In regarding to ALP enzyme the mean concentrations in blood serum of SCA patients were 83.300 U/L in males and 79.360 U/L in females . It is noticed that the greatest level of enzymatic antioxidant represented by GOT was found in males but the lowest concentration was recorded in females. While the highest concentration of GPT was presented in males and the lowest one was measured to be in females. Also the greatest and lowest levels of ALP enzyme was recorded in blood serum of males and females patients respectively. Table (3), illustrates the concentrations belonging to trace elements represented by copper (Cu) and zinc (Zn) in blood serum of SCA patients depending to age factor . It is known that the Cu and Zn are considered as non-enzymatic antioxidant.

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Age(years)	Groups	Cu conc. (µg/dl)	Zn conc.(µg/dl)
1-15	Control (No.=8)	79.670±6.94680	71.358±4.01242
	patients(No.=25)	$153.345{\pm}8.80913^{***}$	65.818 ± 11.31619^{NS}
16-25	Control (No.=6)	63.797±13.17836	82.125±5.10020
	patients(No.=7)	$142.941 \pm 13.66285^{***}$	$49.988 {\pm} 4.59599^{**}$
26-45	Control (No.=22)	87.858±10.36014	105.058 ± 4.19237
	patients(No.=10)	102.336 ± 6.21858 ^{NS}	$70.145{\pm}18.55207^{**}$
46-80	Control (No.=14)	80.103±10.75333	129.273 ± 16.86117
	patients(No.=3)	109.158 ± 7.73067 ^{NS}	$36.950 \pm 1.70097^{**}$

Table 3: Concentrations of zinc and copper in blood serum of SCA patientsaccording to age factor.

Concentration values were expressed as mean \pm SD levels of significance between SCA patients and control group

***P<0.0001, ** P<0.01, * P<0.05, N.S: Non significance

It was found that copper and zinc recorded various concentrations in both SCA patients and healthy groups. The levels of Cu were measured to equal to 153.345, 142.941, 102.336 and 109.158 μ g/dl at first, second, third and fourth age periods respectively in patients with sickle anemia in accordance with age factor. The maximum concentration value belonging to Cu was noticed at first period in SCA patients while the minimum value for concentration was measured at the third period. Concerning zinc levels, it was found the concentrations in SCA patients were 65.818, 49.988, 70.145 and 36.950 mg/dl in first,

Second, third and fourth periods respectively. Also, the greatest value of concentration was recorded at the third age period for whereas the lowest concentration was found at fourth period.

The concentrations of Cu and Zn showed different values in patients infected by sickle cell anemia disease according to sex factor as indicated in table (4).

Table 4: Concentrations of copper and zinc in blood serum of SCA patientsaccording to sex factor.

Sex	Groups	Cu conc. (µg/dl)	Zn conc.(µg/dl)
Male	Control (No.=22)	69.929±6.17020	110.841±11.34225
	Patients (No=20)	$108.725 \pm 7.21339^{**}$	55.213±6.63923 ^{**}
Female	Control (No.=28)	86.345±7.25242	98.078±5.20227
	Patients (No=25)	$124.002 \pm 6.50261^{**}$	68.136±12.51812 ^{**}

Concentration values were expressed as mean \pm SD Levels of significance between SCA patients and control group

***P<0.0001, ** P<0.01, * P<0.05, N.S: Non significance

According to sex factor (male and female), it was noticed that the concentrations belonging to copper in blood serum of sickle cell anemia patients were estimated to

be equal to 108.725 and 124.002 mg/dl in males and females respectively. The maximum value was obtained in females patients while the lowest concentration was measured in males of SCA patients. Also zinc concentrations values in patients with sickle cell anemia were gotten to be equal to 55.213 and 86.136 mg/dl in males and females respectively where the maximum level of concentration was noticed in females but the minimum concentration of zinc was recorded in females.

The hazard of sickle cell anemia disease comes from the genetic and chemical disorder belonging to red blood cells resulting from change of the chemical nature of blood protein which is represented by hemoglobin, so this biochemical status leads to produce inactive RBC and this means the whole circulatory system doesn't work in natural state[17]. Therefore some of enzymatic and non-enzymatic antioxidants will have different changes in their concentrations depending on many factor such a age ,sex, marital status, family history and associated with other disease therefore the severity of SCA will depends on these factors[18]. The current research focused on two factors belonging to patients and healthy subjects are age and sex. It was noticed that the age factor has clear and significant role to estimate the values of concentrations belonging to GOT, GPT and ALP enzymes in all age periods especially in the first stage . Theses results were in agreement with other researches especially concerning level of GPT and ALP enzymes[19,20].

Also, the effect of sex factor was significant especially in the concentration of GOT and GPT but there was no significant difference concerning the ALP level. These findings were in agreement with some other studies [21,22]. Copper and zinc are important biochemical trace elements in the human body so their concentrations are considered as physiological and biochemical markers to follow the severity of diseases including sickle cell anemia. The levels of copper showed high significant increasing in all age periods when treatment groups were compared with healthy individuals (control group) especially in the first and second age stages but there was high significant decreasing in zinc concentrations for all age stages between patients and control groups. According to sex factor, there was a significant increasing in copper levels in patients compared with control groups in both males and females whereas the concentrations of zinc recorded a significant decreasing in patients groups when compared with a healthy persons (control groups) for males and females .These results of Cu and Zn were in agreement with various studies which indicate biochemical correlation between copper and zinc levels in blood diseases and this can be explained by majority fraction of plasma copper is bound to ceruloplasmin leading to increase in

copper levels. At the same time the levels of zinc decreased because of inhibition NADPH and oxidase enzyme lending decrease in ROS generation [23, 24].

5.Conclusions

The current research showed the values of biochemical parameters represented by liver enzymes(GOT, GPT and ALP) and non-enzymatic antioxidants (copper and zinc). It was found that age and sex had a clear effect on the concentration of these parameters in sickle cell anemia patients when compared with control group.

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