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Demographic and Biochemical Study of Beta Thalassemia Major Associated with Liver and Pancreas Disorders in Adult Sample of Patients from Baghdad province

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Abstract: β-thalassemia is an inherited autosomal recessive blood disorder results from decreased or missing synthesis of beta globin chains in hemoglobin. This study was aimed to examine the relationship between liver and pancreas disorders of beta thalassemia major with demographic and biochemical aspects, in adult Iraqi patients**.** Blood samples were collected from 40 patients suffered from beta thalassemia with pancreas disorder as group A, along with 40 patients suffered from thalassemia with liver disorder as group B, and 40 patients suffered from thalassemia without pancreas or liver disorders as group C, from Ibn Al-Baladi Hospital, Baghdad, and 40 samples from age and gender-matched apparently healthy individuals as a negative control (Group D), all subjects with age more than 18 years. Ferritin serum level was determined by using automated immunocheiluminometric analyzer. ALT level was estimated through Atellica™ CH Analyzer, and amylase was evaluated *via* ARCHITECT *c*4000 device. The results revealed that the highest number of thalassemia patients located at the age group (18-28) years with high significant difference (P≤0.01), which was 91 (75.83%), and higher incidence was for male patients 72 (60%) with significant difference (P< 0.05)**.** Also results showed elevation of ferritin level in all patient groups when compared with control, and the highest value appeared in liver disorder patients (4014.9 ng/ml) with high significant difference (P<0.01). The results revealed that ALT level was significantly higher (76.430 U/l in liver disorder patients, as well as results showed that the level of amylase was lower in all patient groups as compared to the control group (84.150 U/l) with high significant difference (P<0.01). This study concluded that there was relationship between liver and pancreas disorders of beta thalassemia major with ferritin, ALT and amylase in adult Iraqi patients.

Keywords: Thalassemia, Liver disorder, Pancreas disorder, ALT, Amylase, Ferritin.

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Introduction

Thalassemia is a common blood disorder caused by impaired globin chain synthesis, adult human hemoglobin (HbA) makeup two α globin chains encoded by the *α-globin* gene (chromosome 16) and two β globin chains encoded by the *β-globin* gene (chromosome 11)(1). Beta-thalassemia major is a severe early-onset form of Beta-Thalassemia characterized by severe anemia requiring regular red blood cell transfusions (2), it consider a critical health problem in Iraq due to mainly the non-availability of equipments and drugs during different periods of turmoil and war and requires an efficient management strategy including public health education initiatives to promote early detection

and treatment (3). Even in nations where chelation is widely available, iron overload is still a serious issue for those with thalassemia major. The main complications that β - TM patients suffer is liver damage. Iron excess is thought to be the primary contributing factor to liver damage(4). Patients with thalassemia major commonly experience pancreatic iron overload, as well as numerous studies have shown that patients with thalassemia have endocrine pancreatic impairment (5). This study was planned to examine the relationship between liver and pancreas disorders of beta thalassemia major with demographic and biochemical aspects, in adult sample of patients from Baghdad province**.**

Materials and methods

This study was based on approval of ethics committee of Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad. Samples were collected from 120 patients, 40 patients suffered from beta thalassemia with pancreas disorder as group A, along with 40 patient suffered from thalassemia with liver disorder as group B, and 40 patient suffered from thalassemia without pancreas or liver disorders as group C, from Ibn Al-Baladi Hospital, Baghdad, and 40 samples from age and gender-matched apparently healthy individuals as a negative control (Group D), all subjects with age more than 18 years.

Two ml of venous blood sample was obtained from all subjects by vein puncture using a sterile disposable syringe under sterile condition, put into a sterile gel vacuum tube, let to coagulation for two hours at room temperature (25°C), then centrifuged at 3000 rpm for 5 minutes, then the serum was separated and kept freezing at (-20°C) until used.

Ferritin serum level was determined by using automated immunocheiluminometric analyzer, ALT level was estimated through Atellica™ CH Analyzer (6).Amylase test was evaluated by using ARCHITECT *c*4000 device (Abbott \ U.S.A) according to(7) .

Statistical analysis

The results of this study were analyzed by using one way classification. General Linear Model was used according to SAS (8) to find the effect of groups on biochemical parameters. The differences between the means were compared using Least Significant Differences Test (L.S.D) Chi-square test was used to find the differences among the numbers according to age and gender.

Results and discussion

Results of distribution of thalassemia patients according to age groups revealed that the highest incidences rate of all patients was at age group (18-28) years with high significant difference ($P \leq 0.01$), which was 91 (75.83%) against age group (30 year and over) which represented 29 (24.17%) as shown in (Table 1). The higher incidences of thalassemia patients was at age group 18-28 that's may be due to the patients who is up to 30 years or more is exposed to many complications, result of the iron overload which effect heart muscle , kidneys and endocrine glands , in addition to the viral infections, which lead to many deaths. Therefore, the patient may not be able to exceed this age, and therefore the number of patients was little in old ages.

Accordance to current result, the study of Lafta *et al*.(9) who found that the number of thalassemia patients at age groups (16-20) and (21-25) years was higher than the patients at age group more than 25 years old.

Regarding to gender, the result of distribution of thalassemia patients found that higher incidence of total number was for male patients 72 (60%) compared to females 48 (40%) with significant difference ($p< 0.05$) as shown in (Table 2). Thalassemia is an autosomal recessive disease with similar incidences in both males and females, but results of this study were found that the number of male patients significantly exceeds the number of females. The reason for this may be due to anemia is more severe in female patients than males and hemoglobin is significantly lower in female patients,subsequns having a history of more frequent blood transfusions compared to male patients, which are the direct cause of heavily iron overloaded, that exposes the body organs such as the heart and kidneys to damage, which lead to inability to survive and the increase in the percentage of mortality compared to male which several studies have reported better survival in male and a lower incidence of complications (in particular heart failure and arrhythmias) so it is reasonable that the number of males was more than female. These results were in agreement with a study by Malallah *et al*.(10) who showed a higher incidence in male than in female patients β-thalassemia. The results of estimation the level of ferritin in serum of patients and control groups showed elevation of ferritin level in all patient groups when compared with control, whereas ferritin level was(1544.6 ± 171.552 ng/ml) in group A , the level was(4014.9 ± 176.290 ng/ml) in group B and (2239.2± 174.212 ng/ml) in group C, while the level of group D was (218.1 ± 15.328 ng/ml) with high significant difference ($P<0.01$) as shown in (Table 3) The results of our study showed that the level of ferritin was significantly higher in all groups of thalassemia patients compared with the control group, and the reason for this may be due to the blood transfusions to hold the hemoglobin level in thalassemia patients at acceptable level (11). Also, the present results can be interpreted by the increased serum ferritin in beta thalassemia major is may result of ineffective erythropoiesis, lack of physiologic mechanism for excreting having too much iron and receiving numerous blood transfusions both cause progressive iron overload. Each transfused unit of red blood cells contains roughly 250 mg of iron, while the body can only excrete up to 1 mg of iron every day (12). The result of increased serum ferritin in the current study agreed with previous studies as result was obtained by Jwaid and Gata (13) they found significantly increase ferritin serum level in β- Thalassemia major patients compared to the control. The results of evaluation the level of ALT in serum of patients and control groups appeared that ALT level in liver disorder patients as group B was significantly higher (76.430 ± 2.802 U/l) when compared with other patients groups and control whereas was (27.513 ± 2.727 U/l) in group A , and (23.333 ± 2.769 U/l) in group C, while the level of group D was (26.290 ±4.083 U/l) with high significant difference ($P<0.01$) as illustrated in (Table4). The significant increase that found by the current results in the level of ALT of thalassemia patients who suffered from liver disorders as group B may be due to liver is a major site of iron deposition related with venous thrombosis, biliary blockage and chronic iron excess. Also, the cause of high level of ALT can be related to elevated serum ferritin levels, which in turn depend on the iron overload brought on by frequent blood

transfusions. In addition, Because of oxidative damage and the direct toxic effects of iron on liver cells, patients with β-thalassemia major who are transfusion reliant had elevated ALT levels (14) (15).Current results came in agreement with earlier study of Marbut *et al.* (16) who referred there is a highly significant increase in the serum of ALT in β-thalassemia males when compared to control males. The results of estimating the level of amylase in the patients and control groups indicated that the level of amylase was significantly lower in all patient groups as compared to the control group (84.150 ± 3.369 U/l) with a significant difference, but the lowest level of amylase was in patients of the group A who suffered from thalassemia with pancreatic disorders, which reached to (26.475± 2.250 U/l) against (62.900± 2.312 U/l) in group B and (49.075 ± 2.285 U/l) in group C with high significant difference (P<0.01) as clarified in (Table 5).

Table (1): Distribution of Thalassemia patient groups according to age

Groups	Age group (years)		
	18-29 year	30 year and over	P-value
Pancreas disorder(A)	29(72.5%)	11(27.5%)	0.004
Liver disorder(B)	34(85%)	6 (15%)	0.000
Thalassemia(C)	28(70%)	12(30%)	0.011
All groups	91(75.83%)	29 (24.17%)	0.000
** (P≤0.01)			

Table (2): Distribution of Thalassemia patient groups according to gender

Groups	Gender group			
	Male	Female	Calculated $\chi 2$	P-value
Pancreas disorder	22(55%)	18(45%)	0.400	0.527 NS
Liver disorder	24(60%)	16(40%)	1.600	0.206 NS
Thalassemia	26(65%)	14(35%)	3.600	0.058 NS
All groups	72(60%)	48 (40%)	4.800	0.028*
* (P≤0.05); NS: Non-Significant				

Table (3): Comparison between ferritin levels in patents and control groups

Group	Mean ± SE of ferritin (ng/ml)
Group: A	1544.6 ± 171.552 c
Group: B	4014.9 ± 176.290 a
Group: C	2239.2± 174.212 b
Group: D	218.1 ± 15.328 d
LSD value	606.535**
P-value	0.0001
Means having with the different letters in same column differed significantly ** (P≤0.01).	

Table (4): Comparison between ALT levels in patents and control groups

Group	Mean ± SE of ALT (U/l)
Group: A	27.513 ± 2.727 b
Group: B	76.430 ± 2.802a
Group: C	23.333 ± 2.769 b
Group: D	26.290 ±4.083 b
LSD value	10.969**
P-value	0.0001
Means having with the different letters in same column differed significantly ** (P≤0.01)	

Table (5): Comparison between Amylase levels in patents and control groups

Group	Mean ± SE of Amylase (U/l)
Group: A	26.475± 2.250 d
Group: B	62.900± 2.312 b
Group: C	49.075 ± 2.285 b
Group: D	84.150 ± 3.369 a
LSD value	6.88 **
P-value	0.0001
Means having with the different letters in same column differed significantly ** (P≤0.01)	

Table (6): Correlation between biochemical parameters with liver and pancreatic disorders

Characters / Groups	FER Con in ng /ml Mean ± Std. Error	ALT Con U/l Mean± Std. Error	Amylase Con U/l Mean± Std. Error
Pancreas disorder(A)	1544.6 ± 171.552 c	27.513 ± 2.727 b	26.475± 2.250 d
Liver disorder(B)	4014.9 ± 176.290 a	76.430 ± 2.802 a	62.900± 2.312 b
Thalassemia (C)	2239.2± 174.212 b	23.333 ± 2.769 b	49.075 ± 2.285 b
Control (D)	218.1 ± 15.328 d	26.290 ±4.083 b	84.150 ± 3.369 a
L.S.D. (P≤0.01)	606.535	10.969	6.88
Different letters mean the differences among level of factors are significantly at the 0.01 level. Similar letters mean the differences among level of factors are no significantly.			

The disorder of amylase level which recorded in this study may be due to the iron accumulate in acinar cells which synthesize amylase, as well as iron overload from long-term transfusion therapy and inadequate iron chelation therapy are the main causes of changes in pancreatic function. Iron buildup in beta-cell islets may increase ROS levels and cell death, resulting in a reduction in insulin output. Patients with thalassemia major frequently experience pancreatic iron overload, and pancreatic iron loading in these patients starts in early childhood, as well as numerous studies have shown that individuals with thalassemia exhibit endocrine pancreatic dysfunction. Frequently observed that the pancreas' endocrine activity is impaired, which may result in severe diabetes mellitus [17] .The present results were agreed with study conducted by Midiri *et al.*[18] They demonstrated that almost all patients with β-thalassemia major had serum levels of amylase and lipase that were below the normal range. The results of the current study showed a remarkable variation in chemical parameters in relation to the groups of thalassemia patients. The results gave an increase in the level of ferritin for all groups of patients compared with the control, while the results of ALT test gained a significant increase for the group of liver disorder patients compared with the rest of the study groups. Also the result of the amylase level test achieved a significant decrease for thalassemia patients who suffer from pancreatic disorders compared to the rest of the study groups as shown in (Table 6). These results indicated damage to the liver, pancreas, and endocrine glands, the reason is due to the storage and detoxifying capacity of ferritin can be exceeded due to iron deposits in thalassemics who have had several blood transfusions. Furthermore, the extra iron completely saturates transferrin. Thus, (free); iron (or non-transferrin bound iron NTBI) starts to build up in the blood and tissues. This (free) iron has the potential to catalyze the development of very harmful substances, such as the hydroxyl radical (OH), which is produced from hydrogen peroxide which hydroxyl radical

considered as highly reactive [19] . The main causes of liver problems in thalassemia include chronic iron excess, biliary blockage, and venous thrombosis. While these factors might cause acute hepatic syndromes, when they become chronic, all three processes can result in fibrosis, cirrhosis, and potentially hepatocellular cancer in thalassemia. Kassab-Chekir *et al*. (20) , Given that the body only has a very limited ability to manage these losses, normal daily iron losses are on the range of 1- 2 mg, while total body iron stores are approximately 4 g.

Conclusion

It can be concluded that according to age groups revealed that the highest incidences rate of all patients was at age group (18-29) against age group (30 year and over) and male more affected than female with beta thalassemia major in this study. In thalassemia patients and for all patient groups, the level of ferritin, ALT and amylase differ compared with the control group

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