A Study of some biochemical parameters in patients with β-thalassemia

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Abstract:

Serum levels of iron,copper,ceruloplasmin and transferrine were estimated in three groups of patients with β- thalassemia: 24 patients have splenectomy thalassemia major, 29 patients have non splenectomy thalassemia major and 19 patients have thalassemia intermedia , data were compared to normal and pathological controls (anemia and minor). There were significant increase in trace element levels in all studied groups of pateints as compared to normal and pathological controls. Also there were a significant increase in ceruloplasmin levels,While the result revealed that there were a significant decrease in transferrine levels in all groups of patients studied as compared to normal and pathological controls. The result also indicate that there is a significant positive correlation between serum ceruloplasmin & copper levels in thalassemia patients while the correlation between serum transferring & iron levels in thalassemia patients are negative.

Introduction:

Thalassemia is a heterogeneous group of genetic disorders in which the production of normal Hb (Hemoglobin) is partly or completely suppressed because of defective synthesis of one or more globin chains[1-3].β-thalassemia is the most familiar type Mediterranean area [4,5],in which the β- globin chain synthesis is impaired [6]. The severity of the disease depends on the amount of HbA (Adult Hemoglobin) and Hbf (fetal Hemoglobin), which present [7].

In Iraq,β- Thalassemia is one important public health problems. This is because of the considerable burden on the children and their families as well as on health services [8].
To keep the Hb concentration in normal range (between 13-16g%),the transfusion therapy should be started when diagnosis is made and the Hb level falls bellow (7 g %) [4, 6, 9].
The excess iron acquired through transfusions result in damage to the liver, endocrine organs and heart,therefore,this patients require chelating therapy to promote the excretion of iron accumulated from transfusions [10].

Trace elements present in the body in very low amounts,some are essential[9],a change in the normal concentration of essential trace elements in the human body might lead to major health disturbances[11] ,including thalassemia,the alteration of elements like iron & copper , is combined with excess amount of hemoglobin subunits enhances the generation of oxygen radicals after a chain of reactions leading to early death of the red blood cells & hemolysis [12]. The relationship between trace elements & their carrier molecules must be kept in mind [13].

Ceruloplasmine (CP),it's a blue copper protein contains (90- 95) % of copper found in the plasma [14,15],one of the main functions ,is transport the copper to the tissues [16].The high level of ceruloplasmine is present in the hemochromatosis [17],while each molecule of transferrin has two iron binding sites [18] ,that is binding up to two atoms of iron / molecule of protein [19] .

Human Transfrrin is a single chain, 80 KDa member of the anion-binding superfamily of proteins [20].Transfrrin is iron transport [21],

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supplies the tissues with it which is needed for their functions & the concentration is control by the iron content of these tissues [22].This study includes determination the level of Fe,Cu,ceruloplasmine & transferring in patients with β- thalassemia & studying the correlation between them.

Materials and Methods:

Patients from Ibn Al-Balladry hospital thalassemia clinic center were studied from April 2004 to September 2004 .The diagnosis of β- thalassemia was established by Hb electrophoresis technique model:Bio Rad Variant.Patients were divided into three groups:those who splenectomy β- thalassemia major (n= 40);range of age (10 to 26) years, non splenectomy β- thalassemia major (n=29);range of age(3 to 16) years & those who thalassemia intermedia (n= 19);range of age (7-30) years .Along side,26 matched healthy subject,aged(3 to 30) years & pathlogical subjects (anemia),(n=18) aged (4 to 30)years & pathological subjects (minor),(n=15),aged (4-30) years, were used as a control groups.

Iron & copper were assayed by an atomic absorption spectrophotometer model Perkin Elmer 5000,while the activity of Ceruloplasmine was determined according to the method of Ravin [23, 24], & the transferrin were assayed according to Ramsey method by measuring TIBC (indirect method) [25, 26].

Statistical methods:

Results were analyzed statistically using student's "t" test [27] to
Determine the level of significance. The difference was considered to be significant only when "p" value was less than (0.05).

Result and discussion:

Tables (1&2) are list the main concentration of Fe & Cu in patients & control groups respectively.

Table (1): Fe levels in sera of normal, pathological controls & patients with β- thalassemia.

Groups	No.	Fe concs. (ppm) mean ± SD
Normal control	26	0.431 ± 0.11
Pathological control:	33	
(1) anemia	18	0.379 ± 0.087
(2) minor	15	0.412 ± 0.1
Patients:	72	
(a) major	53	
(1) splenectomy	24	0.721 ± 0.18
(2) Non splenectomy	29	0.748 ± 0.15
(b) Intermedia	19	0.716 ± 0.088

Table (2): Cu levels in sera of normal, pathological controls & patients with β- thalassemia

Groups	No.	Cu concs. (ppm) mean ± SD
Normal control	26	0.174 ± 0.035
Pathological control:	33	
(1) anemia	18	0.1878 ± 0.041
(2) minor	15	0.188 ± 0.041
Patients:	72	
(a) major	53	
(1) splenectomy	24	0.243 ± 0.0731
(2) Non splenectomy	29	0.252 ± 0.045
(b) Intermedia	19	0.228 ± 0.063

The results showed a highly significant increase ($p<0.001$) in Fe & Cu concentration in all groups of patients that are studied as compared to normal & pathological control groups.Figures(1,2,3&4) show the comparison and distribution of Fe & Cu levels in sera of all groups studied, respectively

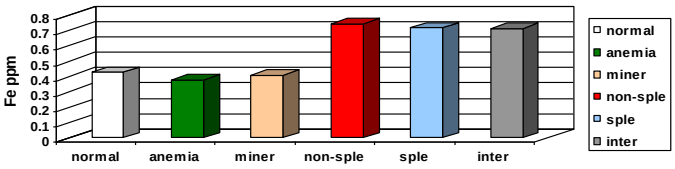

Fig. (1): Comparison of Fe levels in **sera of normal,** pathological controls patients with β- thalassemia.

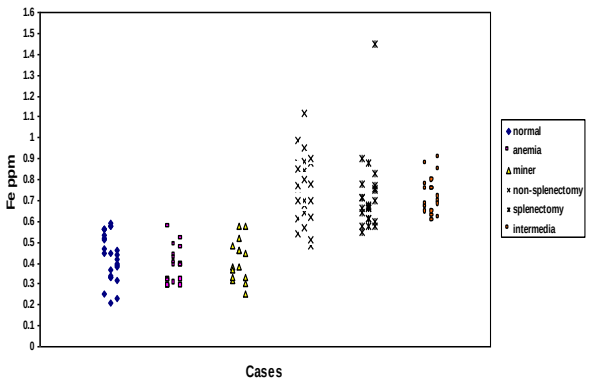

Fig. (2): Distribution of Fe levels in sera of normal, pathological controls patients with β- thalassemia.

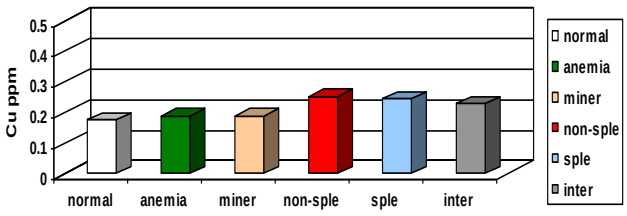

Fig. (3): Comparison of Cu levels in sera of normal, **pathological controls patients** with β- thalassemia.

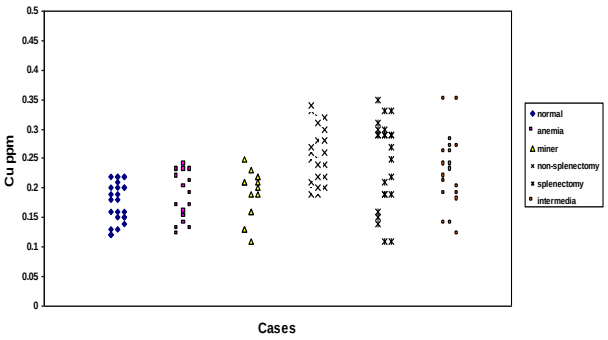

Fig. (4): Distribution of Cu levels in sera of normal, pathological controls patients with β- thalassemia.

These results are agreed with the finding of Keramati (2007),who found an increase in Fe level in patients with thalassemia minor[28].The higher Fe concentration may be result from transfusion therapy & increased iron absorption [9] , but from the results of patients groups which are listed in table (1),it is obvious that the non splenectomy groups were a higher concentration from splenectomy & intermedia groups,this because the number of blood transfusion which is needed higher in non splenectomy than the splenectomy &intermedia groups[1, 29]

This fact is important to monitor the iron over load, while the higher Cu concentration is probably due to parenchymal hepatic damage which is a common side effect in blood transfused patients[30].The presence of zinc deficiency in patients with ß-thalassemia [31] is the other reason for hypercupremia.This inverse relationship between serum Zn & Cu concentrations because of their competition either for the same absorptive binding sites on the intestinal mucosal cells or for similar protein carrier system [32] .

The elevation in Cu concentration is described by other workers, such as Suthipark(1991) ,who studied serum Cu in ß-thalassemia /HbE [33] ,Tatu(1997) and Vantanavicharn(1982) were studied serum Cu in HbH & ß- thalassemia /HbE disease [34, 35] .Bashir(1995) studied serum Cu in ß-thalassemia & sickle cell anemia [36],Hamid (1999)studied Cu in splenectomized group of ß- thalassemia major children [37] & Andona(1976) studied Cu in sickle cell disease [38] .

A similar is reported in Arcasoy's(1975) study ,who studied serum Fe & Cu in sera of homozygous ß- thalassemia & thalassemia intermedia[39] , the present study is agrees with Nammeer's et.al.,(2003) [8] & it doesn't agree with it when iron concentration is compared when splenectomy & non splenectomy are concerned.

Table (3) illustrates the serum ceruloplasmine activity among normal ,pathological control & patient groups .The results show a significant increases ($p < 0.001$) in ceruloplasmine activity in all groups of patients that are studied as compared to normal & pathological control groups. This elevation in ceruloplasmin activity may be due to the decreased ceruloplasmin catabolism,which could in turn account for the increased serum copper level [40] . Although copper- does not affect the rate of synthesis or secretion of apo Ceruloplasmin, but a failure to incorporate copper during biosynthesis results in secretion of an apo-protein that is devoid of oxidase activity and rapidly degraded[41,42].

Table (3): Ceruloplasmin activity in sera of normal, pathological controls & patients with β- thalassemia

Groups	**No.**	CP g% mean ± SD
Normal control	26	0.462 ± 0.11
Pathological control:	33	
(1) anemia	18	0.543 ± 0.10
(2) minor	15	0.509 ± 0.08
Patients:	72	
(a) major	53	
(1) splenectomy	24	0.609 ± 0.15
(2) Non splenectomy	29	0.695 ± 0.16
(b) Intermedia	19	0.664 ± 0.12

Similar rise are indicated in homozygous sickle cell disease in (Hedo & Alken)'s(1993) study [43] but in other disease of microcytic anemia such as Coeliac disease the ceruloplasmin activity is normal [44] .
Figures (5,6) shows the comparision & distribution of ceruloplasmin levels in sera of all groups studied while figure (7) shows the correlation between the activity of ceruloplasmin with Cu levels (r= 0.875) for thalassemic patients .this correlation may be due to the fact that ceruloplasmin is transport protein to copper .

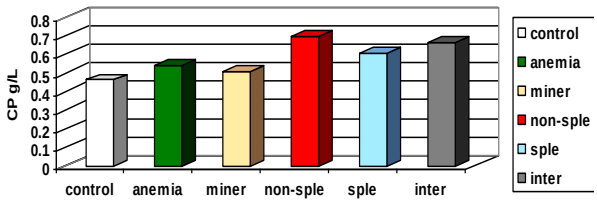

Fig. (5): Comparison of CP levels in sera of normal, pathological controls patients with β- thalassemia.

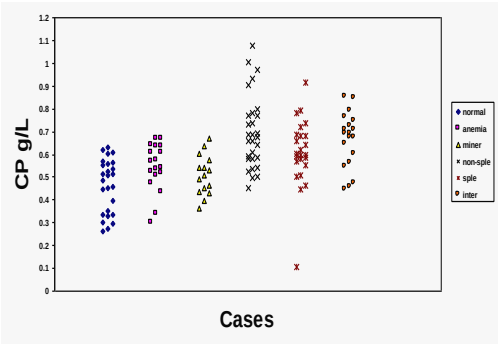

Fig. (6): Distribution of CP levels in sera of normal, pathological controls patients with β- thalassemia.

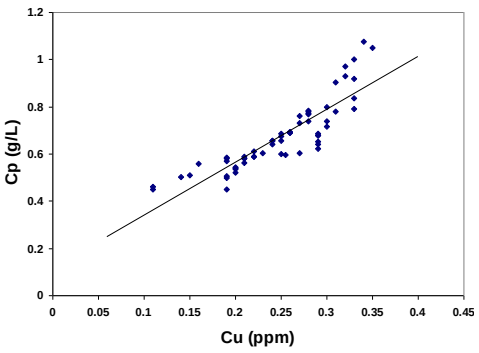

Fig. (7): Correlation between serum CP levels with Cu levels *for thalassemic patients*

Table (4) shows the TIBC(total iron binding capacity) concentration which is measured in all controls & patients groups

Table (4): (TIBC) levels in sera of normal, pathological controls & patients with β- thalassemia

Groups	No.	TIBC µg% mean ± SD
Normal control	**26**	**306.14 ± 21.36**
Pathological control:	**33**	
(1) anemia	**18**	**425.46 ± 28.81**
(2) minor	**15**	**322.26 ± 27.83**
Patients:	**72**	
(a) major	**53**	
(1) splenectomy	**24**	**225.76 ± 24.91**
(2) Non splenectomy	**29**	**217.37 ± 26.93**
(b) Intermedia	**19**	**219.93 ± 31.76**

Figures (8, 9) shows the comparison & distribution of TIBC levels in sera of all groups studied ,respectively.All groups showed highly significantly reduced (p< 0.001) in comparison to normal & pathological controls.This may be due to the TIBC measures the maximum amount of iron that serum proteins can bind & is therefore an indirect way of assessing transferrin levels [9] ,as well as serum TIBC varies in disorders of iron metabolism such as hemo chromatosis [45]. These results are in line with those obtained in study of Karamiam et.al.,(2003)[46], Nammeer et. al.,(2003) [8] .

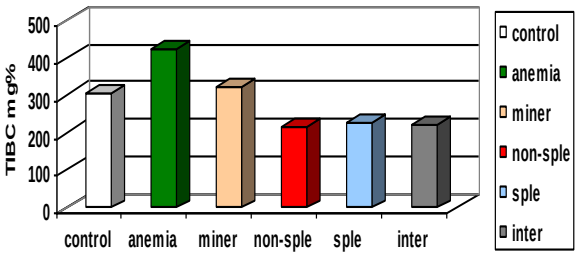

Fig. (8): Comparison of TIBC levels in sera of normal, pathological controls patients with β- thalassemia.

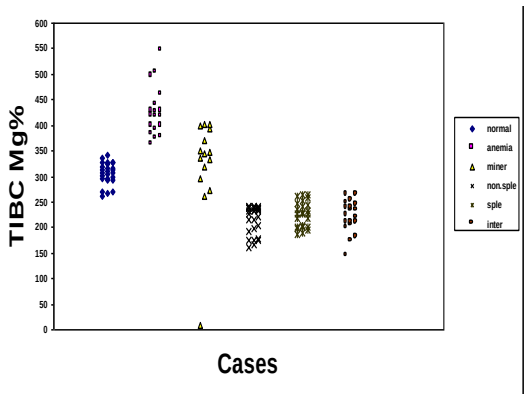

Fig. (9): Distribution of TIBC levels in sera of normal, pathological controls patients with β- thalassemia.

Table (5) shows the transferrin concentration which is measured in all controls & patients groups .

Table (5): Transferrin levels in sera of normal, pathological controls & patients with β- thalassemia

Groups	No.	TF mg% mean ± SD
Normal control	26	255.75 ± 19.8
Pathological control:	33	
(1) anemia	18	367.17 ± 25.8
(2) minor	15	294.22 ± 25.1
Patients:	72	
(a) major	53	
(1) splenectomy	24	177.73 ± 22.0
(2) Non splenectomy	29	176.86 ± 25.8
(b) Intermedia	19	173.93 ± 29.8

Figures(9,10) show the comparison & distribution of transferrin levels in all groups studied All groups showed highly significant reduction ($p<0.001$) as compared to normal & pathological controls.

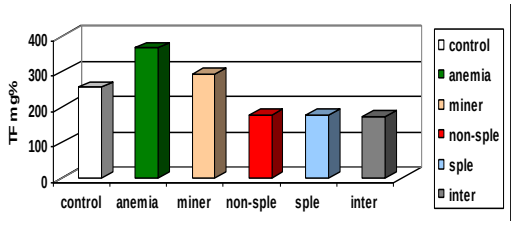

Fig. (9): Comparison of TF levels in sera of normal, pathological controls patients with β- thalassemia.

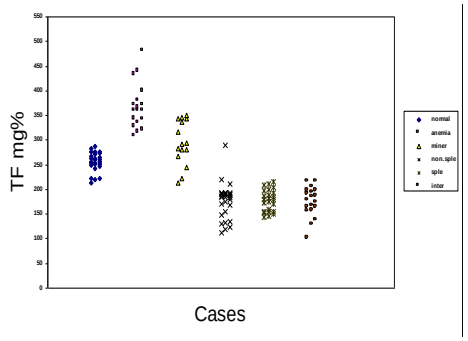

Fig. (10): Distribution of TF levels in sera of normal, pathological controls patients with β- thalassemia.

The reason for this may be the effect of iron overload on transferrin secretion ,in this case the depressed of transferrin secretion is not the consequence of hepatocyte death ,as the phenomenon is confirmed when expressed per cell .since the corresponding mRNA is unaffected ,it may be postulated that iron overload decreases transferrin secretion at some post transcriptional level [47] .

Other reason may be the decreased synthesis of protein which is generally decreased transferrin level [48] .

A similar reduction is shown by Gutteridge's(1994) study [49] & Warrier(1994) who studied the TF in sickle cell anemia [50] .

The correlation between serum transferrin levels with Fe levels for thalassemic patients are shown in figure (11)) (r=0.38337).

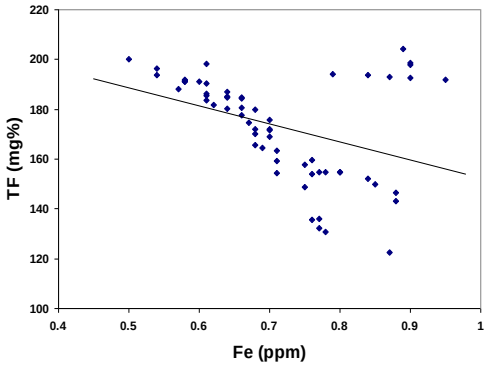

Fig. (11): Correlation between serum TF levels with Fe levels for thalassemic β patients Fe & TF

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دراسة بعض المتغيرات الكيموحيوية لمرضى فقر دم البحر الابيض المتوسط نمط بيتا (β)

اسراء غسان زينل ، ميسون خالد حسين ، شذى عبد الائمة كاظم

الخلاصة

تضمـــنت هذه الدراســه تقدير مستويات النحاس ,الحديد ,السيرولوبلازمين والترانسفرين في مصول مرضـى فقر دم البحر الأبيض المتوسط،(24 مريض رافعي الطحال , 29 مريض غير رافعي الطحال و 19 مريض بفقر الدم الوسطي) و قورنت النتائج مع مجاميع السيطره الاصحاء و مجاميع السيطره المرضيه .

أشارت النتائج إلى وجود زيادة واضحة في مستوى هذه العناصر النزرة في كل مجاميع المرضى المدروسة مقارنة مع المجاميع الضابطة الطبيعية والمرضية.

زيادة واضحة في مستوى السيرولوبلازمين ونقصان واضح في مستوى الترانسفرين في كل مجاميع المرضى المدروسة مقارنة مع المجاميع الضابطة الطبيعية والمرضية , و اظهرت الدراسة وجود علاقة موجبة واضحة بين مستوى السيروبلازمين والنحاس في مجاميع مرضى فقر دم البحر الأبيض المتوسط و هناك علاقة سالبة بين مستوى الترانسفرين والحديد في مجاميع مرضى فقر دم البحر الأبيض المتوسط.