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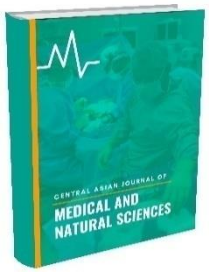

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Evaluate the Presence of Auto-Antibodies in Subjects with B- Thalassemia Major for Early Intervention to Decrease Morbidity and Mortality

1. Thaer Ali Hussein

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[1] Southern Technical University, Al-Nasiriya Technical Institute, Nursing Department. Iraq
thaerali@stu.edu.iq

Abstract: This study was performed of evaluate the presence of auto antibodies and hypercoagulability in subjects with β -thalassemia major. Subjects (60) with –β-thalassemia major were involved in the current work. History background, clinical tests and laboratory investigation including anti-cardiolipin antibodies anti-histone antibodies, ANA and anti-erythropoietin antibodies by ELISA, Ferritin by Cobas e411, protein S, protein C, and anti-thromobin III anti-bodies via ELISA. Findings of the study showed that there is elevated frequency of auto-antibodies in thalassemia major subjects, ANA was positive in 13.3% of cases, anti-histone antibodies was positive in 3.3% of cases, anti-cardiolipin antibodies in 15% and ant erythropoietin in 93.3% of cases. Protein C activity was deficient in 26.6% and protein S was deficient in deficient anti-thrombin III activity. Such abnormalities were not of significance associated to the HCV infection state or to the chelation type if subcutaneous or oral. Deficiency of protein C significantly existed in elder subjects.

Key words: thalassemia, ANA, ACL, Ferritin and HCV.

Introduction

There are various auto-antibodies detected in the thalassemic (THSC) sera subjects getting Fe chelators. The anti-histone presence proposes a change in the immune system response as humors. The case is related to a lupus-like picture regarding complications being clinical as arthritis, auto-immune hemolytic reactions, and lessened functions of kidneys (1). HCV infection is related to auto-immune diseases and an augmented thromboembolic risk cases. Anticardiolipin Ab, lupus anticoagulants and antiphospholipids are detected in THSCs with HCV infection and proved to be involved in inflammatory process (2). It is recognized well that THSCs display an elevated thrombotic cases frequency. Utmost individuals that resist (APCR) are resulting from a singal mutation in factor V amino acid synthesis (3). THSC subjects are subjected to tissue peroxidative injury owing to blood transfusions being continuous. It has been reported that circulating low density lipoproteins in THSC

subjects show marked oxidative modifications that might signify a case resulting in atherogenesis. A significant correlation was fond between oxidized LDL-Ab and triglycerides (1) Resistance to recombinant human erythropoietin therapy was observed in some thalssemic subjects receiving regular transfusion therapy. Antierythropoietin Ab should be evaluated in such subjects and it may play a role in rhEpo resistance (2).

Aim of the work

For evaluating the auto-antibodies presence and hypercoagulability in subjects with B- thalassemia major for early intervention to decrease morbidity and mortality.

Literature review

Thalassemia major

The inherited disorders of hemoglobin synthesis fall into two main groups. Those with an inherited structural alteration in hemoglobin molecule result in its abnormal function or instability. The second group, the thalassemias, are a heterogenous group of disorders branded by an defect being inherited in the synthesis rate of 1 or extra of the hemoglobin peptide chains, even though the affected chain structure remains usually usual. In homozygos β -thalassemia (major), the reduced biosynthesis of p-globin subunit of hemoglobin A, gives a clinically severe form of hemolytic anemia (4). In addition, there is a group of disorders with defective switching of fetal to adult hemoglobin production recognized as hereditary fetal hemoglobin persistence (5). The prevalence of thalassemia in various populations has been estimated in a number of different surveys. A so-called belt of thalassemia spreads along the Mediterranean shores and continues through Arabian countries, Iran, Turkey, India and into Southeastern Asia particularly Cambodia, Thailand, and Southern China (6). Worldwide, about 60,000 children with thalassemia major are borne annually. Around 150 million people globally carry genes of β -thalassemia which are predominantly predominant in Italy (8-10%) and Greece (5-15 %). In certain Greek islands and some villages in Sardinia, the incidence reaches (11-34%), while in Africans and American blacks, the prevalence is approximately (1.5%) In the United States, there are around 1000 cases of β -thalassemia major, most of whom are descendants of Mediterranean or Asian ancestors (7).

Anticardiolipin Antibodies

Anticardiolipin Antibodies were recognized initially in the utmost simple sense as a syphilis test utilizing extract of beef heart and consequently were detected to be heading for in contradiction of the mixture of cardiolipin (8). Of the anione phospholipids, phosphatidylserine seems to be the most antigenic. Phosphatidylserine is normally on the interior surface of the platelats and endothelial cell member ones (9). When the call is activated, phosphaticly serine is redistributed to the cell surface and participates in clot formation**.** Cellular damage may result in inappropriate phosphatidyl serine expression on the cell surface giving rise to antibody format. Lupus anti-coagulant and anti-cardiolipin antibody are related closely, nonetheless people of lupus have the lupus anti-coagulant, and 25 to 61 % have anti-cardiolipin anti-body. Such anti-bodies also are able to be detected in individuals who don't have lupus**.** The prevalence of anticardiolipin Antibodies is about 10-14% among normal healthy individuals (10). Anticardiolipin Antibodies are also found in different conditions as infections like syphilis, Lyme disease, infectious mononucleosis, drug induced conditions as with procainamide, phenothiazines and valpoate and malignancies like myeloma, renal cell carcinoma and thymoma. Anticardiolipin antibodies were reported in the sera of subjects with different auto-immune disorders. In SLE about 15-50% of the subjects have anticardiolipins and there was a close correlation with lupus anticoagulants (9). Also anticardiolipin was observed in the sera of subjects with other auto-immune disorders as myasthenia gravis, Behchet syndrome and Sjorgeon disease . It was found that close

relation exists concerning the presence of anticardiolipin antibodies and hyperviscosity as risk factors of ischemic stroke. A possible explanation is through the interference with activation of protein C and thrombomodulin (8)

Anticardiolipin Antibodies in Thalassemia:

Anti-cardiolipin anti-bodies and lupus anti-coagulants have been perceived in subjects with infection of HCV and are related to elevated thromboembolic events. With elevated incidence of thrombotic cases and the HCV infection in THSCs, anticardiolipin antibodies would possibly have a role (11). The mechanism of ingiury in the thrombembolic cases in THSCs just similar to that described in other diseases in which anticardiolipin antibodies were detected as SLE, ITP, myostheia grauis, IMN and others. The mechanism is through interactions with the endothelial release of prostaeyelins, activation of protein C and thombomodulen interference of antithrombin III activity and endothelial release of plasminogen activator (12). A strong association was found between the presence of anticardioplin antibodies and HCV infection. It was reported that anti cardiolipin antibodies were higher in THSC subjects infected with HCV than the non infected ones**.** THSC subjects are subjected to tissue peroxidative injury owing to nonstop blood anticardiolipin antibodies and other auto-antibodies as oxidative low density lipoprotien antibodies show marked modifications in the THSC subject's sera (13).

Anti-histone Antibodies:

There are vanous auto-antibodies found in the sera of THSC subjects receiving Fe chelactors. The anti-histone anti-bodies presence proposes a change in the immune system response as humoral; this case is associated with several clinical manifestations including nephritis arthritis and auto-immune hemolytic vacations(11). Antinucleur antibodies, as well as antidouble stranded DNA are detected with anti-histone antilriles showing a positive correlation with it**.** Chelation therapy constitutes a very important factor in the treatment regime of THSC subjects in a trial to control hemosiderosis and its complications. They receive ether des feral, oral chelators as defripone or L1. It was reported that anti-histone antibodies were detected in the sera of chelated subjects more than the non chelated ones and that their presence elevated with the use of defripone rather than desferral (12).

Subjects and Methods

The study has been conducted on sixty subjects with thalassemia major attending the new children hospital of Al- Haboby in city of Nasiriya, Iraq (between April 2021 to July 2022). for follow up. Cases were 38 males and 22 females.

Sampling:

5 ml whole blood obtained by vein puncture, allowed to clot and sera were separated for analysis and aliquots stored at -70 till analysis time for work.

Laboratory methods:

Serum antibodies measured by Elisa technique and Ferritin measured using fully-automated by ElectroChemiLuminescence (ECL) immunoassay on the coobas e411analyzer (Roche Diagnostics) according to manufacturing protocol. Type of study Cross-sectional clinical Laboratory study.

Inclusion criteria:

Subjects were categorized into groups based on:

(1) Type of chelation therapy whether they are suing Desferral (n=28) or Defripone (n=32)

(2) HCV+ve (n=45) or HCV-ve (n=15) cases

All subjects were subjected to:

Examination:

Anthropometric measurements:

- Physical examination including height, weight and BMI

Clinical Examination

- Cardiac examination
- Joint examination

Laboratory Investigations:

- HB%
- Serum Ferritin

Results

Table (1): Shows the clinical data of the studied cases concerning age, weight, height and BMI

Table1: Study population clinical data

	Age (y)	Wt. (kg)	Ht. (cm)	BMI (kg/m^2)
No. of cases	60	60	60	60
Range	10 – 15	20 – 40	75 - 142	11.7 – 62.2
Mean ± SD	12.2 ± 1.88	29.55 ± 5.05	120.92 ± 14.66	20.88 ± 6.6

Table (2): shows a comparison of age and growth parameter between subjects using Defripone (32) and subjects using Desferal (28).

No difference of significant was there in age and growth parameters between both groups indicating matched groups.

Table2: Clinical data based on the chelation therapy type

	Deferiprone (n = 32)	Desferioxamine (n = 28)	P value
Age (years)*	12. 19 ± 1.82	12.21 ± 1.99	0.957 (NS)
Weight (kg)*	29.41 ± 4.85	29.71 ± 5.35	0.816 (NS)
Height (cm)*	122.34 ± 15.96	119.29 ± 13.13	0.425 (NS)
BMI (kg/m^2)*	20.79 ± 8.72	20.97 ± 2.79	0.917 (NS)

*** Data presented in mean ± SD**

NS: No-significant difference statistically

Table (3): Show age and growth comparison parameters between THSC cases who are HCV+Ve (45) and those who are HCV-ve (15). The age of HCV-ve cases was significantly lower than HCV+ve cases of *P* value 0.039.

Table 3: Clinical data according to HCV infection

	HCV (-)ve (n = 15)	HCV (+)ve (n = 45)	P value
Age (years)*	11.33 ± 1.72	12.49 ± 1.87	0.039 (S)
Weight (kg)*	27.12 ± 6.12	30.17 ± 4.55	0.001 (S)
Height (cm)*	110.7 ± 18.13	124.32 ± 11.68	0.102 (NS)
BMI (kg/m^2)*	24.22 ± 11.29	19.57 ± 3.57	0.022 (S)

*** Data presented in mean ± SD**

NS: Statistically non-significant difference

S: Statistically significant difference

Table (4) Fig 1 compares the serum ferritin levels among cases according to their chelation therapy. Cases on oral chelator defripone showed a significatally higher serum ferritin levels than those on desferioxamine therapy (P=0.044). All the 60 THSC cases were splenectomized. We further subdivided them according to the duration since splenectomy into less than and equal to 8 years (35 cases) and more than 8 years (25 cases).

Table 4: Ferritin levels according to the type of chelation therapy

	Deferiprone	Desferioxamine	P value
No. of cases	32	28	0.044 (S)
Range	131 – 4450	790 – 4400	
Mean ± SD	2640.03 ± 1004.6	2197.18 ± 963.45	

S: Statistically significant difference

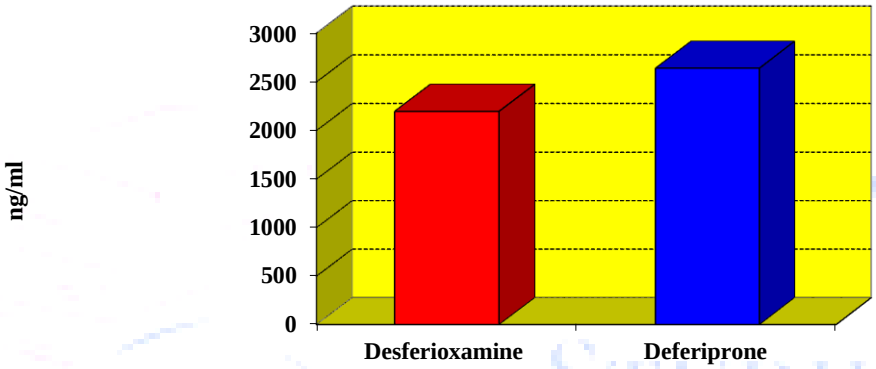

Figure (1) : **Ferritin levels according to the type of chelation therapy**

Table (5) Show the non significant difference relation between the BMI and the duration since splenectomy.

Table 5 : BMI according to duration since splenectomy

	≤8 years	>8 years	P value
No. of cases	35	25	0.349 (NS)
Range	11.73 – 62.22	16.1 – 24.41	
Mean ± SD	21.78 ± 8.36	19.61 ± 2.28	

NS: Statistically significant difference

Table (6): Demonstrate the results of antibodies seropositivity among case, and erythropoietine antibodies positive in 56 (93.3%) of cases.

Table 6: Distribution of positive antibodies among the 60 cases

	+ve n (%)	-ve n (%)
ANA	8 (13.33)	52 (86.67)
Anti histone	2 (3.33)	58 (96.67)
AntiCL	9 (15.00)	51 (85.00)
Anti EP	56 (93.33)	4 (6.67)

*** Data presented in no. of cases (%)**

Table (7) shows the distribution of antithrombotic factors deficiency among cases. Deficiency of Protein C was noticed in 16 cases (26.7%) whereas no deficiency was observed for antithrombin III.

Table 7: Distribution of anti-thrombotic factors deficiency among cases

	Deficient n (%)	Normal n (%)
Protein C	16 (26.67)	44 (73.33)
Protein S	8 (13.33)	52 (86.67)
Anti Th III	0 (0.00)	60 (100.00)

Table (8) Fig 2 shows the auto antibodies positivity according to HCV seropositivity. No significant difference was noted regards HCV seropositivity.

Table8: Positive antibodies according to HCV infection

	HCV (-)ve (n = 15)	HCV (+)ve (n = 45)	P value
ANA*	1 (6.67)	7 (15.56)	0.661 (NS)
Anti histone*	0 (0.00)	2 (4.44)	1.000 (NS)
AntiCL*	1 (6.67)	8 (17.78)	0.531 (NS)
Anti EP*	15 (100.00)	41 (91.11)	0.550 (NS)

*** Data presented in no. of cases (%)**

NS: Statistically non-significant difference

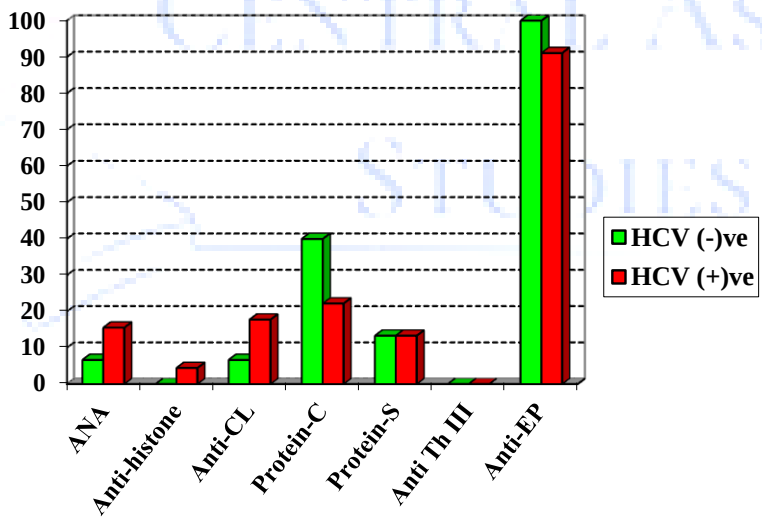

Figure 2 : Distribution of positive antibodies and anti-thrombotic factors deficiency according to HCV infection

Table (9) presents the relation between HCV status and the antithrombotic factor deficiency. The results were not statistically significant.

Table 9: Distribution of anti-thrombotic factors deficiency according to HCV infection

	HCV (-) ve (n = 15)	HCV (+) ve (n = 45)	P value
Protein C deficiency*	6 (40.00)	10 (22.22)	0.312 (NS)
Protein S deficiency *	2 (13.33)	6 (13.33)	0.661 (NS)
Anti Th III deficiency *	0 (0.00)	0 (0.00)	

*** Data presented in no. of cases (%)**

NS: Statistically non-significant difference

Table (10) Fig 3 no significant differences were found concerning the existence of auto antibodies in THSC subjects on different chelation modalities.

Table 10: Positive antibodies according to the chelation therapy

	Deferiprone (n = 32)	Desferioxamine (n = 28)	P value
ANA*	6 (18.75) %	2 (7.14) %	0.384 (NS)
Anti histone*	2 (6.25) %	0 (0.00) %	0.532 (NS)
AntiCL*	4 (12.50) %	5 (17.86) %	0.828 (NS)
Anti EP*	28 (87.50) %	28 (100.00) %	0.156 (NS)

*** Data presented in no. of cases (%)**

NS: Statistically significant difference

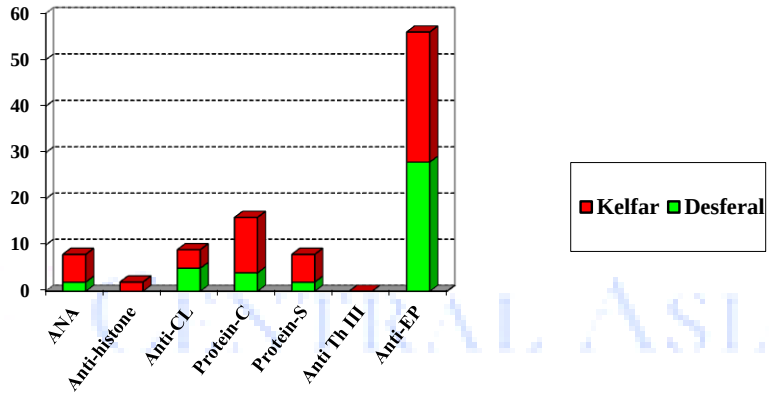

Figure 3 : Distribution of positive antibodies **and anti-thrombotic factors deficiency according to chelation treatment**

Table (11) shows the presence of anti thrombotic factors deficiency among cases of different chelation methods, no significant differences were detected.

Table11: Distribution of anti-thrombotic factors deficiency according to the chelation therapy

	Deferiprone (n = 32)	Desferioxamine (n = 28)	P value
Protein C deficiency*	12 (37.50) %	4 (14.29) %	0.083 (NS)
Protein S deficiency*	6 (18.75) %	2 (7.14) %	0.384 (NS)
Anti Th III deficiency*	0 (0.00)	0 (0.00)	

*** Data presented in no. of cases (%)**

NS: Statistically non-significant difference

Table(12) shows the impact of the duration since splenectomy was not significant regarding the seropositivity of the studied auto antibodies in cases with thalassemia major.

Table12: Distribution of antibodies positivity according to duration since splenectomy

	≤8 years	>8 years	P value
ANA*	3 (8.6) %	5 (20.00) %	0.369 (NS)
Anti histone*	0 (0.00) %	2 (8.00) %	0.331 (NS)
AntiCL*	5 (14.3) %	4 (16.00) %	1.000 (NS)
Anti EP*	33 (94.3) %	23 (92.00) %	1.000 (NS)

*** Data presented in no. of cases (%)**

NS: Statistically non-significant difference

S: Statistically significant difference

Table (13) we can notice than protein C deficiency was significantly more prevalent among cases that performed splenectomy more than 8 years ago.

Table 13: Distribution of anti-thrombotic factors deficiency according to duration since splenectomy

	≤8 years	>8 years	P value
Protein C deficiency *	5 (14.3) %	11 (44.00) %	0.023 (S)
Protein S deficiency *	3 (8.6) %	5 (20.00) %	0.369 (NS)
Anti Th III deficiency *	0 (00.00)	0 (00.00)	

Discussion

The study was conducted on 60 subjects with major thalassemia of 12.2±1.88 mean age and 1.7: 1 male: female ratio.

Subjects were categorized into groups based on the type of chelation used, those on defripone (n=32) and those on Desferioxamine (n=28). Another classification is according to HCV infection, those who are HCV+Ve (n=45), HCV-Ve (n=15) and the last classification was according to the duration since splenectomy, we have two groups those who had splenectomy 8 years and their number is thirty five and those who had splenectomy > 8 years and their number is twenty five. Among our 60 thelassemic subjects, ANA was positive in 8 (13.33%) of them. This result is near to those obtained by many authors. (Taher *et al.*, 2008) reported a 13% (10/75) incidence of ANA in ploy transfused thelassemic subjects(3), and (Urbanus *et al.*, 2008) found ANA to be positive in 12 out of their 83 thalassemia major subjects (14.5%) (13). Our is near to the study done by (Chou *et al.*, 2012) who reported ANA positively in 9 out of their 90 subjects (10%) (5). Only one of the ANA positive cases was HCV seronegative (1/15, 6.67%) while the others were HCV sera positive (7/45, 15.56%) this result in less the hat obtained by Siagris *et al.*, 2004 who reported that ANA positivity was found in 11.42 (26.2%) of thelassemic subjects with chronic C virus hepatitis infection, this able to be attributed to the younger age of our study group with less all allontigenic stimulation by frequent blood transfusion. Two of our cases (3.33%) had Positive anti-histone antibodies both of them were on oral Fe chelater deferiprone and were ANA positive. One of them had also anticardiolipin antibodies(14). Anticardiolipin antibodies (IgM) positive in 9 subjects (15%) and no history of thrombosis was given by the subjects No significant relation was present between it and anti HCV antibodies positivity. IgM anticondiolipin antibodies were detected in 6% of cases of thalassemia major that are no statically significantly relationship between anti-HCV anti-bodies and IgM anti cardiolipin anti-bodies. Moreover, none of their subjects had thromobotic appearances. anticardiolipin antibodies were detected in 42.7% of case but they measured the IgG type (which is more prevalent then the IgM type) and it was not statistically related to HCV infection . Also an older study by Grordano *et al.*, 1998 no significant relation was found between HCV infection and anticardiolipin antibody positivity and none of the positive subjects for anticardolipin antibodies termed any thrombotic manifestations(15). These cases included all who were positive to other performed antibody testing fifty percent of them were on desferioxamine therapy and the other half was an oral Fe chelatar deferiprone. HCV status had no effect on the presence of anti erythropoietin antibody. Non of our patient was on treatment by recombinant human erythropoietin.

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