

The role of antioxidants protein in regulated thalassemia patients state in relation with humeral immune system

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Abstract_This study was designed to determine the role of interaction between humeral immune system and antioxidants protein in regulation thalassemic patient statement.

Seventy tow blood samples from patient admitted to the regulation thalassemia center of AL-zahraa hospital in AL- Najaf city and other 60 samples were taken from healthy persons as controls group. These patients and control were not affected by any kind of infection during study period and at different ages group.

The results revealed the significant different $P < 0.05$) between patient and control group and explained clearly increase in level of immunoglobulin (IgG, IgM & IgA) and antioxidant protein (Ceruloplasmin and Haptoglobin) in thalassemic patient which also undergo reduce in level of complement component (C3, C4) in contrast to control group and though results of this study appear clearly that thalassemic patient explain significant high serum level of the plasma protein (Ceruloplasmin and Haptoglobin) in contrast to control and these protein in these person play vital role in maintenance of iron homeostasis which regard one important way to prevent complication iron overloading that occurs because chronic blood transfusions .

Key word_ Haptoglobine , ceruloplasmin, antioxidant protein, immune response, iron overload

1 introduction

Thalassemia describes a group of inherited blood disorder, transmitted to the offspring from their parents and now considered an important health problem in all parts of the world especially in arable country as a result of relatives married. Thalassemias are classified according to the globin that is affected, hence the names *alpha* and *beta* thalassemia depended on the fact that adult hemoglobin is made up of three components: alpha globin, beta globin, and hem [1].

Beta-thalassemia is one of most common disorders worldwide, caused by the reduced (β^+) or absent (β^0) synthesis of the β -globin chains of the hemoglobin (Hb) tetramer [2]. Iron is an essential element for several biological processes, including cell growth and differentiation, electron transfer reactions, oxygen transport activation and detoxification [3]. Iron is also implicated in the regulation of inflammatory processes and host immunosurveillance by modulating proliferation, differentiation, activation, and apoptosis of T, B, and NK cells [4]. Complications due to iron overload remains high because repeated blood transfusions, ineffective erythropoiesis and increased gastrointestinal iron absorption in these patient, which lead to iron overload in the body with its subsequent pathophysiologic complications in heart, liver and endocrine glands [5].

There are plasma proteins that are involved in iron homeostasis such as Ceruloplasmin (CP), Ferritin and Haptoglobin (Hp). CP is synthesized mainly in the liver as a single polypeptide chain and secreted into the plasma with six to seven atoms of Cu bound per molecule, plays a role in several biological functions including copper transport, coagulation, angiogenesis, ferroxidase activity, and defense against oxidative stress [6]. The antioxidant properties of CP are attributed to its ability to prevent lipid oxidation, scavenge superoxide, and sequester free copper ions [7]. Furthermore, CP has been reported to possess a ferroxidase enzymatic activity that plays a major role in iron metabolism mostly because of its catalytic oxidation of Fe^{+2} to Fe^{+3} or by incorporation into the Fe storage protein ferritin [8]. Ferritin is a key protein of iron metabolism that is capable of sequestering large amounts of iron, and thus serves the dual function of iron detoxification and iron storage [9]. It is likely that the protein is involved in host defense and repair processes mediated by the

immune system [10]. Haptoglobin (Hp) is a polymorphic $\alpha 2$ -glycoprotein that exists in three main phenotypes (Hp 1-1, Hp 2-1, and Hp 2-2) possessing different structural and functional properties [11]. The plasma concentration of Hp increases several fold in the event of an inflammatory stimulus such as

infection, injury and malignancy, whether local (vascular) or systemic (extravascular). In circulation, free hemoglobin Hb immediately binds with the plasma protein Hp forming Hp-Hb complexes that get removed quickly from circulation to prevent loss of iron and kidney damage and are scavenged by the CD163 on hepatocytes, tissue monocyte and macrophage [12]. It is thus considered a powerful antioxidant as a result of its ability to prevent hemoglobin-driven oxidative damage [13]. Both plasma protein CP, Hp appear to play significant role in the homeostasis of iron. Therefore this study was conducted to evaluate the possible interplay between iron overload and ceruloplasmin-Haptoglobin activity on one hand and evaluation of the immune response efficiency on the other, in group of Beta-thalassemia patients.

2 Material and Methods

* Study group: 72 patients admitted to the thalassemia center of AL-zahraa hospital, were included in this study. The age patient vary (16-21) and none of the patients was affected by any kind of infection during study period. In addition, 60 healthy persons have been treated as controls.

* Blood samples: peripheral blood was withdrawn from patients by sterile syringe usually in the range of (5 ml). The blood was transferred into tubes under aseptic conditions used for serum collection and kept in -20°C until the time of assay. Blood samples from healthy were assayed identically.

* The serum levels of the immunoglobulins IgA, IgG, IgM, serum complement (C) components, C3, C4 and haptoglobin were detected by Signal radial diffusion test procedure based on manufacture instruction "Biomaghrab" [14]. Serum iron and ceruloplasmine were determined by automated chemistry analyzer (Hitachi 912, Roche, Germany). Serum ferritin levels were determined by immunoassay analyzer (Elecys, Roche, Germany).

* Data analysis was carried out using the statistical software package of SPSS ver. 17.0 (SPSS Inc, Chicago, IL). Statistical comparison between groups was assessed by the Kruskal-wallis, and results were presents as mean \pm SD. statistical significant was set at $p \leq 0.05$.

3 Results and discussion

The results showed significant elevation of the immunoglobulin level, which were (1668.8 ± 44.56), (259 ± 18.31) and (320.5 ± 10.9) for IgG, IgA, IgM respectively, these data associated largely with significant decrease in level of serum component C3 (71.5 ± 7.22) and C4 (18.6 ± 2.1) of thalassimic patient as compared with control group as shown in tables (1 and 2).

Table (1) :Immunoglobulin (IgG,IgM,IgA) levels in Thalassemic patients and control group.

	N No. of status	Ig	IgM	IgA
Th Thalas emic p.	72 72	1668.8 ± 44.56	25 259.8 ± 18.31	32 320.54 ± 10.96
C Contro l	60 60	79 794.2 ± 26.11	12 122.90 ± 21.44	22 228.35 ± 12.4

The values are mean ±SD . P ≤0.05

Table (2) :Complement component (C3 , C4) levels in Thalassemic patients compared with control group.

	No.of status	C3 C3	C4 C4
Thalasmic p.	72	71.5 ± 7.22	18.6 ± 2.16
Control	60	86.3 ± 8.53	27.14 ± 4.04

The values are mean ±SD . P ≤0.05

Elevated IgG levels indicate prolonged antigenic stimulation with exogenous or autogenous , while elevated IgM level possibly reflect a primary antigenic challenge or persistence of antigen due to repeated transfusions and infections [15] . According to hypothesis including that iron overload on skin stimulated of IgA production as a mucocutaneous antibody[16] .On the other hand Iron overload was suggested by some investigators as an important contributing factor in altering the immune parameters in thalassemia patients [17] . It has been suggested that iron overload results in increased migration of T helper cells to the gut and lymph nodes and this causes an increase in serum immunoglobulin levels in thalassemia patients [18] .

The fact that humoral immune system and sufficient amount of immunoglobulins are the main components of the immune system to prevent infections in patients with beta thalassemia, the patients' contact with viral and bacterial infections caused by frequent blood transfusions makes the immune system constantly stimulated to produce more amounts of immunoglobulins and complement but depletion of early complement components may be evidence of consumption by antigen-antibody complexes or may be related to iron overload or overuse of complements in order to remove antigen [19 ; 20].

Many studied explained increased level of immunoglobuline and decreased levels of classic pathway components [20 ;21]. And the differing immunoglobulin serum levels in major beta thalassemic patients could be due to heterogeneity of different studies in aspects including age groups, race, socioeconomic status, nutrition, and difference in the care provided for the patient to control anemia and varied measures of ferritin, ignorance of the patients [22] .Accumulation of iron results in progressive dysfunction of liver causes hepatic fibrosis and cirrhosis this may lead to depression of complement levels in thalassemia has been assumed to be the result of failure of synthesis of components in the liver which regard principle location for synthesis complement compound C3 [5] .

The result of this study demonstrated for the first time a close association between serum antioxidant protein and activity of humeral immune system that effect clearly with ferrous load observed in thalassemic patient who

explained significant high serum level of CP and HP in compared to health control as shown in table(3).

Several study revealed that most thalassemic patients are under greater oxidative stress as a results of decreased plasma antioxidant capacity and increased markers of oxidative damage [23 ;24] . This status lead to the toxicity of iron overload as a consequence of life-long blood transfusions and increased intestinal absorption of iron [25] . In addition to the high levels of serum iron and ferritin , under such conditions of disturbed iron metabolism , therefore the functional activity of Cp altered in a way to oppose and counteract the consequence of iron overload .

Serum concentration of CP increase during infection, tissue injury, and certain malignant disorders [26] , this supporting the possible role of CP in host protective function and the natural killer cell (NK) lymphocytes are known to have a key role on cell-mediated cytotoxicity ,the cytotoxic damage to targets cells usually occur by releasing breakdown enzymes or other toxic substances [27] . Therefore , secretion or shedding of NK cell-derived CP may also contribute to cell-mediated host defense processes. Indeed, CP was shown to express some bactericidal activity [28] .

Table (3) :Ceruleplasmin and Haptoglobin in Thalassemic patients compared with control group.

	No. of status	Ce S. Fe µg /dl	S.Ft µg /l	Cp mg /dl	Ha Hp
Th Thalase mic pa.	72 72	230.5 ± 55.2*	2890 ±1246*	59.3 ± 17.9*	30 306.5 ±42.16*
Co Control	60 60	65.7 ± 22.6	40.3 ± 26.6	41.7± 13.6	2 217.14 ± 40.02

*:high significant p≤0.05 , Fe : iron S.Ft: serum firitin

Many studies have reported that Hp are directly or indirectly involved in disease processes influenced by iron metabolism such as hemochromatosis, atherosclerosis and cardiovascular disease (Banha *et al.*,2008). In thalassemia, increased levels of Hp-Hb formation therefore rapidly deplete Hp in circulation .Hp-Hb complex is irreversible and that Hp molecules participating in Hp-Hb complex formation are not recycled Consequently, free Hb is either converted to free heme that rapidly clears by hemopexin or filters through the kidney causing renal damage.The specific antioxidant functions of the protein at extravascular sites are: activation of neutrophils; maintenance of reverse cholesterol transport; inhibition of cyclooxygenase (COX) and lipooxygenase (LOX) in addition Hp play numerous roles in both the cellular and humoral activities, including prostaglandin synthesis, leukocyte recruitment and migration, the generation of cytokine patterns following injury and infection, and tissue repair (Van Vlierberghe *et al.*,2004 ; Tseng *et al.*,2004). The antioxidative property of Hp and the rate of clearance of free Hb from circulation is phenotype-dependent , It has been suggested that Hp2-2 phenotype has lower Hb-binding capacity than Hp1-1 or Hp2-1 and that clearance of the Hp1-1-Hb complex is more rapid than that of the Hp2-2-Hb complex (Wobeto, *et al.*,2008; Van Vlierberghe *et al.*,2004). Related to this issue is the finding that different Hp phenotypes differ in their ability to protect against oxidative stress induced by extracellular Hb on intact RBCs, with Hp1-1 found to be the most effective (Pillary,2007). The observed high levels of Cp and their significant correlation with serum ferritin among Hp thalassemic patients might be related to antioxidative property of Hp, which is mainly attributed to its high serum concentration and to its increased affinity to free Hb in circulation (Okazaki, *et al.*,1997;Gueye , *et al.*,2006) . Taken together, quantitative and qualitative properties of Hp play a major role in handling and prevention of iron toxicity in thalassemia.

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