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Studying of Oxidative Stress and Some Biochemical Parameters in Patients with β-Thalassemia Major in Kirkuk City

Nazar Ahmed Naji[a*], Sayran Sattar Saleh[b], Galawesh Noori Taher[c]

[a,b]*Department of Chemistry / College of Science / Tikrit University, Iraq*

[c]*Department of Nursing/ Instituted Kirkuk Technical/ Northern Technical University, Iraq*

[a]*Email: nan.abed@yahoo.com* , [b]*Email: Sayran_university@yahoo.com*, [c]*Email: galawesh@yahoo.com*

Abstract

This research include studying the oxidative stress state and measuring some biochemical parameters in the blood of β-thalassemia major which include: Acetylcholinesterase (AChE), Aspartate aminotransferase(AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP),Catalase(CAT),Glutathione (GSH), Malonadialdehyde (MDA),Total bilirubin (Tb), Ceruloplasmin (Cp),Nitric oxid (NO) and Peroxynitrite($ONOO^-$). The study was done on (80) thalassemic patients (42) were male , (38)female and (40) normal healthy subjects as control group (21) were male and (19)female ,the age of patients and control group ranged from 3-30 years for both sexes.The results showed that there was a significant increase in the activity of AChE, AST, ALT, ALP, Cp,ONOO-and Tb in β-thalassemia major compared with healthy group for both sexes, these result were indicated that these parameters may be good biochemical markers for this disease .Beside of that, the results showed there was a highly oxidative stress in the patients for both sexes, so there was a significant increase in the activity of oxidant indicator of MDA, with a significant decrease in NO and GSH levels in thalassemic patients compared with healthy group for both sexes.

Keywords: Enzyme; Oxidative stress; β-thalassemia major; MDA,NO.

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* Corresponding author.

1. Introduction

Thalassemia Syndrome is among the most common genetic disorders of hemoglobin Synthesis worldwide [1]. the disorder was termed thalassemia, from the greek word , Thalassa which means (sea) and emia which means (blood) [2]. That refer to disorders associated with reduction synthesis of alpha (α) or beta globin (β) poly peptide chains caused to chronic hemolytic anemia since birth [3].

Thalassaemia causes varying degrees of anemia, which can range from mild anemia to significant life threatening anemia.These hematologic disorders range from asymptomatic to severe anemia that can caused significant morbidity and mortality[4]. β thalassemia are the most important types of thalassemia because they are so common and usually produce severe anemia in their homozygous and compound heterozygous states . Beta thalassemia major also called (Cooley anemia) characterized by severe illness with long-term, transfusion-dependent anemia and entails a risk of iron overload and multiorgan involvement [5].

Oxidative stress is defined as the shift in the balance between the oxidants and antioxidants in favor of oxidants . The regulation of reducing and oxidizing, state is critical for cell activation, proliferation and organ function .It occurs when the generation of free radicals and active intermediates in a system override , the system ability to neutralize , and eliminate them [6]. In patients with beta thalassemia major where frequent blood transfusions are required due to severe anemia, oxidative stress occurs as a result of increased levels of lipid peroxides and free-radical intermediates, as well as the decrease in total antioxidant capacity. Use of iron chelatory agents in combination with antioxidants can be helpful in the regulation of the antioxidant status in patients with beta thalassemiamajor [7]. This oxidative stress and a possible consequential accelerated apoptosis may contribute to shortened life span of erythrocytes. Malondialdehyde (MDA), a product of lipid peroxidation is generated in excess amounts in supporting the fact that large amount of membrane bound iron is present in thalassemic erythrocytes [8].

Cholinesterase is a family of enzymes that catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation. It involves two types:

(Acetylcholinesterase (AChE) and Pseudocholinesterase (PChE) the difference between the two types has to do with their respective preferences for substrates that AchE ehydrolyzes acetylcholin faster, while pchE hydrolyzes butyrylcholine faster [9]. Different aminotransferases show different tissue distribution: aspartate aminotransferaseactivity is high across most tissues, whereas alanine aminotransferase activity is highest in theliver. The alkaline phosphatase affects the inflammatory responses and may play a directrole in preventing organ damage [10]. Catalase, widely distributed in all cells, is present in high amounts in erythrocytes. It is responsible for detoxification of hydrogen peroxide in the cells [11]. Many foregoing studies have confirmed that most beta-thalassemia patients are under greater oxidative stress as evidenced by decreased plasma antioxidnt capacity, increased markers of oxidative damage. This status of oxidative stress is attributed to the toxicity of iron overload as a consequence of life-long blood transfusions, increased intestinal absorption of iron, and to the greater intravascular hemolysis, it is thus possible that the functional activity of Cp, which is a major

plasma antioxidant, is altered in a way to oppose and counteract the consequences of iron overload [12].

Pathological processes in thalassemia accelerate the destruction of (NO), and limit the compensatory increase in(NO) production[13]. ONOO- is a powerful oxidantcapable of oxidizing low density lipoproteins, of causing vascular dysfunction and is responsible for nitration of tyrosine residues in proteins. Therefore, the presence of nitrotyrosine in the plasma is considered to be an indirect measure of $ONOO^-$ [13].

The aim of the present investigation was to study the relationship between oxidative stress by measuring the MDA level, which is the marker of oxidative stress in thalassemia patients and study also measures some the enzymatic antioxidant and biochemical parameters level in thalassemic blood sample.

2. Materials and methods

The study was done on (80) sample had β-thalassemia major (42) were male and(38) female and (40) were healthy subjects as control group(21) male and (19) female the age of thalassemic patients ranged from 3- 30 years for both sex. Blood samples were collected from Azadi Teaching Hospital, Kirkuk, Iraq. All patients are diagnosed by specialist pediatrician. Different questions asked for patients and control groups that include medical history, Consanguinity of parents, Age at diagnosis of thalassaemia, no, of blood transfusion/year, no, of DFO doses/week, Splenectomy, weight and height. The biochemical test included each of AChE, MDA ,CAT ,Cp ,NO , $ONOO^-$ were manually measured while AST, ALT,ALP and Tb were measured using the standard kits from Biolabo.

Table 1: Methods used to estimate the biochemical parameters.

Biochemical parameters	The method
AChE	S-Acetylthiocholine Iodide method[14]
AST and ALT	Reitman and Frankel method[15]
ALP	Kind and King method[16]
MDA	Guidet, B.; shah method[17]
CAT	Abi,H. method[18]
Cp	Sunderman and Nomato method[19]
NO	Dervisevic and his colleagues method[20]
$ONOO^-$	modified method.[21]

3. Statistical analysis

In this study the results include Mean ± SD and significant differences (p value) between groups that examined by a available statistical (SPSS 17.0), significant differences was estimated as the p value was equal or less than 0.01 [22].

4. Results and Discussion

4.1. Acetylcholinesterase (AChE)

AchE activity was found to be highly significant increase (P≤0.01) in patients with β-thalassemia major compared with control group as shown in tables (2) in both sexes. These results were agreed with previous results of the researchers [23]. May be attributed to lipid peroxidation, an index of oxidative stress.

AChE has also been reported to be associated with stress responses and related to inflammation. In addition the results are consistent with previous studies which indicated that the level of enzyme increase, during apoptosis of the cells, as the oxidative stress stimulates the cortex of the thymus gland to secrete the enzyme, and induce AChE expression [24]. Several studies showing a high enzyme activity in patients with neurogical diseases for example, the high activity of enzyme in the saliva of Parkinson disease [25].and Al-zheimer patients was observed, this is due to the active role of enzyme in the formation and dissolution of the nerves or may result in damage to brain and nerve tissue, or to the immune role played by molecular neurotransmitter Ach ,and therefore has a role in many neurogical disease [26]. The increase in enzyme activity can be attributed to the conditions of depression, anxiety, boredom and sadness, this confirms the relationship between this disorder and psychiatric health and psychiatric disorder [27]. The effect of estrogen on the brain and its stimulation to pull of choline and further build the acetylcholine, may be the reason for the higher activity of enzyme in male than female [23] .

Table 2: The activity of AChE in the Blood of Patients and Control for both sexes.

Parameters	**Gender**	**Control**	**Patients**	**P≤**
Mean± S.D. AchE (µmol/ml/min)	**General**	4.39± 1.03	6.21±1.18	0.01
	Male	4.598±1.176	6.200± 1.384	0.01
	Female	4.154±0.807	5.414±0.921	0.01

4.2. Levels of 0xidative Stress Marker(MDA) With Antioxidant parameters (GSH ,CAT, Cp)

MDA is good indicator of oxidative damage .In addition, MDA represents the end product of polyunsaturated fatty acid oxygenation is commonly used as the lipid peroxidation level marker and the level of the presence of oxidative stress [28].

The results in tables (3) showed that there was a significant increase (p<0.01) in the levels of MDA in patients with β-thalassemia major. Our study confirm that, in thalassemia there is excess production of reactive oxygen intermediates, such as superoxide anion (O2 –), hydroxyl radical (OH·), singlet oxygen andhydrogen peroxide (H2O2) within the erythrocytes, all these events leads to oxidative stress. This oxidative stress and a possible consequential accelerated apoptosis may contribute to shortened life span of erythrocytes. MDA, a product of lipid peroxidation is generated in excess amounts in supporting the fact that large amount of membrane bound iron is present in thalassemic erythrocytes [29]. Iron are also implicated as causative agents in excessive generation of free radical which are capable of causing oxidative damage to erythrocytes. Our results agree with

those of ElalfyMS and his colleagues (2013)[30], and Hasan F AL –Azzawie and his colleagues (2017)[31] ,This oxidative stress will cause growth failure as well as liver, cardiovascular, endocrine and neurological complications in thalassemia major. Peroxidative damage of lipids is indicated by the increase in serum MDA and decreased antioxidant defence mechanism play an important role in the pathogenesis of _ thalassemia major[32]. It requires adequate treatment in thalassaemic so that the early deaths especially from iron induced cardiomyopathies

As a result of continuous blood transfusions, the patients might be subjected to peroxidative tissue injury by the secondary iron overload. These findings might support the idea that iron overload in thalassemia leads to an enhanced generation of reactive oxygen species and oxidative stress. It also correlate with the study of Elham Abed Mahdi in 2014 [33],GSH is a major intracellular reducing agent which is very sensitive to oxidative pressures and it has a substantial factor in protecting body tissue and cells against the ROS damage. GSH protects plasma membrane from lipid peroxidation, regulation of gene expression, neutralizes the oxidative factors and prevents its formation [34].

The results of the current study indicate a significant decrease ($P \leq 0.01$) in the level of GSH as compared with control as shown in tables (3) .Our results are in good agreement with the result of study done by Muaid SAS and his colleagues (2015) [35] ,and Muanprasat C and his colleagues (2013) [36]. Where they confirmed that GSH level will be low in β –thalassemia major .

Catalase, widely distributed in all cells, is present in high amounts in erythrocytes. It is responsible for detoxification of hydrogen peroxide in the cells. In present study, catalase was significantly decrease ($P \leq 0.05$) in the levels of CAT in thalassemia patients as compared with healthy controls as shown in tables (3) .This could be due to increase in the lipid per oxidation product malondialdyhyde, which can form cross links, therapy inactivating several membrane bound enzymes[11]. Our results are in good agreement with the study done by Elham Abed Mahdi in 2014 [33], Choudhary ,M,(2017) [37]and Hasan F AL –Azzawie and his colleagues (2017)[31] , in which they found that, a greater amount of hydrogen peroxide might produce directtoxic damage to catalase, the concentration of this is considerably reduced in conditions of high oxidative stress. On the other hand,the decrease in CAT activity in patients may be due to the decrease in nicotinamide adenine dinucleotide phosphatase (NADPH) that is crucial for the maintenance of CAT activity because CAT monomer contains a high affinity binding site for NADPH. A second contributing cause may be at play. It is possible that iron may deplete H2O2 through Fenton chemistry, which would result in loss of induction of CAT expression by H2O2. Iron converts hydrogen peroxide to hydroxyl radical, peroxyl radical, and hydroxyl anion. It is notable that production of hydroxyl and peroxyl radicals may accentuate lipid peroxidation chain reactions, this would explain the association of this disorder with lipid peroxidation [38].

While its not agreed with the research of Qaiser, S and his colleagues (2015) [39] , Boudrahem. Addour and his colleagues (2014) [40], andSandra,S and his colleagues (2015) [41] ,in which they suggested that, the peroxidative status generated by (ROS) in beta thalassemia major patients may lead to significantly increased in catalase activity.

The results in tables (3) showed that there was a significant increase ($p<0.01$) in the levels of Cp in thalassemic patients as compared to control .The high serum levels of Cp observed in our patients might be attributed to several iron overload related mechanism:

1- It is possible that the levels were increased to prevent the formation of (ROS) through the Fenton reaction. This is achieved by the antioxidative capacity of Cp through the oxidation of Fe2+to Fe3+, thus preventing oxidative damage of lipids, proteins and DNA [42].

2- Oxidation of the ferrous form of iron to the ferric form enhancesits binding with transferrin and thus its transport to storage and/or utilization sites.

3- It is possible that Cp levels were increased in response to hypoxia and ineffective erythropoiesis.

4- It is possible that Cp is increased in response to accumulated iron in tissues, thus enhancing efflux of iron into transferrin in plasma. Several studies have provided evidence supporting the function of Cp in mobilization of iron between tissues and plasma [43].

Table 3: The level of (GSH, MDA,CATandCp)in the Blood of Patients and Control for both sexes .

Parameters	Gender	Control	Patients	P≤
Mean± S.D MDA µmol/L	**General**	3.415±0.750	5.39±1.39	0.01
	Male	0.787± 3.504	5.125±1.100	0.01
	Female	3.318±0.715	5.687±1.610	0.01
Mean± S.D GSH µmol/L	**General**	8.67±1.08	4.65±1.17	0.01
	Male	0.934±8.623	4.646±1.234	0.01
	Female	8.723±1.245	4.657±1.104	0.01
Mean± S.D CAT K/ml	**General**	0.198±0.029	0.157±0.038	0.01
	Male	0.123±0.173	0.152±0.087	0.01
	Female	0.194±0.130	0.148±0.133	0.01
Mean± S.D Cp g/L	**General**	0.1503±0.0462	0.308±0.123	0.01
	Male	0.1439±0.0442	0.3002±0.105	0.01
	Female	0.157±0.0462	0.317±0.140	0.01

4.3. liverFunctionTest (ALT,AST,ALPandTotal Bilirubin) Concentration

From the data presented in table(4),it appear that there is a highly significant increase ($p\leq0.01$) in the activity of

liver function enzymes (ALT, AST, ALP) in patients with β-thalassemia major Moreover, there is a high significant increase ($p \leq 0.01$) in the concentration of total bilirubin, as compared with the control group for both sexes .

Table 4: The level of (ALT,AST,ALPand TB)in the Blood of Patients and Control for both sexes .

Parameters	**Gender**	**Control**	**Patients**	**P≤**
Mean± S.D ALT IU/L	**General**	21.98± 6.99	55.5±19.07	0.01
	Male	6.42± 21.24	57.38±50.35	0.01
	Female	22.79±7.67	53.53±50.36	0.01
Mean± S.D AST IU/L	**General**	23.00±5.72	52.6±17.08	0.01
	Male	5.52±22.48	56.57± 17.32	0.01
	Female	23.58±6.04	48.13± 20.54	0.01
Mean± S.D ALP IU/L	**General**	113.3±21.8	173.4±32.1	0.01
	Male	20.04±107.95	169.62±33.27	0.01
	Female	119.16±22.76	177.58±30.27	0.01
Mean± S.D TB mg/dl	**General**	0.868±0.154	3.226±0.978	0.01
	Male	0.881±0.928	3.317±0.895	0.01
	Female	0.852±0.203	3.126±1.065	0.01

When certain types of cells are damaged they may leak enzymes into the blood stream, where they can be measured as indicators of cell damage. ALT , AST and ALP are such enzymes.

ALT is highly specific for hepatocellular damage, and could be due to liver damage secondary to iron overload, the level of it is increased in any hepatic injury or it may be high before the appearance of hepatic diseases like jaundice. AST displays its powerful activity in an alkaline environment and is particularly concentrated in liver, bones, and muscles. Thus, high levels of (AST) in the blood suggest medical conditions that affect the heart, liver, or muscles. Higher levels are seen in extrahepatic bile obstruction, intrahepatic cholestasis, infiltrative liver disease and hepatitis [44].

Alp is mainly expressed in variety of tissues, in bones , Liver. The elevations of serum Alp activity (hyper alkaline phosphatasemia) , is seen with more specivie disorders such as , malignant biliary obstruction , biliary cirrhosis , hepatic lymphoma , liver disease , hepatitis , paget disease , bone cancers and cholangitis [45].

In the current study, we have found the elevated levels of ALP as well. This is mainly caused by leakage of the enzymes from cytoplasmic and mitochondrial compartments of injured hepatocytes to the plasma. It can be concluded that the high levels of hepatic enzymes are possibly due to the hepatic injury, caused by iron overload in thalassemic patients receiving multiple blood transfusion. The results of the present study are thus concurrent with the results of other studies [46,47]. Moreover, there is a high significant increase ($p\leq 0.01$) in the concentration of total bilirubin in thalassaemic patients as compare with control subjects .This could be due to destruction of RBCs and hemolysis of immature RBCs in spleen that occurred in thalassaemic patients and the liver's decreased ability to conjugate the bilirubin. Iron overload could potentially induce hepatic toxicity, and consequently increased bilirubin concentrations . This result was in agreement with other studies [48,49] .

4.4. Nitric Oxide and Peroxynitrite Concentration

Results in table (5) have shown highly significant increase ($p\leq 0.01$) in the concentration of peroxynitrite, and highly significant decrease ($p\leq 0.01$) in the concentration of nitric oxide in the blood of **β**-thalassemia major patients ,compared with control group .

Table 5: The level of (NO and $ONOO^-$) in the Blood of Patients and Control for both sexes .

Group **Parameter**	**Gender**	**Control**	**Patients**	**P≤**
Mean ± S.D. NO nmol/ml	**General**	11.60± 3.32	5.15±1.45	0.01
	Male	11.548±2.991	4.896± 1.029	0.01
	Female	11.647±3.732	5.966±0.884	0.01
Mean ± S.D. $ONOO^-$ µmol/L	**General**	57.8±13.5	68.8±15.9	0.01
	Male	58.95±13.81	70.82±17.90	0.01
	Female	56.51±13.32	66.59±13.27	0.01

Hemoglobinopathies characterized by chronic hemolysis are currently considered sources of strong oxidative stress. Free heme and the red cell membrane elements that are produced during hemolysis have a negative effect on(NO) and arginine availability, which in turn promotes vasoconstriction. They also lead to further endothelial dysfunction, resulting in a more pronounced (NO) reduction [50]. Hemolysis and repeated blood transfusions cause decreases in nitric oxide levels and iron overload in various organs, including the brain. Increased iron in the brain leads to oxidative stress and possible irreparable brain tissue damage, causing cognitive impairment. In hemoglobinopathies, NO binds very rapidly to deoxyhemoglobin, forming a stable Hb (Fe+2)-NO complex. NO also reacts with and converts oxygenated hemoglobin to methemoglobin and nitrate (NO3 -). Pathological processes in thalassemia accelerate the destruction of (NO), and limit the compensatory increase in(NO) production [51]. We found that serum NO levels in beta-thalassemia major patients were significantly lower than those in control, that is significantly correlated with oxidative stress . Our results are in good agreement

with the study done by Nihayat Bayraktar and his colleagues (2008) [52], in which they found that, Lowered level of NO might be explained by hemolysis-associated endothelial dysfunction, thalassemia major are probably associated with more severe degrees of hemolysis. The body's immunosurveillance may prove ineffective if the generation of peroxynitrite is enhanced tremendously, as seen in chronic inflammation and in injured tissues. Once peroxynitrite level increases, the damage and nitration would be inevitable and the immunoregulatory network would be activated resulting in the production of autoantibodies. Cellular metabolism and ionization radiation produces both reactive nitrogen and oxygen species. These radicals and their subsequent intermediates may react with cellular macromolecules and induce a variety of chemical alterations leading to autoantibodies [53].

5. Conclusion

1- Thalassaemic patients were have a higher AchE activity compared to the control group .

2- Iron overload gives rise to oxidative stress through free radical formation.

3- Oxidative stress and reduced antioxidant defense mechanism play an important role in complications of β-thalassaemia major patients.

4-There is a direct effect of iron overload on liver injury.

5-There was significant increase in concentration of Serum $ONOO^-$,and reduction in serum of NO for thalassemic patients .

6. Recommendations

1-The possibility of using the enzymes AChE, AST and APT, as biochemical parameters to follow the severity of the disease.

2-Conducting studies in the β-thalassaemia major to estimate more biochemical parameters and study its relation with the disease.

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