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Biochemical Assessment of Iron Overload Effect on Human Organs (Liver, Heart, Brain) In Patients with Beta-Thalassemia Major and Thalassemia Intermediate

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ABSTRACT

Back ground: The aim of this study was to evaluate the effect of over iron on the functions of some organs in the body (liver, heart, and brain) in patients with hemoglobinopathies (thalassemia major and thalassemia intermediate).

Material and Method: This was a case-control study included 136 participants (105 patients and 31 controls). The patients were divided into two subgroups: 89 beta-thalassemia major, and 16 beta-thalassemia intermediate, who was visited the center of genetic blood diseases, Nasiriya, Thi Qar - Iraq. The level of iron, ferritin, ALT, AST, albumin, high sensitive troponin, myoglobin, CK-MB and CK-BB was measured by standard methods.

Result: The study was revealed, a significant increase in serum iron and ferritin levels in the patient's group when compared with the control. It was concluded that the toxic effect of high levels of iron might lead to tissues damage and hence to organs dysfunction.

Conclusion: It has been proven that when iron rises in the plasma of patient; it causes damage in the function of these organs (liver, heart, and brain), due to iron over load.

Keywords: *Iron overload, Organs dysfunctions, liver, heart, brain, thalassemia*

INTRODUCTION

Iron excess damages the heart, thyroid, liver, pancreas, and central nervous system. The overproduction of reactive oxygen species in the presence of excess iron is the primary cause of this organ dysfunction. Iron overload syndromes are pathological disorders that indicate bodily iron overload, and iron accumulation causes organ dysfunction such as fibrosis, cell damage, and carcinogenesis (Kohgo et al., 2008).

The liver is the principal location for iron storage in patients with hemochromatosis or transfusion dependent anemia, and liver iron concentration correctly represents total body iron reserves. The liver is the only organ which synthesizes ferritin and transferrin, and also the principal organ for iron accumulation the liver, iron is normally protein-bound, and free ferrous iron is extremely poisonous.

Excess iron catalyzes the formation of free radicals, that has been linked to lipid peroxidation and hepatotoxicity (Al-Moshary et al., 2020)

Cardiac hemochromatosis commonly known as primary iron overload cardiomyopathy, is a major and possibly avoidable cause of heart failure. Disorders of iron excess can be inherited or acquired. The cellular iron homeostasis is governed by cardiomyocyte feroportin, and extent of heart function is affected by the location of myocardial iron accumulation (Aronow, 2018).

There is widespread recognition of the extremely harmful effects of excess iron particularly in the brain, as such, several studies have started to address the effects of high iron intake or high peripheral iron status on neurophysiological mechanisms, pathological development, and cognition (Ferreira et al., 2019).

MATERIALS AND METHODS

A case-control study involved 136 Patients (58 males and 78 females). Their ages were ranged between 5-45 years old. Oral consent of the patients and control subjects was obtained from patients or their parents. These patients were registered as thalassemia patients in the Center of Genetic Hemoglobinopathies, Nasiriya, Thi Qar, Iraq. The patients were 89 βeta-thalassemia major, 16 βeta-thalassemia intermedia. Also, the study was involved 31 apparently healthy people as the control group. Statistical analysis data were analyzed using statistical package for social science version 21 (SPSS).

RESULTS

Comparison Between Control And Thalassemia Major

Comparison between control and thalassemia major patients was shown in table1. The result of study was revealed highly statistical significance increases for means according to the findings of serum iron and ferritin in the thalassemia major (209 ±79.2, 2970.9 ± 1843.3) when compared with control (115 ± 51.5, 41.9 ± 32.7), receptively; P value < 0.001. A previous study (Sewwandi Karunaratna et al., 2017) in SriLankan patients with β-thalassemia, was reported that patients' mean of serum ferritin concentration was significantly higher than control. Furthermore, the findings of this study was agreed with previous studies conducted in Saudi Arabia(Badawi et al., 2019), Pakistan (Riaz et al., 2011), and India (Mishra & Tiwari, 2013) and Egypt (Mathematics, 2016)

The results of study were shown highly statistical significance increases of serum ALT and AST in the thalassemia major group 24.5 ± 22.5 and 40.5 ± 41.7, when compared with control group (15.1 ± 9.7 and 22 ± 8.9); P value were 0.026 and 0.017, respectively. These results were in agreement with a study conducted by (Kasarala & Tillmann, 2016), who proved that several biochemical markers, including AST, ALT, and albumin, are widely known to reflect hepatic function. According to the findings, increased AST and ALT values may reflect the degree of liver damage caused by iron overload. The level of AST was higher than ALT in thalassemia major patients. The results of this study also was similar to a study (Terzi et al., 2016), who was repored that the level of AST was higher than ALT in thalassemia major patients. Also, the findings of this study were agreement with previous research findings (Al-Moshary et al., 2019), a similar association was discovered in children to youth thalassemic illness. Bangladeshi research found that increased serum AST and ALT levels in beta-thalassemia patients indicate impaired liver and muscle function. Such findings were also discovered in Jordan, where a favorable association between serum ALT and AST levels and serum ferritin concentration was discovered in beta-thalassemia patients versus controls was discovered.

This study demonstrated that albumin synthesis was decreased in thalassemia major as the severity of liver damage due to iron overload or toxity, where the means of albumin in thalassemia major group for (total, males and females) were statistically in comparison with the control; P values were 0.001, 0.001, and 0.001, respectively. These findings were agreed with previous research findings of (Wahidiyat et al., 2018) (Terzi et al., 2016), It has been proven that

when iron excess increases, liver damage increases and albumin synthesis decreases. The coefficient connection between liver by MRI value and albumin appeared positive, as opposed to transaminases, throughout albumin metabolism, only a small fraction of produced albumin being retained in the liver, with the vast majority being released into the bloodstream. Any hepatocyte injury can reduce albumin production, which can then be observed in the blood.

This study revealed statistical significance differences of serum troponin (cTnI) of control group 0.03 ± 0.01, when compared with the means of serum troponin in the thalassemia major group 0.071 ± 0.06, P value was 0.001. When troponin levels are equal to or higher than ≥ 0.30 ng/mL, it indicates the possibility of an acute myocardial infarction. The results of current study were similar to previous study (Shodikin et al., 2016), who reported, when ferritin levels in the blood were elevated in the major thalassemia groups was highly statistical significant 4292.5 µg/L in comparison with the control group 136.2 µg/L, p value = 0.0004. When ferritin levels rise, troponin levels rise as well. The average cTnI level in the MBT group reached 0.22 ng/mL, while it was 0.20 ng/mL in the control group.

There were highly statistical significance increases of serum myoglobin and CK-MB in thalassemia major group in total, males and females groups in comparison with control; P values were < 0.001 and < 0.001, < 0.001 and 0.007, < 0.001 and < 0.001; respectively. A previous study (Shahramian et al., 2015) was demonstrated that after the age of eight in patients with thalassemia, cardiac involvement occurs as a result of repeated red blood cell transfusion. The main reason for frequent transfusion in β -thalassemia is ineffective and rising erythropoiesis of bone marrow, which leads to higher in plasma volume and high cardiac output, eventually leading to cardiomyopathy. In these cases, cardiac involvement occurs without the presence of an infarction. All patients of B-thalassemia major were chronically anemic. Cardiomyopathy cause of iron excess is the greatest cause of death in patients with thalassemia major (Heris et al., 2021).

Also, the result of study was revealed highly statistical significance increases of CK-BB in thalassemia major group for total, males and females in comparison with control; P values were 0.003, 0.006, and 0.03; respectively. There is no previous study for this analysis.

TABLE 1: Comparison between control and thalassemia major

Variables	Total (120)			Males (52)			Females (68)		
	Thalassemia Major (n= 89)	Control (n= 31)	P value ANOVA	Thalassemia Major (n= 30)	Control (n= 13)	P value ANOVA	Thalassemia Major (n= 50)	Control (n= 18)	P value ANOVA
Age (year)	14.3 ± 8.3	18.9 ± 8.5	0.12	16.6 ± 8.4	17.3 ± 8.2	0.75	12.5 ± 7.7	14.1 ± 8.8	1.000
Iron (µg/dL)	209 ± 79.2	115 ± 51.5	< 0.001	203.1± 60.8	118.3 ± 52.5	< 0.001	214.8 ± 91.3	112.7 ± 52.2	< 0.001
Ferritin (ng/mL)	2970.9 ± 1843.3	41.9 ± 32.7	< 0.001	3460 ±1974	54.7 ± 42.8	< 0.001	2589 ± 1654	32.7 ± 19.4	< 0.001
ALT (U/L)	24.5 ± 22.5	15.1 ± 9.7	0.026	26.9 ± 22.8	13.8 ± 7.0	0.04	22.6 ± 22.3	16 ± 11.1	0.23
AST (U/L)	40.5 ± 41.7	22 ± 8.9	0.017	47.6 ± 49.9	20.7 ± 8.6	0.059	34.8 + 33.5	23 ± 9.3	0.14

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Albumin (g/dL)	2.2 ± 0.7	4.2 ± 0.6	0.001	2.1 ± 0.6	4.3 ± 0.8	0.001	2.1 ± 0.7	4.1 ± 0.9	0.001
Troponin (ng/mL)	0.071 ± 0.06	0.03 ± 0.01	0.001	0.071 ± 0.024	0.03 ± 0.01	< 0.001	0.07 + 0.08	0.03 ± 0.01	0.041
Myoglobin (ng/mL)	5.13 ± 2.1	2.0 ± 0.7	< 0.001	5.14 ± 2.3	2.3 ± 0.68	< 0.001	5.1 ± 2.0	1.9 ± 0.6	< 0.001
CK-MB (ng/mL)	0.23 ± 0.20	0.05 ± 0.02	< 0.001	0.28 ± 0.26	0.06 ± 0.02	0.007	0.19 ± 0.1	0.04 ± 0.02	< 0.001
CK-BB (ng/mL)	1.22 ± 0.51	0.9 ± 0.1	0.003	1.18 ± 0.25	0.96 ± 0.16	0.006	1.2 ± 0.6	0.91 ± 0.1	0.03

Comparison between control and intermedia thalassemia

The comparison between control and intermedia thalassemia was shown in table 2. The result of study of was shown highly statistical significance increases of serum iron between the control and intermedia thalassemia for total 115 μg/dL, and 171.1 ± 85.3 μg/dL, respectively; P value was 0.007. Also the results showed that there were statistical significance increases in the mean of serum ferritin between in intermedia thalassemia for (total, males and females) in comparison with control, where control were 41.9 ± 32.7, 54.7 ± 42.8, and 184.3 ± 96.0); while intermedia thalassemia were 1736 ± 1436, 1604 ± 1032, and 1816 ± 1682, P values were < 0.001, < 0.001, and < 0.001; respectively. Iron overload is a well-known characteristic of intermedia thalassemia. The pathophysiology is primarily linked to increased iron absorption from the gastrointestinal tract and recurrent blood transfusions. Iron absorption by intermedia thalassemia is 3–10 times more than compared normal people, and rises following splenectomy (Shah et al., 2014).

The results of study were shown no statistical significance increases means of ALT and AST of intermedia thalassemia for total, males and females in comparison with the control; P value ALT were 0.17, 0.43 and 0.28; AST 0.18, 0.86, and 0.16; respectively. There was a previous study (Salama et al., 2015), that disagree with these results, as demonstrated in thalassemia patients with higher ALT or AST compared with normal controls; P values were 0.007, and 0.004, respectively.

On the other hand, the results of study revealed were highly statistical significance decrease means of albumin between the control and intermedia thalassemia for total, males and females; P values were < 0.001, 0.001, and 0.001, respectively.

The results of study were highly statistical significance increases means of serum troponin, myoglobin, and CK-MB in intermedia thalassemia group for total and females in comparison with the control, P value of total were: 0.008, < 0.001, and < 0.001, females: 0.006, < 0.001, and 0.006; respectively. Also results showed that there were statistical significance increases in the means of males of myoglobin and CK-MB; P. values were < 0.001 and 0.006. While troponin of males was showed no statistical significance differences; P value was 0.56.

Also, the results showed that there were statistical significance increases means of CK-BB in intermedia thalassemia group for total and females in comparison with the control, P values were 1.2 ± 0.5, and 1.2 ± 0.6; respectively. Except males for CK-BB, which showed no statistical significance differences; P value was 0.093.

TABLE 2: Comparison between control and intermedia thalassemia

Variables	Total 47			Males (19)			Females (28)		
	Intermedia thalassemia (n=16)	Control (n=31)	P value **ANOVA**	Intermedia thalassemia (n= 6)	Control (n=13)	P value **ANOVA**	Intermedia thalassemia (n=10)	Control (n=18)	P value **ANOVA**
Age (Year)	18.1 ± 12.1	18.9 ± 8.5	0.807	13.8 ± 9.2	17.4 ± 8.2	0.40	20.8 ± 13.3	14.1 ± 8.8	0.8
Iron (µg/dL)	171.1 ± 85.3	115 ± 51.5	0.007	149.3 ± 65.6	118.3 ± 52.5	0.28	184.3 ± 96.0	112.7 ± 52.2	0.01
Ferritin (ng/mL)	1736 ± 1436	41.9 ± 32.7	< 0.001	1604 ± 1032	54.7 ± 42.8	< 0.001	1816 ± 1682	32.7 ± 19.4	< 0.001
ALT (U/L)	21.1 ± 20.7	15.1 ± 9.7	0.17	17.1 ± 9.9	13.8 ± 7.6	0.43	23.6 ± ± 25.3	16 ± 11.1	0.28
AST (U/L)	29.9 ± 30	22 ± 8.9	0.18	21 ± 10.5	20.7 ± 8.6	0.86	35.9 ± ± 36.5	23 ± 9.3	0.16
Albumin (g/dL)	2.2 ± 0.7	4.2 ± 0.6	< 0.001	2.3 ± 0.7	4.0 ± 0.5	0.001	2.2 ± 0.7	4.4 ± 0.9	0.001
Troponin (ng/mL)	0.04 ± 0.02	0.03 ± 0.01	0.008	0.05 ± 0.02	0.03 ± 0.001	0.56	0.05 ± 0.02	0.03 ± 0.01	0.006
Myoglobin (ng/mL)	4.8 ± 0.8	2.0 ± 0.7	< 0.001	4.6 ± 0.6	2.3 ± 0.68	< 0.001	4.9 ± 0.1	1.9 ± 0.6	< 0.001
CK-MB (ng/mL)	0.24 ± 0.24	0.05 ± 0.02	< 0.001	0.23 ± 0.19	0.06 ± 0.02	0.006	0.2 ± 0.2	0.04 ± 0.02	0.006
CK-BB (ng/mL)	1.2 ± 0.5	0.9 ± 0.18	0.013	1.11 ± 0.1	0.96 ± 0.16	0.093	1.2 ± 0.6	0.9 ± 0.1	0.046

Comparison among different patient groups

The results of study were revealed no statistical significance differece in serum iron, when compared thalassemia major group (209 ± 79.2) with thalassemia intermedia (171.1 ± 85.3), P values was 0.08. While, ferritin level in thalassemia major 2970.9 ± 1843.3 ng/mL was highly statistical significance increase than in thalassemia intermedia 1736±1436 ng/mL; P value was 0.01.

The means iron and ferritin in thalassemia major were greater than that of thalassemia intermedia, because thalassemia major patients had taken more bags blood during the year than thalassemia intermedia. Thalassemia intermedia patients usually do not require regular blood transfusions (Shah et al., 2014). This finding agreed with (Ismail et al., 2019); who were observed that ferritin in thalassemia major was statistical significance high than in thalassemia intermedia; P value was < 0.01. The liver is the major iron storage organ, readily absorbing excess transferrin and non-transferrin associated iron; also it mobilizes iron quickly and efficiently in periods of demand or in reaction to iron chelation. The heart, from the other hand, has robust safeguards in place to prevent excessive

transferring-mediated absorption (Walker, 2002).

The results of the presented study showed that there were no statistical significance differences in serum albumin, troponin myoglobin, CK-BB and CK-MB in thalassemia major patients when compared with thalassemia intermediate; P value > 0.05. When the ability for iron attachment is exhausted, labile unbound iron species start to circulate, resulting in pathologic myocardial iron overload. Even so, cardiac iron uptake lags behind that many other extrahepatic organs, such as the pancreas, are affected. As a result, most children may have significant hepatic iron overload while showing no signs of cardiac iron overload (Wood et al., 2008).

TABLE 3: Comparison between thalassemia major and ıntermedia thalassemia

Variables		Intermedia thalassemia (n=16)	Thalassemia Major (n=89)	P value
Age (year)		18.1 ± 12.1	14.3 ± 8.3	0.11
Sex	males	6 (13.33 %)	39 (86.6 %)	0.64
	females	10 (16.66 %)	50 (83.33 %)	
	Total	16 (19.99 %)	89 (84.77 %)	
Iron (µg/dL)		171.1 ± 85.3	209 ± 79.2	0.08
Ferritin (ng/mL)		1736 ± 1436	2970.9 ± 1843.3	0.01
ALT (U/L)		21.1 ± 20.7	24.5 ± 22.5	0.58
AST (U/L)		29.9 ± 30	40.5 ± 41.7	0.33
Albumin (g/dL)		2.2 ± 0.7	2.2 ± 0.7	0.42
Troponin (ng/mL)		0.04 ± 0.02	0.071 ± 0.06	0.17
Myoglobin (ng/mL)		4.8 ± 0.8	5.13 ± 2.1	0.68
CK-MB (ng/mL)		0.24 ± 0.24	0.23 ± 0.20	0.94
CK-BB (ng/mL)		1.2 ± 0.5	1.22 ± 0.51	0.87

Comparison between males and females of patients groups

The comparison between males and females of intermedia thalassemia patient's groups were shown in table 4. The results of study were no statistical significance differences for all parameters, when comparison between males and females, except troponin; P values were > 0.05; which may be due to low sample size. While, the results demonstrated that only ferritin level in males in thalassemia patients was higher than in females. A previous studies and inconsistent with this study, that showed the level of ferritin in males was higher than in (Faruqi et al., 2015).

TABLE 4: Comparison between males and females patients groups.

Variables	Intermedia thalassemia (n=16)			Thalassaemia Major (n= 89)		
	Females (n=14)	Males (n=7)	P value	Females (n=50)	Males (n=39)	P value
Age (year)	20.8 ± 13.3	13.8 ± 9.2	0.2	12.5 ±7.7	16.6 ± 8.4	0.2
Iron (µg/dL)	184.3 ± 96.0	149.3 ± 65.6	0.4	214.8 ± 91.3	203 ±60.8	0.10
Ferritin (ng/mL)	1816 ± 1682	1604 ± 1032	0.7	2589 ± 1654.9	3460.6 ± 1974.9	0.04
ALT (U/L)	23.6 ± 25.3	17.1 ± 9.9	0.5	22.6 ± 22.8	26.9 ± 22.8	0.3
AST (U/L)	35.9 ± 36.5	20 ± 10.5	0.32	34.8 ± 33.5	47.3 ± 49.9	0.1
Albumin (g/dL)	2.2 ± 0.7	2.3 ± 0.7	0.06	2.1 ±0.7	2.1 ±0.61	0.1
Troponin (ng/mL)	0.05 ± 0.02	0.04 ± 0.02	0.04	0.07 ±0.07	0.07 ±0.02	1.0

Myoglobin (ng/mL)	4.9 ± 1.0	4.6 ± 0.6	0.57	5.1± 2	5.1 ± 2.3	0.9
CK-MB (ng/mL)	0.2 ± 0.2	0.23 ± 0.19	0.9	0.1 ±0.1	0.2 ± 0.2	0.52
CK-BB (ng/mL)	1.2 ± 0.6	1.19 ± 0.1	0.6	1.2 ±0.6	1.1 ± 0.2	0.56

CONCLUSION

Iron overload may cause liver damage and hence causes an increase in liver enzymes such as ALT and AST and a deficiency of albumin. Also, for the heart, iron overload causes an increase in the level of troponin, myoglobin, CK-MB may be due to heart damage. An excess of iron causes an increase in the level of CK-BB isoenzyme may due to brain damage.

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