Relationship of TNF-$_{\alpha}$ gene expression with serotonin and ferritin levels in Beta thalassemia major patients

Maryam M. Nasser[1], Muthana I. Maleek[2], Zainulabdeen AL-badri[3]
[1]Department of biology, College of Science, University of Wasit, Iraq.
[2]Department of biology, College of Science, University of Wasit, Iraq.
[3]College of Medicine, University of Wasit, Iraq.
Email: marymohh95@gmail.com
Email: mmaleek@uowasit.edu.iq
Email: zian98.muthana@gmail.com

Abstract

Objective: Beta-thalassemia syndrome are a collection of genetic blood disorders characterized by condensed or absent beta globin chain synthesis, causing in reduced Hb in red blood cells (RBC), decreased RBC production and anemia. Thalassemia major refers to a severe phenotype which occur when patients are homozygous or compounds heterozygous for β chain mutation, patients usually present with symptoms within the first two years of life. Aim: The study aimed to evaluate serotonin level and its effect on β-thalassemia patients and investigate the production of the inflammatory cytokine tumor necrosis factor alpha (TNF-α) in serum and their relationship with ferritin serum level in transfusion dependent β-thalassemia patients. Methods: Measurements of serotonin and TNF-α was by using enzyme-linked immunosorbent assay (ELISA). Ferritin was measured automatically by Cobas e 411 device .The study has been carried on patients with β- thalassemia major disease registered at Thalassemia hematology center in Al-Kut women &children Hospital in Wasit province / Iraq. A total 85 samples were included in this study, 55 patients with β- thalassemia major and 30 samples as a control and evaluate result used P≤0.05. Results: Results showed reduced serotonin level in β-TM patients and revealed a significant correlation at (P≤0.02) when compared with control. Serum ferritin level highly increased in blood transfusion dependent β-TM patients and its presented significant correlation (P≤0.001) in patients when compared with control group. Highly TNF-α level increased in β-thalassemia patients and it's correlated positively in patients when compared with healthy controls. Serotonin, ferritin and TNF-α revealed no significant correlation (P>0.05) with age when compared between the two age groups of β-thalassemia patients less and more than 15 years old.

Keywords: B-thalassemia patients, serotonin, ferritin, TNF-α

1. Introduction

Beta-thalassemia is a chronic and genetically determined hematological disorder considered by severe hemolytic anemia as a result of lacking synthesis of b chains of the hemoglobin. The anemia demands regular blood transfusions to maintain life (Yesim *et al.*, 2005). Thalassemia major refers to a severe phenotype which happens when patients are homozygous or compounds heterozygous for β chain mutation, patients usually present with symptoms within the first two years of life (Jha & Jha, 2014). Serum ferritin level is one of the important requested investigations in equally primary and secondary care. Even as low serum ferritin levels always indicate reduced iron stores, elevated serum ferritin levels could be the result of several different a etiologies, including iron overload, inflammation, malignancy, liver or renal disease, and the lately defined metabolic syndrome (Cullis *et al.*,2018) . The estimation of serum ferritin levels is the most commonly employed test to evaluate iron overload in Beta - thalassemia Major. Serotonin or (5-Hydroxytryptamine) is a biogenic amine best renowned for its role as a neurotransmitter. Serotonin is a molecule with varied effects in the central nervous system also in the periphery. It acts as a hormone, a neurotransmitter, and a mitogen and is abundant in the animal kingdom (Zadeh *et al.*, 2008). It is widely accepted that serotonin (5-HT), a neurotransmitter involved in the ruling of emotion, mood, sleep and aggression, plays a key role in the beginning and course of depression (Maes & Meltzer, 1995) & (Neumeister *et al.*, 2004). Patients with chronic physical diseases typified by thalassemia are exposed to emotional and behavioral problems .While several studies have reported the association between thalassemia major and depression (Messina *et al.*, 2008) & (Mikelli & Tsientis, 2004). Tumor necrosis factor-alpha (TNF-α) is one of the important cytokines. It`s acting recognized roles in regulating innate immunity, adaptive immunity, autoimmunity, and inflammation. A deregulation of TNF gene expression and signaling can occasion chronic inflammation, which may result in a development of autoimmune diseases and tissue damage (Brzustewicz & Bryl, 2015). TNF- α as an example of cytokine , Cytokines are broad and small proteins, molecules and hormone-like proteins (5-20kDa)

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playing a very important role in many cellular processes, such as growth, migration, differentiation, and apoptosis (Badowska–Kozakiewicz, 2013).

2. Methodology

A cross sectional study design has been conducted on β-thalassemia major patients who are registered in Thalassemia hematology center in Al Kut women &children Hospital in Wasit province / Iraq, for regular blood transfusion and treatments in the period from February 2022 to May 2022.The study involve 85 samples, 55 specimens of blood samples have been collected from β-thalassemia major patients, in age ranged from 2-34 years and 30 blood samples have been collected from totally healthy individuals , who have no history of thalassemia disease or any other hematology disease and clinical complications , in age ranged from 18-45 years.

3. Blood collection

3 ml of venous blood were collected by vein puncture from β- thalassemia major patients and control in gel tubes arranged and labeled, and left to clot in room temperature for 30 minutes, and then it was centrifuged for 5 minutes at 4000 RPM for serum separation. , hemolysis has been avoided by taking the necessary precautions and ignoring hemolysis samples. Serum was collected and distributed by micropipette into eppendorf tubes and give the same number, and the samples were frozen in (-20°C) in deep freezer until the assay was done. Those specimens were then processed by using enzyme-linked immunosorbent assay (ELISA) to investigate serotonin level and TNF-α level in β- thalassemia major patients and control.
Ferritin level in was measured automatically by Cobas e 411 device.

4. Results and Discussion

This study included 55 patients of β-thalassemia major with the percentage (64.7 %), and 30 control of healthy individuals with the percentage (35.3%), Table (1). The distribution of patients according to gender is 23 female with the percentage (41.8%) and 32 male with the percentage (58.2 %).
The samples we have collected show that the frequency of male thalassemia patients were higher than female thalassemia patients, this study is in agreement with previous studies which suggest that the percentage of male patients were higher than female (Laghari *et al.*,2018)& (Khan *et al.*,2015).

Table (1): Demographic patient's data .

Item	No.	%
Patients	55	64.7
Control	30	35.3
Blood group of the patients		
A	12	21.8
AB	7	12.7
B	16	29.1
O	20	36.4
Gender of the patients		
Female	23	41.8
Male	32	58.2

While other studies show that there is no significant difference between genders because thalassemia it is a genetic disease that is transferred from parents to children and to both gender similarly. This result is agreed with previous studies (Al-attar & Shekha ,2014). The distribution of patients according to blood group is (12) of A blood type with the percentage (21.8 %), (7) of AB blood type with the percentage (12.7 %), (16) of B blood type with the percentage (29.1 %) , and (20) of O blood type with the percentage (36.4 %) , Table (1) . Thalassemia patients need frequent blood transfusion so it is necessary to determine the frequency of ABO blood groups and any complication related with transfusion of blood with each blood group type. Previous study shows that in both genders of thalassemia patients, blood group O is the highest percentage in thalassemia patients, followed by B blood group in males. However, the 2nd one in female patients is A blood group. This study also found that the largest proportion of Beta-thalassemia major patients was of group O+, followed B+, then A+. While, the lowest percentage of ABO blood group in thalassemia patients is AB blood group (Marbut *et al.*, 2018).

Correlation of biochemical parameters in patients and control.

The results revealed a highly significant increasing in concentration of TNF-α level (2.6±0.8) in β-TM patients and the data in table (2) shows significant correlation (P≤0.001) respectively in patients when compared with healthy controls.

Table (2): Correlation of TNF-α, serotonin and ferritin in patients and control.

Item	patients and control	N	Mean±SD	Std. Error Mean	*P value
TNF-α	Patients	55	2.6±0.8	0.1	0.001
	Control	30	1.9±0.5	0.09	
Serotonin	Patients	55	2.4±0.7	0.1	0.02
	Control	30	2.8±0.6	0.1	
Ferritin	Patients	55	3362.6±3.1	428.7	0.001
	Control	30	237.6±7.2	13.2	
*p = p≤0.05\					

The result of this study is in agreement with other studies that demonstrated a high significant increasing in TNF-α level in β-TM patients compared with control, this result may be due to iron overload and antigenic motivation encouraged by chronic transfusion therapy (Hassoon ,2021). It was

proposed that the increase in TNF-α could be caused by macrophage activation due to iron overload and the antigenic stimulation induced by chronic transfusion therapy. The activated macrophages were selectively phagocytosing apoptotic erythroid precursors, thereby contributing to ineffective erythropoiesis (Angelucci et al., 2002).

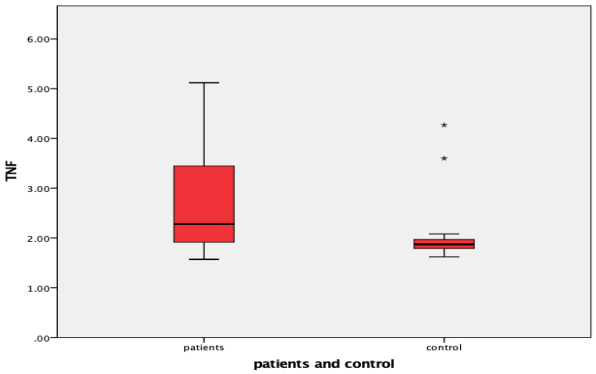

Figure (1): Boxplot of TNF-α level in patients and control.

Our result is also agreed with (Tanyong et al., 2015) where confirmed serum level of TNF-α was statistical significantly higher in post-splenectomized thalassemic patients than in healthy controls and non-splenectomized patients, which specified that TNF-α could act an important role in the pathogenesis of the disease. In addition, EPOR protein in erythroid progenitor cells was inhibited by TNF-α , this submits that TNF-α produced a reduction of both EPOR protein expression and EPO-induced cell proliferation of thalassemic erythroid progenitor cells, which could be implicated in the mechanism of ineffective erythropoiesis in β-thalassemia/Hb E patients .

Evaluation of Serotonin in β- thalassemia major patients shows a significant decreased serotonin level (2.4±0.7), and the results in table (2) reveals a significant correlation (P≤0.02) respectively in patients when compared with healthy controls. Several studies demonstrated that thalassemia patients suffer from depression. A study conducted in Egypt, its results revealed the prevalence of Psychological depression between patients with Thalassemia of both types (Major and intermediate) at an average level, there were significant differences at (0.000) due to the variance of Thalassemia type in Depression in patients with Thalassemia in favor of patients for Chronic thalassemia (Al-Husseini & Mohamed ,2019). A Previous study demonstrated that the effects of serotonin (5-HT) on anxiety and depression are facilitated by a number of 5-HT receptors, including autoreceptors which action to inhibit 5-HT release, the results propose that strategies intended at blocking 5-HT1B autoreceptors might be beneficial for the treatment of anxiety and depression (Nautiyal *et al.*, 2016).

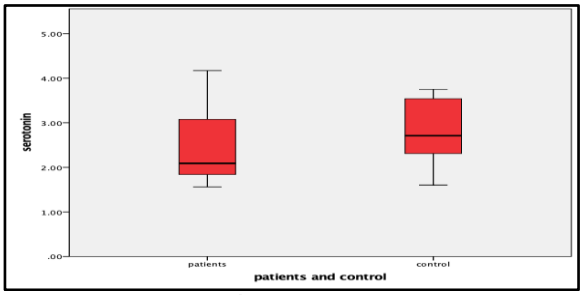

Figure (2): boxplot of serotonin level in patients and control.

Estimation of serum ferritin in thalassemia patients demonstrated a significant increase in β-TM patients (3362.6±3.1), and the data in table (2) reveals a significant correlation (P≤0.001) respectively in patients group when compared with control group. . Iron overload in patients with β-TM is a direct consequence of frequent blood transfusion (Shenoy *et al.*, 2014). The increased level of serum ferritin in our study is in agreement with previous study achieved by (Saad et al., 2021) that verified the high ferritin levels (1812±1024.9 ng/mL) found in the study support the statement that the management of β- thalassemia major patients was sub optimal, meanwhile the values were double of the recommended ferritin values of 1000 ng/mL .

Correlation between biochemical parameters according to age of the patients.

Table (3), showed two age groups of β-thalassemia patients more and less than 15 years old .The data presented in the table above revealed that TNF-α have no significant correlation (p > 0.05) with age when compared between the two age groups of β-thalassemia patients less than 15 years, the mean is (2.4±0.7) and more than 15 years, the mean is (2.8±0.8).

Table (3): Correlation between TNF-α, Serotonin and ferritin according to age of the patients.

Item	age in years	N	Mean ±SD	Std. Error Mean	P value
TNF-α	< =15	27	2.4±0.7	0.15	0.1
	>15	28	2.8±0.8	0.1	
Serotonin	< =15	27	2.3±0.7	0.1	0.6
	>15	28	2.4±0.7	0.1	
Ferritin	< =15	27	3561.5±3.0	593.7	0.5
	>15	28	3170.5±3.3	626.1	

A previous study conducted to investigate the relationship between pro- inflammatory markers and age in the natural aging process, confirmed that there was no significant difference between the studied groups. In addition, there was no correlation between TNF-α and age in any of the three analyses of 110 participants, male, and female (Milan-Mattos *et al.*, 2019).

The data presented in the table (3) showed that serotonin have no significant correlation ($p > 0.05$) with age when compared between the two age groups of β-thalassemia patients less than 15 years , the mean is (2.3±0.7) and more than 15 years , the mean is (2.4±0.7) . The adolescent with beta-thalassemia major is undergoing both physical and psychological problems; one of them is depression because of suffering a chronic illness. A study conducted in Indonesia on a number adolescents aged 13–20 years with a beta-thalassemia major , the results of this study showed that adolescent with beta-thalassemia major age 13–20 years had 45.7% minimal depression, 20% mild depression, 25.7% moderate depression, and 8.6% severe depression (Az-zahra et al.,2019). German study concluded large negative effects of age in 5-HT-2A receptors, moderate negative effects of age in 5-HT transporters and small negative effects of age in 5-HT-1A receptors (Karrer et al., 2019).

Ferritin have no significant correlation ($p > 0.05$) with age when compared between the two age groups of β-thalassemia patients less than 15 years , the mean is (3561.5±3.0) and more than 15 Years , the mean is (3170.5±3.3) , as presented in table (3). It was found that serum ferritin levels in β- thalassemia patients are higher than the same populace with the normal situation, and this increase has no correlation with age or gender. After analyzing the results, appears that there is a negative relation between the mean ferritin level of serum and BMI, which incomes that higher ferritin levels correlate with lower BMI (Yousefian et al., 2022). These results are not in accordance with the findings that show a positive correlation of ferritin with age increases. The evidence specifies that iron buildup in β-TM is age-related, representing that it happens even in β-TM patients not on transfusion routine (Saad et al., 2021). Serum ferritin levels can be affected by a number of factors, for example the age when primarily diagnosed, the age when firstly consuming blood transfusion and the age when for the first time treated with iron chelating agent (Jana et al., 2016).

References

Al-attar, M. S.; & Shekha, M. S. (2014). The Prevalence of Thalassemia in Erbil Province. University OfSalahaddin-Hawler Iraqi Kurdistan Journal, 2(1), 46–55.

Al-Husseini Abdel-Fattah Mohamed Attiwa, M., & Mohamed. (2019). The level of Psychological Depression among patients with Thalassemia in Cairo in some variables. Psychological Studies, 29(1), 89-128.

Angelucci, E., Bai, H., Centis, F., Bafti, M. S., Lucarelli, G., Ma, L., & Schrier, S. (2002). Enhanced macrophagic attack on beta-thalassemia major erythroid precursors. haematologica, 87(6), 578-583.

Az-zahra, W. F., Mardhiyah, A., & Nurhidayah, I. (2019). The Depression Categories of Adolescent with Beta-Thalassemia Major. Journal of Nursing Care, 2(1).

Badowska–Kozakiewicz, A. M. (2013). Biologiczna rola czynnika martwicy nowotworów α w fizjologii i patofizjologii. Prz Menopauzalny 2013; 2: 136, 141.

Brzustewicz, E., & Bryl, E. (2015). The role of cytokines in the pathogenesis of rheumatoid arthritis–Practical and potential application of cytokines as biomarkers and targets of personalized therapy. Cytokine, 76(2), 527-536.

Cullis, J. O., Fitzsimons, E. J., Griffiths, W. J., Tsochatzis, E., Thomas, D. W., & British Society for Haematology. (2018). Investigation and management of a raised serum ferritin. British journal of haematology, 181(3), 331-340.

Hassoon, I. J. (2021). Study of Some Innate Immune Parameters Associated with β-Thalassemia in Basrah Province, MSc-thesis submitted College of Science, University of Basrah.

Jana, A., Gupta, D., Bhattyacharyya, M., & Sarkar, B. N. (2016). Correlation between nutritional status and clinical parameters among thalasaemic patients–A study of West Bengal. Hum Biol Revi, 5(3), 349-360.

Jha, R., & Jha, S. (2014). Beta thalassemia-a review. Journal of pathology of Nepal, 4(8), 663-671.

Karrer, T. M., McLaughlin, C. L., Guaglianone, C. P., & Samanez-Larkin, G. R. (2019). Reduced serotonin receptors and transporters in normal aging adults: a meta-analysis of PET and SPECT imaging studies. Neurobiology of aging, 80, 1-10.

Khan, M. S., Ahmed, M., Khan, R. A., Mushtaq, N., & Shah, M. W. (2015). Consanguinity ratio in b-thalassemia major patients in District Bannu. J Pak Med Assoc, 65(11), 1161-3.

Laghari, Z. A., Baig, N. M., Charan, T. R., Lashari, K. H., & Suhag, R. (2018). Distribution of ABO blood groups and rhesus factor in ß-thalassemia patients at Thalassemia Care Center Nawabshah, Pakistan. Sindh University Research Journal-SURJ (Science Series), 50(01), 123-128.

Maes M, Meltzer H Y (1995). The serotonin hypothesis of major depression. In Bloom F E, Kupfer D J (eds), Psychopharmacology: The Fourth Generation of Progress. Raven Press, New York .

Marbut, S. M. M., Hamdi, M. A., Jumaa, A. M., & Salman, B. A. (2018). Distribution of ABO blood groups in beta thalassemia patients dependent on blood transfusion In Bagdad city. Journal of Madenat Alelem University College, 10(2), 1-11.

Messina G, Colombo E, Cassinerio E, Ferri F, Curti R, Altamra C,CappelliniMD(2008). Psychosocial aspects and psychiatric disorders in young adult with thalassemia major. Intern Emerg Med 3(4):339–343.

Mikelli A, Tsientis J (2004). Brief report: depressive symptoms and quality of life in adolescents with β-thalassemia. J Adolesc 27:213–216.

Milan-Mattos, J. C., Anibal, F. D. F., Perseguini, N. M., Minatel, V., Rehder-Santos, P., Castro, C. A., ... & Catai, A. M. (2019). Effects of natural aging and gender on pro-inflammatory markers. Brazilian Journal of Medical and Biological Research, 52.

Mohammad-Zadeh, L. F., Moses, L., & Gwaltney-Brant, S. M. (2008). Serotonin: a review. Journal of

veterinary pharmacology and therapeutics, 31(3), 187-199.
Nautiyal, K. M., Tritschler, L., Ahmari, S. E., David, D. J., Gardier, A. M., & Hen, R. (2016). A lack of serotonin 1B autoreceptors results in decreased anxiety and depression-related behaviors. Neuropsychopharmacology, 41(12), 2941-2950.
Neumeister, A., Young, T., & Stastny, J. (2004). Implications of genetic research on the role of the serotonin in depression: emphasis on the serotonin type 1 A receptor and the serotonin transporter. Psychopharmacology, 174, 512-524.
Saad, B. H., Abdul-AM, A. H. H., Hussein, A. M. B., & Mazin, J. M. (2021). The study of serum ferritin level as a predictor of growth retardation in thalassemia-major. Archivos Venezolanos de Farmacología y Terapéutica, 40(5), 492-501.
Shenoy, N., Vallumsetla, N., Rachmilewitz, E., Verma, A., & Ginzburg, Y. (2014). Impact of iron overload and potential benefit from iron chelation in low-risk myelodysplastic syndrome. Blood, The Journal of the American Society of Hematology, 124(6), 873-881.
Tanyong, D. I., Panichob, P., Kheansaard, W., & Fucharoen, S. (2015). Effect of tumor necrosis factor-alpha on erythropoietin-and erythropoietin receptor-induced erythroid progenitor cell proliferation in β-thalassemia/hemoglobin E patients. Turkish Journal of Hematology, 32(4), 304.
Yesim A, Serpil E, Nagihan B, Deniz Y, Ufuk S (2005) Psychosocial implications of thalassemia Major. Pediatr Int 47:84–89.
Yousefian, S., Mirialiabad, G., Saleh, R., & Khedmati, M. (2022). Association of Body mass index and serum ferritin level in pediatrics with Beta-thalassemia major disease. Iranian Journal of Pediatric Hematology and Oncology, 12(1), 34-40.