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Effect of sex class on the levels of some biochemical variables in thalassemia patients

Khalaf N. Mohammed, Mossa M. Marbut

Biology Department, College of Sciences, Tikrit University, Tikrit, Iraq https://doi.org/10.25130/tjps.v24i6.428

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Corresponding Author:

Name: Mossa M. Marbut

E-mail: mossa1955p@yahoo.com

Tel:

ABSTRACT

he study was conducted at Azadi Teaching Hospital - Thalassemia Center for the period from 1/10/2017 to 1/4/2018. It included 60 patients including 30 males and 30 females, as well as control group which included 20 Healthy people were all 10 to 21 years of age. The following variables were studied: ferritin, GSH, insulin-like growth factor-1 (IGF-1) Malondialdehyde (MDA) Interleukin-6 (IL-6), and Cortisol. The analysis of correlation coefficients and simple linear regression was carried out to determine the effect of the variables among them. The results were as follows: Thalassemia patients recorded the lowest levels of GSH, IGF-1 and cortisol at 2.2 μ mol / L and 50.4 ng / ml and 10.11 μ g / dL respectively, while the same group recorded the highest levels of ferritin, MDA and IL- 6 was 3083 ng / ml, 941 mmol / L and 368 pg / mol. There were significant effects of both sexes in the group of thalassemia patients in both ferritin, GSH, IGF-1, MDA, and IL-6 with 2573, 3592 μg / dL, 2.821, 1.573 μmol / L, 63.3, 37.47 ng / ml and 1163.1 719.3 mmol / L 300.9 and 435.6 pg / mol for males and females, respectively. The results of the correlation coefficient analysis showed existence of and low correlations in the negative and positive direction between the study variables of the two groups of patients and control. The results of the simple linear regression analysis showed positive and negative regression ratios of IL-6 and IGF-1 growth factor on the rest of the study variables.

Introduction

Thalassemia is a genetic disease transmitted from parents to children across genes and affects the production of hemoglobin in the human body leading to a severe anemia called thalassemia, the most prevalent disease in the world, especially in the Middle East and Southeast Asia, is often diagnosed Thalassemia in the first six months after birth may be fatal if the patient does not receive a proper treatment[1] where the infected children need three weeks to be able to survive and lead a normal life. This disease was first diagnosed by Dr. Kuli[2].

People with Thalassemia, especially the major ones, need long-term chronic treatment. The treatment is continuous and continuous blood transfusions to keep high levels of hemoglobin in order to reach oxygen throughout the body, as well as treatment with Desifral, a major turning point in patients Thalassemia This treatment leads to the elimination

of excess iron to remove excess iron as well as prevent its deposition in the body of the patient [3]. Malondialdehyde as biomarker for oxidative stress is only formed by fatty acids with three or more double bonds and is used as a measure of lipid per oxidation.

The reaction is initiated by an existing free radical or by metal ions[4].

Recent studies have shown that there is a relationship between thalassemia and natural growth factors in the human body, as people with thalassemia have a growth rate less than normal and the hormones that affect growth are insulin-like growth factor (IGF)[5] IGF- I is a polypeptide hormone consisting of 70 amino acid and a partial weight estimated 7649 Da and has an indirect impact on the growth of many cells and tissues of the body and a component of a large family very similar among them, which is very similar to the proportion of insulin so called the term "insulin-like" Mainly excreted from liver that

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controlling the nutritional status of the body excretion, and some other hormones such as growth hormone GH which is the most influential and hormone insulin and Althairoxin sexual steroids[6]. Thalassemia patients have an effect on immune factors. Examples of this are changes in the level of interleukins and IL-6 Interleukin-6 is cytokine a

factors. Examples of this are changes in the level of interleukins and IL-6 Interleukin-6 is cytokine, a typical phenotype that regulates a variety of physiological events in vertebrates, such as cell proliferation, differentiation, survival apoptosis[7]. Plays IL-6 roles in the immune endocrine, nervous system and blood system, and metabolism metabolism Several immune cell types are reported to produce IL-6 including T cells, B cells, polymorphointear cells, eosinophil, monocytes / macrophages, mast cells and dendritic cell cell types The other Known as IL-6 are cartilage cells cellderived cells endothelial cells skeletal muscle cells soft cells, particle cells, thyroid cells fibroblasts, meningocytes, keratinocytes, some tumor cells, adipose tissue cells, glial cells[8].

Cortisol is known as the main stress hormone produced in the body. These glucocorticoid plays a central role in maintaining blood pressure and energy levels and serves many other functions. Considering changes in hormone production and circulation levels not only provides a look at stress levels, but allows one to study the physiological changes that may result from this hormone. Cortisol is one of the hormones produced in the adrenal glands of the kidney via the HPA axis[9].

The present study aims at identifying the effect of the gender of thalassemia patients on the levels of iron overload, MDA, GSH, IGF-1, IL-6, and cortisol, as well as the Pearson correlation coefficient and the simple linear regression coefficient between these variables.

Materials and methods

The study was conducted at Azadi Teaching Hospital - Thalassemia Center for the period from 1/10/2017 to 1/4/2018. The study was conducted on 60 patients including 30 males and 30 females. All patients were diagnosed by a pediatrician. (20) male and female healthy group (control group) who were found to be uninfected after the laboratory tests by the specialist doctor and have no family history of genetic blood diseases and all ages between 10-21 years.

Sampling: Several considerations were taken during sample collection, eg Disposable syringes, as well as Heptin sterilization. Samples were collected between 8 am and 10 am. Blood was drawn from the vein using a 5 ml syringe, the blood was drawn in a plain tube for 30 minutes and then placed in the centrifuge for 10 minutes at 3000 cycles per minute to separate the serum. The samples were divided into five tubes to measure: ferrite and insulin-like growth hormone (IGF) 1), interleukin (IL-6), cortisol hormone and oxidative stress, and the serum was maintained by freezing at a staircase Temperature (-20) m o in the

main blood bank to maintain the effectiveness of the enzyme until the appropriate tests.

The exams

Serum Ferritin Determination: - The level of iron in the serum was assessed using the colorimetric method with ferene and using a kit equipped by the Italian company Giesse.

Ir $(\mu g / dl) = \text{sample} / \text{standard} \times 100$

MDA concentration in the serum: - was determined by the specific method by measuring the amount of MDA, which is one of the main products of lipid peroxidation. This method is performed through the interaction of lipid peroxidation, (TBA). This reaction is carried out in an acidic medium, the output is colored, and its absorbance strength is at 201 nm (154). The concentration is calculated by the following equation:

MDA (mmol / lit) = Absorbance \times 106 \times 103 \times Vs / (1056 \times 105) \times Vt

Vs = Volume of sample (0.1 ml), Vt = Total volume (1.5 ml)

Determination of serum glutathione (GSH): The concentration of glutathione in the serum was measured using the method used by the researchers (Burtis & Ashood) ⁽¹⁵³⁾.

Hormone Insulin Like growth: The IGF concentration was estimated by the kit equipped with the DRG kit, which operates on the ELAISA (450 nm).

IL-6 levels: The levels of IL-6 in the serum were quantified using the Sandwich ELISA test (60) with thalassemia and 20 healthy individuals and according to the instructions in the Sigma-Aldrich examination kit.

Cortisol concentration: Cortisol is used to estimate the hormone cortisol. Several of the prepared analyzes were used by the German company Bioactive Diagnostica. This method is based on Sorbent Enzyme-Linked Immune Assay Immunotherapy. This technique is the immunocompetitive assay of Immune Competitive by adding Cortisol Compared with the anti-cortisol monoclonal antibody, cortisol in the human sample competes with cortisol for comparison to sites associated with unrestricted cortisol and cortisol. Comparative enzyme is then washed by washing buffer. J is inversely proportional to the concentration of cortisol in the sample is added and then after that Stop Solution to drill the reaction is to stop the reaction is then after reading the length of the wavelength of 450 nm using Micro Plate Reader.

Statistical Analysis: - The statistical analysis was performed using Chi-Square. For numerical variables, it was described using the standard deviation of the mean. The comparison between totals was performed using a t-test between two groups or the ANOVA test during the comparison between more than two groups Use the correlation coefficient test to find the possible relationship between two variables. All tests were

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conducted in the current study at a significant level (0.05).

Result

The test results showed in table (1) Existence a significant difference between the two study groups for ferritin levels at a probability level of 0.0004. The highest rate was 3083 ng / ml and the control group was 133.9 ng / ml. The results of the T test for the two groups of study (patients and control) and the phenomenon in Table (1) showed a significant difference between them at a probability level of 0.0003, during which the group of patients the highest rate of 941 mmol / L, while the control group rate was 421 mmol / L. The results indicate a higher MDA level in patients with thalassemia compared to healthy subjects. The results showed that there was a significant difference between them and a probability level of 0.0003 according to the results of the test in the T test method, in which the control group surpassed the highest serum GSH concentration of 4.48 µg / dL, while the group of patients was 2.2 µg/

For IGF-1 the general rates of the study groups and the phenomenon in Table (4-10) also, it is noted that a significant difference between them was recorded according to test in T test method, during which the control group achieved the highest rate of 200.3 ng / ml. The results of the IL-6 showed a significant difference between the two study groups (patients with thalassemia and control) during which the highest adjusted group of patients reached 368 pg / mol. The results of the test T test showed that there was a significant difference between them, during which control group achieved the highest rate of Cortisol hormone, which reached 13.56 μg / dL.

Table (1) Rates of variables for the two study groups

Variables	Patient	control	P value
Groups			
Ferritin (ng/dL)	3083± 2038	133.9± 47.8	0.0004**
MDA (mmol/L)	941± 300	421± 150	0.0003**
GSH (μmol/L)	2.20 ± 1.33	4.48 ± 1.18	0.0003**
IGF-1 (ng/ml)	50.4 ± 22.9	200.3± 106	0.0003**
IL-6 (pg/mol)	368± 171	238± 114	0.0002**
Cortisol (µg/dL)	10.11 ± 4.55	13.56± 3.92	0.004**

** and * significant at the probability level of 1 and 5%, respectively.

The results of Table (2) showed significant differences between the averages according to the test in ANOVA method. The female class in the mean group of patients was the highest mean of the iron

level which reached 3592 ng / ml by a significant difference from the rest of the averages followed by males in the same group, while the female class within the control group of the lowest mean, which was 113.9 ng / ml without significant difference from the category of males for the same group, as these results show that women with thalassemia increases the levels of vertebrate more than in males.

Shows serum MDA levels of the two groups of patients and controls and their effects on sex. The highest mean of MDA was 1163.1 mmol / L for males in the group of patients and significantly higher than the other averages according to the ANOVA test results. Females within the lower control group, which reached 308.7 mmol / L. The results showed that males with thalassemia were at higher levels of MDA than in females.

Table 2 shows the effect of sex in the levels of GSH in the groups of patients with thalassemia and control. The results show significant differences between the averages according to ANOVA, 5.383 μmol / L, while the female group in the lowest group of patients was 1.573 μmol / L. It was found that females were more affected than males.

The results showed a significant effect of IGF-1 in the serum group of patients with thalassemia and control. The male group in the mean control group reached 206 ng / ml by a significant difference from the rest of the averages according to the ANOVA test results, followed by the female group within the same group, while the female group was among the lowest group of patients, which reached 37.47 ng / ml.

For levels of IL-6 and affected by the sex factor in the groups of patients and control, Table 2 showed significant differences between their mean according to the results of the test in ANOVA, In the mean group of patients with a mean average of 435.6 pg / mol by a significant difference from the rest of the averages, while the lowest averages 190.7 pg / mol of the male category within the control group.

For cortisol hormone levels, the results of the ANOVA test shown in Table 2 showed no significant effect of the sex factor in the levels of this hormone, although it was found in the females of the study groups at levels higher than in males. The highest mean was 13.87 μg / dL in the control group without significant difference for the male group within the same group, while the mean mean was 9.64 μg / dL for the male group in the group of patients without significant difference from the female class within the same group.

Groups	Patient		control	
Gender	Male	Female	Male	Female
Variables				
Ferritin (ng/dL)	b2573± 1760	a3592± 2195	$c153.9 \pm 48.0$	c113.9± 40.5
MDA (mmol/L)	a1163.1± 185	b719.3± 216.6	c532.5± 104.1	d308.7± 93.6
GSH (μmol/L)	c2.821± 1.560	d1.573± 0.625	$a5.383 \pm 0.675$	b3.579± 0.81
IGF-1 (ng/ml)	c63.30± 11.45	d37.47± 24.36	a206± 67.5	b194.6± 63.6
IL-6 (pg/mol)	b300.9± 109.8	a435.6± 194.4	d190.7± 66.1	c285.1± 134.5
Cortisol (µg/dL)	b9.64± 4.97	b10.59± 4.175	a13.26± 3.63	a13.87± 4.37

The results of correlation analysis between the studied variables of the group of patients shown in Table 3 showed a low correlation between the levels of dichloride and positive levels of GSH and IGF-1 with correlation coefficients of 0.349 and 0.353 respectively, Interlockin-6 was a correlation coefficient between -0.305. The rest of the correlation between the variables of the study did not reach the limits of the statistical significance and ranged between negative and positive.

Table (3): Analysis of correlation coefficient between the study variables for the group of thalassemia nations

study variables for the group of thandssemma					patients
Variable	Fe	GSH	IGF	MDA	IL-6
GSH	0.211				
IGF	-0.273	0.215			
MDA	-0.057	0.349*	0.353*		
IL-6	0.068	0.060	-0.188	-0.305*	
Cortizol	0.190	-0.070	0.047	-0.004	-0.066

** and * significant at the probability level of 1 and 5%, respectively.

The results of correlation coefficient analysis between the study variables of the control group (control) are shown in Table 4. There is a negative correlation between the levels of the clotathione at the 1% probability level with the levels of the insulin-like growth factor -0.657 and positive at the 5% With dihydide monomers at 0.497. A significant correlation was also shown in the negative trend of the levels of the insulin-like growth factor with the levels of MDA with a correlation coefficient of -0.754. The correlation between the variables of the study did not reach the threshold of statistical significance and ranged between negative and positive. The results showed that there was no significant correlation between IGF-1 and the variables studied.

Table (4) Analysis of the correlation coefficient between the study variables of the control group

the study variables of the control group					
Variable	Fe	GSH	IGF	MDA	IL-6
GSH	0.297				
IGF	-0.405	-0.657**			
MDA	0.210	0.497*	-0.754**		
IL-6	-0.209	-0.422	0.405	-0.236	
Cortizol	-0.11	-0.169	-0.052	0.217	0.405

** and * significant at the probability level of 1 and 5%, respectively.

IL-6 regression: - The results of a simple linear regression analysis of the interleukin-6 (IL-6) on the other studied variables in the thalassemia group in figures 1-5 shows that the regression relationships

were positive with both Fe and GSH. Indicates that an increase in the level of IL-6 by 1 pg / mol leads to an increase of 0.403 ng / ml and 0.5111 mol / L for Fe and GSH respectively, while the regression ratios of IL-6 were negative with all other variables, the increase in the level of IL-6 by 1 pg / mol results in a decrease of 0.02294 ng / mol, 0.5585 mmol / L and 0.001832 μg / dL in insulin-like growth hormone levels, Dehydrate and cortisol respectively.

The results of the model match, determined by the value of the R-sq parameter, show that the linear model was suitable for interpreting IL-6 regression results with both the Fe and Cortisol variants. R-sq of these relationships was less than the correct one, while the linear model was inappropriate with both GSH and IGF and MDA because the values of the coefficient of selection were greater than the correct one. This indicates the need to use quadratic or cubical regression equations for the purpose of obtaining highly acceptable results according to statistical indicators, the values were bananas This is consistent with the results of the analysis of variance and the test of averages of some high values of standard deviation due to some variation in the study samples.

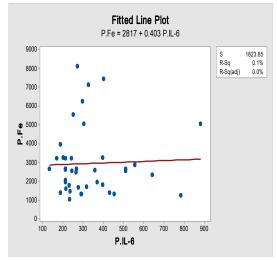


Fig. (1) regression of the IL-6 on Fe.

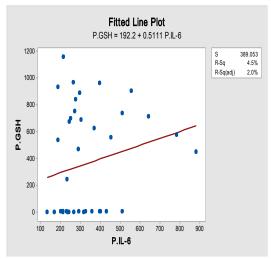


Fig. (2) regression of IL-6 on GSH

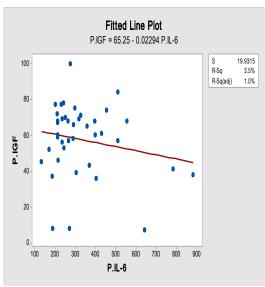


Fig. (3) regression of IL-6 on IGF.

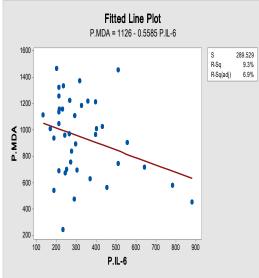


Fig. (4) regression of IL-6 on MDA.

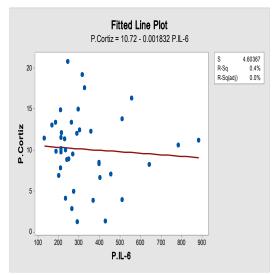


Fig. (5) regression of IL-6 on cortisol.

IGF regression: - The results of the simple linear regression analysis of the insulin-like growth factor (IGF-1) on the rest of the study variables are shown in Figures 6-9, showing that the regression relationships were positive with both MDA and cortisol, The level of insulin-like growth factor (IGF-1) of 1 ng / mol increased to 5.283 mmol / L and 0.01060 μg / dL for MDA and cortisol respectively, while the regression ratios of IGF-1 were negative with other variables, The increase in IGF-1 levels of 1 ng / mol results in a reduction of 25.19 ng / ml and 5.366 mol / L in levels of ferrite and glutathione on Respectively.

The results of the model match, which is determined by the value of the R-sq, indicate that the linear model was appropriate for interpreting IGF-1 regression results with cortisol. R-sq for this relationship was less than the correct one, while the linear model was inappropriate with Both Ferritin (Fe) and GSH and MALDA because the values of the coefficient of selection were greater than the correct one. This indicates the need to use slope equations of the quadratic or cubic order for the purpose of obtaining highly acceptable results according to the statistical indicators. It is also noted that the values were distributed Homogeneous in a The regression forms have been overcome with some extreme readings. This is consistent with the results of the analysis of variance due to the existence of some high values of standard deviation due to some variation in the study samples.

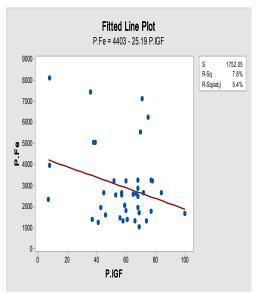


Fig. (6) regression of IGF-1 on Fe.

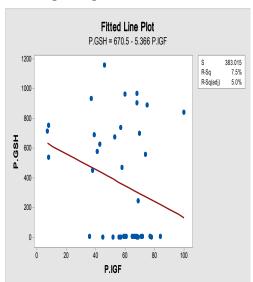


Fig. (7) regression of IGF-1 on GSH.

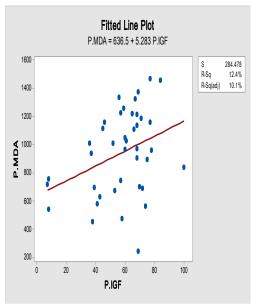


Fig. (8) regression of IGF-1 on MDA.

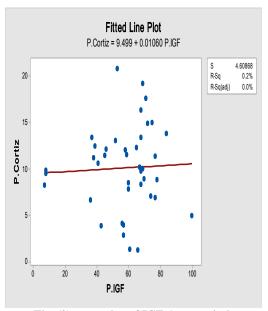


Fig. (9) regression of IGF-1 on cortisol.

Discussion

The increase in iron levels in patients with thalassemia attributed to the large number of blood transfusions to them, which leads to raise serum level and have harmful effects on tissues, glands, liver and some other members by activating free radicals, which in turn caused Oxidative stress[10]. Oxidative stress is a component of inflammatory mechanisms that contribute to anemia. The degree of oxidation is closely related to the state of inflammation[9] These mechanisms include depletion of the reduction capacity with deformation of the structure of the erythrocyte membrane, thus shortening the life span [11]. Thus, hepcidin production, which inhibits intestinal absorption of iron and mobilization of iron stores by binding with ferroprotin on the cell membrane[12], will be increased. Iron accumulation different tissues leads to several problems including hypothyroidism, and thyroid glands[13,15] indicates that the average for ferritin was 3196 and 3212 ng / ml for male and female patients with thalassemia respectively, 122.19 and 121 ng / ml. (13) had higher levels of ferritin in males than females with thalassemia, with averages of 549.02 and 472.95 ng / ml, respectively. The results are consistent with the results of the study of (13) who received a significant difference of levels of the two ferrites at rates of 510.98 and 177.97 ng / ml for both thalassemia patients control and groups respectively[16] also reported that the ferritin levels of the thalassemia and control groups were 3558.43 and 59.87 ng / ml, respectively.

Malondialdehyde (MDA): Increased levels of MDA indicate an increase in hyperoxicity, which causes increased oxidative stress in Thalassemia patients. Excess iron in Thalassemia patients also stimulates fat oxidation processes. The internal blood cells of thalassemia generally respond to oxidative stress through its interaction with hydrogen peroxide to

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form a hydroxyl root that breaks down small cellular molecules [24, 25]. got on similar results, with mean 4.87 and 3.79 μmol / L for males and females with thalassemia, respectively. Also obtained (25) on MDA levels of 4.34 and 1.88 μ mol / L for thalassemia and control respectively with a high moral difference between the two. The MDA rates for both thalassemia and control groups were 2.807 and 1.009 nmol / ml, respectively[26]. This was acceptable to [32] with levels of 1.211 and 1.615 μmol / L in male patients with thalassemia and control respectively, 0.84 and 1.846 μmol / L in female thalassemia patients and control Respectively.

These results for GSH indicate a significant consumption of antioxidants, which is parallel to high levels of ferrite, which is one of the main reasons for increasing oxidative stress. Glutathione prevents damage to cellular components. From the results of the previous studies,[18] got on GSH rates of 12.43 and 3.36 U / L for control groups and thalassemia patients, respectively. [17] reported a negative effect on thalassemia at levels of clotathione, with rates of 804.4 and 285.2 μmol / L for control groups and thalassemia patients respectively with a high significant difference. There was also a significant difference between the groups of patients with thalassemia and control who had 5.3 and 9.6 μM for both groups respectively[19].

The developmental delay in thalassemia patients has been shown to be multivariate[20] and that despite normal blood transfusions and deferzoxamine treatment, these patients have developmental delays and bone abnormalities[21]. In general, thalassemia patients suffer from developmental delays due to contributing to the lack of puberty mutation and lack of IGF-1 synthesis, which may be secondary to IGF-1 disorder and / or undernourishment, possibly due to hyperglycemia[22]. Previous studies in this subject indicated[23] that IGF-1 values were 61.33 and 126.93 ng / ml for both thalassemia and control groups respectively. ¹⁴ had IGF-1 values of 138.2 and 345.5 ng / ml for both groups of patients and control respectively.

The increase in IL-6 indicates that weak neutrophil functions and phagocytic macrophage and killing functions, produce some cytokines. IL-6 is known to be an important component of the pro-inflammatory response and its high serum level may be related to pathophysiology of thalassemia. Elevated levels of IL-6 may be due to excessive stimulation of plaques, which may cause deformities in iron metabolism[28].

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The results of many researchers indicate that patients with thalassemia have high levels of IL-6 compared to healthy people, he got [27] rates of 40.5 and 51.5 pg / ml for patients with thalassemia and control group respectively and a significant difference between them.

Thalassemia hormones are linked to receptors within the pituitary gland to produce more hormones, which work specifically on target organs, for example, the adrenal gland to produce cortisol, or gonads to produce sex hormones [29]. These results were consistent with the findings of ¹³ patients with no significant sex effect on cortisol levels in thalassemia patients, with rates of 5.38 and 4.77 µg / dL for males and females with thalassemia, respectively. These results are consistent with most previous studies on the effect of thalassemia on cortisol levels and lowering them to relatively low levels. (13) showed a significant difference between cortisol levels in the thalassemia groups and controls with mean values of 4.58 and 8.73 µg / dL Respectively. The increase in iron levels is one of the main reasons for the decline of cortisol hormone, as the results of the study[30] anatomy that the deposition of iron in the adrenal glands occurred greatly in the zona fasciculata produced Cortisol. [31] showed significant differences between the groups of thalassemia patients and control of cortisol level at 10.9 and 12.7 μg / dL, respectively.

Of the previous studies about correlation, [14] got on significant associations of BMI with ferritin, IGF-1, and IGF-1 with ferritin. (13) also indicated that the association of cortisol with ferritin within the group of patients did not reach the limits of statistical significance. The results showed that there was no significant correlation between IGF-1 and the variables studied, including Fe in the group of thalassemia patients.

Thalassemia was found to have a negative effect on the studied biomarkers and significant differences in the control group. Significant effects were found in the thalassemia group in both ferritin, GSH, IGF-1, MDA and IL-6. It is recommended to follow the levels of ferritin in patients with thalassemia and to find effective treatment methods to prevent its accumulation, to raise awareness of the seriousness of the disease and to warn of its genetic causes, including the marriage of kindred, the need to conduct periodic examinations and follow the levels of various vital indicators in patients with thalassemia.

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تأثير فئة الجنس على مستويات بعض المتغيرات الكيموحيوية في مرضى الثلاسيميا

خلف نایف محمد ، موسی محمود مربط

قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

بهدف معرفة تأثير فئة الجنس لمرضى الثلاسيميا على بعض المتغيرات الحيوية أجريت الدراسة في مستشفى آزادي التعليمي – مركز الثلاسيميا للفترة من 2017/10/1 ولغاية 2018/4/1، وشملت 60 مريضا منهم 30 ذكور و 30 اناث، فضلاً عن مجموعة السيطرة التي تضمنت 20 شخصا أصحاء وكانت جميع الاعمار ما بين 10 –21 سنة، وتمت دراسة المتغيرات التالية: الفيريتين GSH والكلوتاثيون GSH وعامل النمو الشبيه بالأنسولين IGF ومالون ثنائي الألديهايد MDA والانترلوكين (1-16) Interleukin والكورتيزول Cortizol. كما اجري تحليل معاملي الارتباط والانحدار الخطي البسيط لمعرفة تأثير المتغيرات فيما بينها. وكانت النتائج كما يلي: سجلت مجموعة مرضى الثلاسيميا أدنى المعدلات لمستويات المعروتيزول حيث بلغت 12.2 μg/dL والانحدار الخطي التوالي، بينما سجلت المجموعة ذاتها اعلى المعدلات لمستويات الفيريتين و MDA و السال و 10-10 سو/m و 10-13 و 10-14 و 10-14