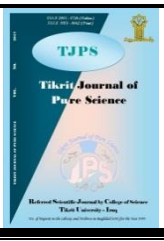
Tikrit Journal of Pure Science (2023) 28 (6): 1-10
Doi:

TJPS

IASJ
المجلات الاكاديمية العراقية

Tikrit Journal of Pure Science
ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)

Journal Homepage: http://tjps.tu.edu.iq/index.php/j



Evaluation of the level of 5'-nucleotidase and some biochemical parameters in the sera of beta-thalassemia patients

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Keywords: 5'-Nucleotidase, Oxidative stress, Glutathione, Ferritin, Beta-thalassemia.

ARTICLEINFO.

Article history:

-Received: 17 Oct. 2023

-Received in revised form: 06 Dec. 2023

-Accepted: 07 Dec. 2023

-Final Proofreading: 24 Dec. 2023

-Available online: 25 Dec. 2023

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ABSTRACT
One of the hydrolytic enzymes that catalyzes the hydrolysis of 5' -nucleotides into ribonucleosides and phosphate is the 5'nucleotidase, which is found in several organs. Oxidative stress shows depletion of antioxidants, such as glutathione (GSH), and the increased concentration of product of lipid peroxidation, such as MDA. The study comprised a total of 54 subjects including patients with beta thalassemia (n=27) and healthy volunteers (n=27) matched by age and gender. Hematology (Hb and % PCV), 5'-nucleotidase, Ferritin, GSH and MDA were measured using different methods and devices. The 5'nucleotidase, Ferritin, and MDA levels were significantly increased (p<0.001) in beta-thalassemia patients compared to the controls. As opposed to that, the Hb, % PCV and GSH levels were significantly decreased (p<0.001) in patients with beta-thalassemia. There was a negative significant correlation (r = -0.146, -0.245, p<0.05) between Hb and 5'- NT enzyme and PCV% with 5'- NT enzyme, respectively. While Hb had a high significant correlation (r = 0.956, p<0.05) with PCV%. In addition, 5'- NT enzyme had a positive significant correlation with GSH, MDA and Ferritin (r = 0.584, 0.442, 0.735, p<0.05), respectively. The present findings indicated an increase in the level of effectiveness of the 5'nucleotidase enzyme, ferritin and GSH in β thalassemia patients while decreasing in MDA.

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تقييم مستوى 5' - نوكليوتيداز وبعض المتغيرات البيوكيميائية في امصال مرضى بيتا ثلاسيميا

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قسم تقنيات المختبرات الطبية- معهد تقني بعقوبة- الجامعة التقنية الوسطى- بغداد- العراق

الملخص

أحد الإنزيمات التي تحفز التحلل المائي لـ 5 'نيوكليوتيدات في الريبونوكليوسيدات والفوسفات هو 5' نوكليوتيديز، الموجود في عدة أعضاء. يظهر الإجهاد التأكسدي استنفاد مضادات الأكسدة مثل الجلوتاثيون وزيادة تركيز ناتج بيروكسدة الدهون مثل المالونداياليديهايد. تضم الدراسة مجموعة من 54 شخصًا بينهم مرضى بيتا ثلاسيميا (27) ومتطوعون أصحاء (27) بأعمار وأجناس مختلفة. أجريت قياسات بطرق وأجهزة مختلفة لمجاميع الدراسة والتي تشمل الهيموغلوبين، حجم الخلايا المضغوطة و الفيريتين أضافة الى انزيم الـ 5 'نيوكليوتيديز و الكلوتاثايون والمالونداياليديهايد. زادت مستويات انزيم الـ 5 'نيوكليوتيديز والفيرتين والمالونداياليديهايد بشكل كبير عند مستوى احتمالية ($p < 0.001$) في مرضى بيتا ثلاسيميا مقارنة بالأصحاء. في حين انخفضت مستويات الهيموغلوبين وحجم الخلايا المضغوطة والكلوتاثايون بشكل كبير عند مستوى احتمالية ($p < 0.001$) في مرضى بيتا ثلاسيميا. لوحظ وجود ارتباط سلبي ($r = -0.146, -0.245.\ p<0.05$) لكل من الهيموغلوبين و حجم الخلايا المضغوطة مع انزيم الـ 5 'نيوكليوتيديز, في حين إن الهيموغلوبين له ارتباط ايجابي مع حجم الخلايا المضغوطة. تشير النتائج التي توصلنا إليها إلى زيادة في مستوى فعالية إنزيم الـ 5 'نيوكليوتيديز في مرضى الثلاسيميا نوع بيتا مع زيادة كل من الفيريتين والمالونداياليديهايد.

الكلمات المفتاحية: إنزيم الـ 5 'نيوكليوتيديز، الاجهاد التأكسدي، الكلوتاثايون، الفيريتين، بيتا ثلاسيميا.

Introduction

As one of the most prevalent inherited hematologic illnesses, thalassemia is characterized by significant disruptions in the synthesis of β chain hemoglobin (Nickavar et al., 2017). Increased alpha/beta ratio and decreased or suppressed production of -globin chains are characteristics of beta-thalassemia (Carsote et al., 2022). The Mediterranean, Africa, and Southeast Asia regions all have high rates of thalassemia syndromes (Weatherall, 2010). The majority of hereditary monogenic diseases are beta-thalassemia syndromes. These are heterogeneous diseases brought on by decreased or missing beta-globin synthesis, which results in an imbalance of the globin chains. Beta-globin is a key component of adult hemoglobin A (HbA, α2β2) (Demosthenous et al., 2019).

Pyrimidine 5' nucleotidase (P5' N-1, also known as uridine-5'-monophosphate hydrolase-1) catalyzes the dephosphorylation of the pyrimidine 5' monophosphates, uridine triphosphate UMP and cytidine monophosphate CMP to the corresponding nucleosides. It belongs to hydrolase class of enzymes (EC 3.1.3.5) (David et al., 1989; Al-Taii, 2015). It is generally divided into two types of symmetry, the cytoplasmic analog (dissolved in cytoplasmic) and the symmetric associated with the wall membrane bound isoenzyme. This classification is based on the presence of the enzyme within the cell. The role of 5'-nucleotides cytoplasmic enzyme is associated with intracellular Adenosine triphosphate (ATP) degradation by Adenosine monophosphate (AMP) to adenosine or by Inosine monophosphate to Inosine. The enzyme of pyrimidine 5'-nucleotidase is among the widespread enzymes in animals, plants, microscopic life, as well as some types of primates and parasites. It is present in many tissues of the human body. The 5'-nucleotidase can be used alone or in conjunction with other enzymes to detect hepatic metastases. It has been demonstrated that 5' NT has more diagnostic utility than other liver enzymes, particularly in liver metastases (Al-Mudhaffar & Al-Salihi, 1980). About 92% of patients with obstructive jaundice, 70% of patients with parenchymal liver disease, and 81% of patients with hepatic metastases have elevated levels of 5'nucleotidase activity. Moreover, it has been observed that serum 5' NT is clinically effective for differentiating between hepatobiliary and osseous disorders, with hepatobiliary diseases showing the sole increase in enzyme activity (Rathnakumar, 2000). The aim of the present study is to evaluate the serum activity of 5'NT and some biochemical parameters levels in β- thalassemia patients.

MATERIALS AND METHODS

Blood samples were collected at the Thalassemia Diseases Major Department Center in the province of Diyala. The subjects were classified into two groups:

1. **Healthy Group:** it consisted of 27 apparently healthy individuals, males and females whose age ranged between 23-35 years.
2. **Patients Group:** it consisted of 27 thalassemia blood transfusion dependent patients, males and females whose age ranged between 13-32 years.

Samples were collected between (8 am) and (10 am). Blood was drawn from the vein using a 5 ml syringe. Then, 2 mL of the blood was transferred to a tube containing EDTA, and the remaining blood was gently agitated with a blood shaker to prevent clotting. After that, the remaining blood had been allowed to clot at room temperature for 10-15 minutes and centrifuged at 4000 rpm to extract the serum. The serum was then transferred into a fresh tube and frozen at (-20 °C). Then, the remaining blood had been allowed to clot at room temperature for 10-15 minutes.

Hematology (Hb and % PCV) measurements were applied by using Horiba Medical technology, which provides a large range of hematology analyzers and automated instruments to realize blood analysis (blood cells identification and counting) (Woolley, Timothy MSc, CSci, FIBMS; Davies, Bethan Jade MSc; Rutter, Emma MSc; Probert, Charlotte; Fitzgerald, Leanne MSc; Relf, 2017). This would provide a significant improvement in differentiating blood cells. The 5'NT activity was measured in serum according to Fiske and Subbarow's method (Lowry & Lopez, 1946). The principle of this method is based on the estimation of the number of inorganic phosphate micromole resulting from the reduction of 5'-AMP in the interaction medium using Fiske and Subbarow's detector (Goldberg, 1973; Pratibha et al., 2004). The Cobas device and Elecsys technology were used to measure ferritin. The malondialdehyde was determined spectrophotometrically according to the modified method of Satoh (Knight et al., 1988). It is based on the principle of auto-oxidation of unsaturated fatty acids, involving the formation of semi stable peroxides, which then undergo a couple of reactions to form malondialdehyde (MDA) (Kadir et al., 2018). GSH was determined by using the modified method of Sedlak and Lindsay (1968) by reducing the thiol group of GSH to form a colored product.

Statistical Analysis

Using descriptive statistics and an independent t-test, every statistical analysis was carried out. Statistics were deemed significant at $P < 0.05$. The software SPSS version 26.0 (SPSS Inc., Chicago, Illinois, U.S.A.) was used for all statistical analyses.

Results and Discussion

A total of 27 patients (males and females whose age ranged between (13-32) years) were enrolled in this study. Table (1) shows the difference at the indicative level of 0.05 between the average healthy hemoglobin (13.95 ± 1.51) and Patient's hemoglobin (7.59 ± 1.22), as well as PCV % in healthy (43.59 ± 4.40) and (24.43 ± 3.96) in patients at an indicative level of ($P< 0.001$). Also, from the same table, the level of ferritin serum in patients (3523.21 ± 507.31) with β -thalassemia was higher in healthy people (82.63 ± 6.77).

Table 1. Means of Hb, PCV% and Serum Ferritin for the Healthy group and Patients of β -Thalassemia

Parameters	Healthy (N=27)	Patients (N=27)	*P value*
Hb g/dl	13.95 ± 1.51	7.59 ± 1.22	0.001
PCV %	43.59 ± 4.40	24.43 ± 3.96	0.001
Serum Ferritin (ng/ml)	82.63 ± 6.77	3523.21 ± 507.31	0.001

The low levels of antioxidants can give rise to high oxidation products. In table (2), there was a decrease in the value of (GSH) for patients with β-thalassemia (1.56 ± 0.43) µmole /ml compared to the healthy group (7.44 ± 0.22) µmole /ml. While the value of (MDA) increased in the patients group (1.51 ± 0.08) compared to the healthy group (0.85 ± 0.13). However, the effectiveness of 5'- NT was high in the patients group (10.60±3.76) compared to the healthy group (0.439 ± 0.063).

Table 2: Means of GSH, MDA and 5'- NT for the Healthy group and Patients of β -Thalassemia

Parameters	Healthy (N=27)	Patients (N=27)	*P value*
GSH * 10^{-5} (µmole /ml)	7.44 ± 0.22	1.56 ± 0.43**	0.001
MDA (µmole/L)	0.85 ± 0.13	1.51 ± 0.08**	0.001
5'- NT (U/L)	0.439 ± 0.063	10.60 ± 3.76**	0.001

Table 3: Pearson's correlation coefficient among the Hb level, PCV% and Serum 5'- NT enzyme in patients with thalassemia

Parameters		Patients group		
		Hb (g\dL)	PCV%	Serum 5'- NT
Hb (g\dL)	r	1	0.956**	-0.146
	p- value		0.000	0.469
PCV%	r	0.956**	1	-0.245
	p- value	0.000		0.218
Serum 5'- NT	r	-0.146	-0.245	1
	p- value	0.469	0.218	

** Correlation is highly significant at the 0.05 level.

Table (3) and Figure (1) show the correlations among the Hb level, PCV% and Serum 5'- NT enzyme in the patients group. There was a significant negative correlation ($r = -0.146$, $p<0.05$) between Hb and 5'- NT enzyme. So, there was a significant negative correlation ($r = -0.245$, $p<0.05$) between PCV% and 5'- NT enzyme. While Hb has a high significant positive correlation ($r = 0.956$, $p<0.05$) with PCV%.

Table 4: Pearson's correlation coefficient among GSH, MDA and Ferritin with Serum 5'- NT enzyme in patients with thalassemia

Parameters		Patients group		
		GSH (µmole /ml)	MDA (µmole/L)	Serum Ferritin (ng/ml)
Serum 5'- NT	r	0.110	0.158	0.068
	p- value	0.584	0.442	0.735

** Correlation is highly significant at the 0.05 level.

Table (4) and Figure (2) show the binding coefficient for Serum 5'- NT enzyme with GSH, MDA and ferritin. The results showed a positive correlation (r = 0.110, 0.158, 0.068. $p<0.05$) between this enzyme and these parameters (GSH, MDA and ferritin). However, their relationship was not strong because there was no significant difference between them, as the p-values were (0.584, 0.442, 0.735), which are greater than 0.05, for the GSH, MDA and ferritin, respectively.

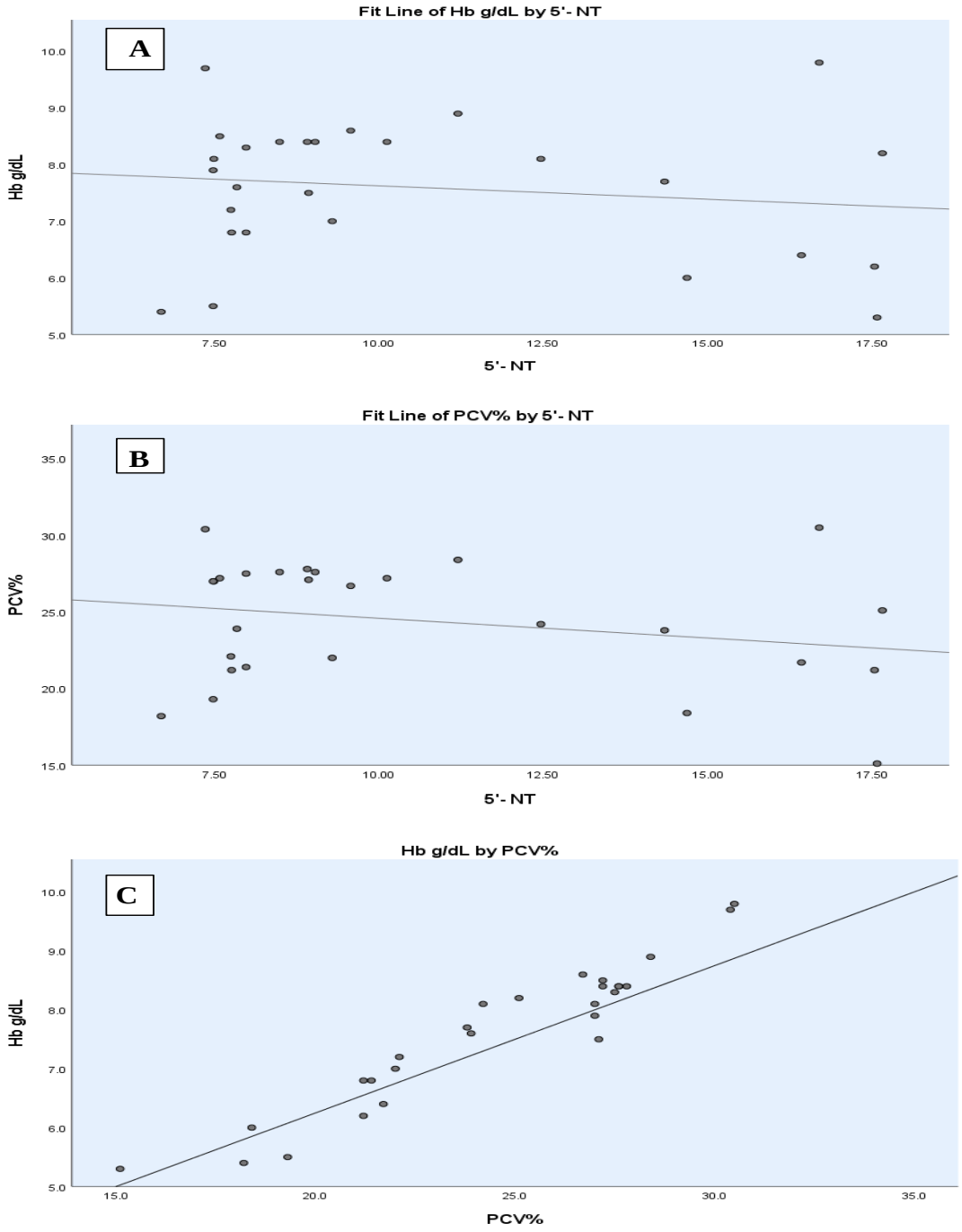
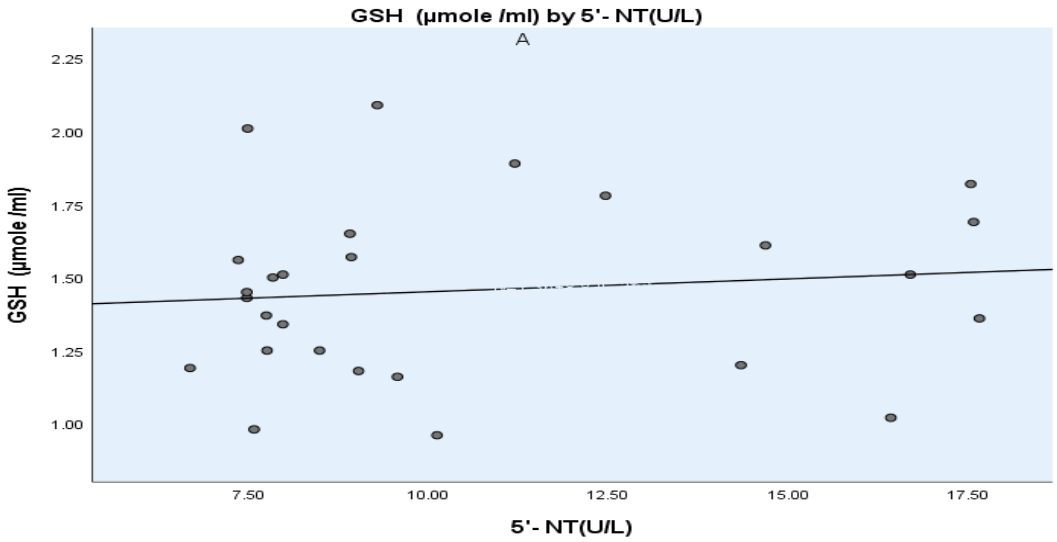
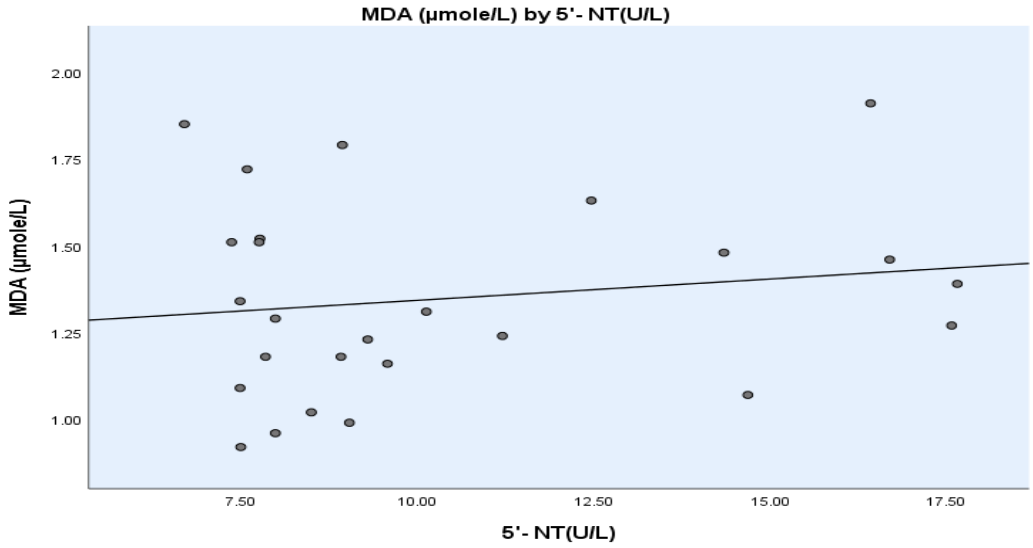


Figure 1: The correlation of 5'- NT with (A) Hb (B) PCV% and (C) Hb with PCV%


GSH (µmole /ml) by 5'- NT(U/L)
A
GSH (µmole /ml)
2.25
2.00
1.75
1.50
1.25
1.00
7.50
10.00
12.50
15.00
17.50
5'- NT(U/L)


MDA (µmole/L) by 5'- NT(U/L)
MDA (µmole/L)
2.00
1.75
1.50
1.25
1.00
7.50
10.00
12.50
15.00
17.50
5'- NT(U/L)

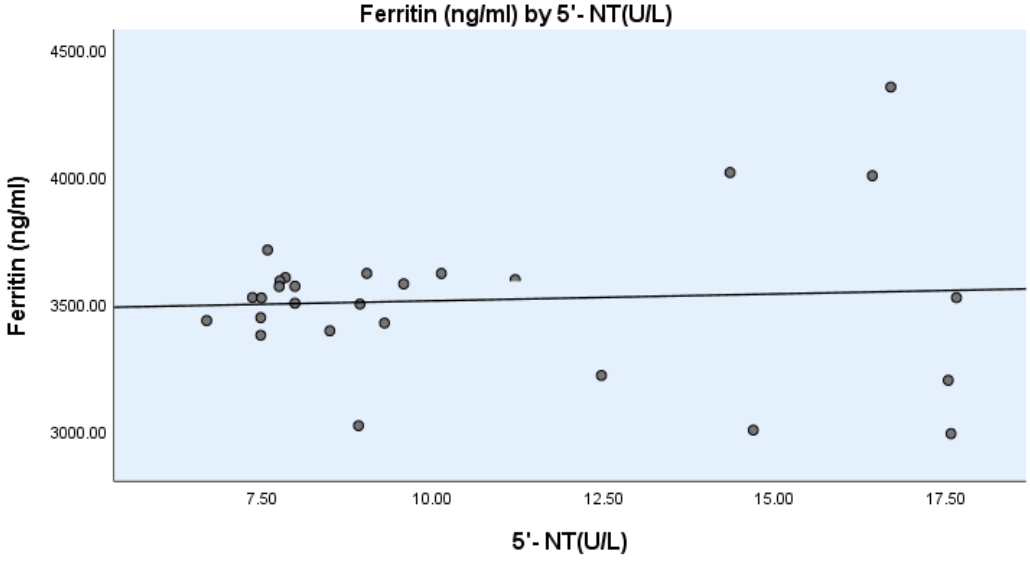

Ferritin (ng/ml) by 5'- NT(U/L)
Ferritin (ng/ml)
4500.00
4000.00
3500.00
3000.00
7.50
10.00
12.50
15.00
17.50
5'- NT(U/L)

Figure 2: The correlation of 5'- NT with (A) GSH (B) MDA and (C) Ferritin

Because parents received little information about the disease, blood transfusions were only utilized when patients displayed clinical symptoms brought on by severe anemia or simply to maintain life. This explains the reason behind the low hemoglobin levels in these patients. These results are consistent with those found by (Adaay et al., 2011; Yang et al., n.d.). The low concentration of Hb in β- thalassemia patients is caused by a genetic mutation in the DNA of hemoglobin-forming cells, genetically transmitted from parents to children. This genetic mutation disrupts normal hemoglobin production (Hemoglobin A). Therefore, the low levels of hemoglobin and the high rate of red blood cell damage lead to symptoms of anemia (thalassemia) (Huang et al., 2019).

The protein known as ferritin, which stores iron, deposits it in a safe form and then transports it to where it is needed, does so in a non-toxic manner. Patients with excessive iron have a noticeably elevated ferritin level (3523.21 ng/ml) in comparison with healthy group (82.63 ng/ml). This result is in agreement with that found by (Miess et al., 2018). This may help distinguish them from iron deficiency patients since both conditions result in low red blood cell counts (Dehghani et al., 2021; Şen et al., 2015). High iron stored is produced when damage occurs in one of the body's organs, such as the liver and spleen.

In addition, there was an increase in the levels of lipid peroxides (MDA) (1.51 µmole/L) and a decrease in the levels of GSH (1.56 µmole /ml) in the thalassemia patient group compared with the healthy group whose levels were as (0.85 µmole/L) and (7.44 µmole /ml) for MDA and GSH, respectively. The reduced oxygen-derived free radical (ODFR) scavenging capacity in cellular and extracellular fluids makes tissues more susceptible to the damage of ODFR and is a sign of elevated oxidative stress in patients (Mohammed & Marbut, 2019). Antioxidants, such as GSH, become depleted as a result of increasing oxidative stress, decreased synthesis from the sick liver, and antioxidant intake. Because it represents the most prevalent natural antioxidant in human bodies, glutathione is chosen for this investigation. Glutathione plays a critical role in the reaction catalyzed by the selenium-containing enzyme GSH peroxidase, which reduces hydrogen peroxide and organic peroxides (such as lipid peroxides). These results are consistent with the findings of several studies (Knight et al., 1988; Miess et al., 2018; Nasar et al., 2018).

In table 2, the effectiveness of enzyme 5'-NT had increased in the group of patients with beta thalassemia (10.60U/L) compared to the group of healthy individuals (0.439 U/L). This result is in agreement with that found in the studies of (Ahmed, 2015; Bogusławska et al., 2022; Mehsen et al., 2019; Nasar et al., 2018; Vyas et al., 2019), which reported an increase in this enzyme in different liver diseases, alcoholic and drug induced cirrhotic patients, hemolytic anemia, Hepatobiliary Diseases, breast Tumors and in smokers. The results suggested that these alterations in the activity of enzymes from the purinergic system are associated with an increase in platelet aggregation. The 5'-NT enzyme family play a crucial role in the maintenance of thermogenesis and vascular hemostasis. According to the regulation of platelet aggregation, increasing ROS production may be the cause of the increased activity of this enzyme (Srihirun et al., 2015). This result found in the current study contradicts that found by (David et al., 1990). This may be due to the presence of α-thalassemia cases among patients. Also, the present results are inconsistent with earlier data found in literature (Warang et al., 2012). This is because they used the enzyme Pyrimidine 50 nucleotidase of type I (P50N-1) which lacks the most common defect in cell nucleotide metabolism that leads to hereditary non spherocytic hemolytic anemia (HNSHA). The present study also found significant correlations between 5'- NT enzyme and both of Hb and PCV%, as well as between Hb

and PCV% (Fig. 1). In contrast, there were no significant correlations between 5'-NT and both of Hb and PCV%. While there was a positive and high correlation between hemoglobin and PCV% for patients with thalassemia. This result is evident in Table 3, which shows a positive (r) value and approaches number one. It is a strong package relationship and the significant value is less than 0.05. (Fig. 1), as well as in table 4 and (Fig. 2). In addition, 5'- NT enzyme had a positive significant correlation with GSH, MDA and Ferritin ($r = 0.584, 0.442, 0.735, p<0.05$), respectively.

Conclusions

The thalassemia patients group was shown to significantly differ from the control group and to have a detrimental impact on the examined biomarkers. Based on the results of this study, it can be concluded that the level of the 5'-NT enzyme for the group of thalassemia patients increased, while there was an increase in the lipid peroxidation MDA and ferritin for the same group. This indicates a defect in the liver of thalassemia patients because increased lipid peroxide led to the loss of cell membrane function and thus increasing the flow of enzyme and ferritin from liver cells and spleen. As for GSH, its concentration decreased in the thalassemia patients group, it had reduced properties and played a central role in metabolic pathways, in addition to being considered an antioxidant in the body.

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