Assessment of the Effectiveness of Prolidase and Study of the Impact of Certain Antioxidants in Individuals Suffering from Beta Thalassemia in Anbar Province

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Abstract: Thalassemia is a common hereditary disease that is transmitted from parents to their children. As a result, many individuals do not follow the recommended guidelines, particularly before marriage. Early screening is considered an important measure to raise awareness among individuals for the early detection of thalassemia. The current study included 60 individuals (males and females) diagnosed with thalassemia, aged between 5 and 20 years, identified by specialists in hematology at Al Ramadi Hospital in Al Anbar Governorate, and 30 individuals (males and females) aged 5 to 20 years who are not affected by thalassemia, serving as a control group. Samples were collected at Al Ramadi Hospital for Women and Children / Thalassemia Unit.We discussed the effect of the disease on the activity of the Prolidase, which was found to significantly increase when exposed to this pathological condition (thalassemia). Therefore, we can rely on the estimation of elevated prolidase activity as a biomarker for the onset of health deterioration in individuals affected by thalassemia, thus preventing progression to advanced stages of the disease. The study aimed to estimate the effectiveness of the Prolidase in patients with thalassemia and compare it with a control group to understand the reason for the elevated enzyme activity in the patients. The measurement of levels of certain antioxidants, including (CAT, D3, MAD, GSH). The results showed a significant decrease in levels of (D3, GSH) at a significance level of ($P \leq 0.05$) in patients compared to the control group. At the same time, there was a significant increase in levels of (MAD, CAT) at a significance level of ($P \leq 0.05$) in the serum of patients compared to healthy individuals.

1 INTRODUCTION

Thalassemia is a hereditary blood disorder that leads to the production of abnormal hemoglobin due to a defect in one or more of the (alpha and beta) globin chains found in hemoglobin. This results in an imbalance in the production of these chains, causing a disturbance in the normal structure of hemoglobin, especially when one parent is a carrier of the disease, enabling transmission to the next generation [1]. When there is a disruption in the synthesis of one of the globin chains (alpha or beta), this leads to a reduced level of hemoglobin in red blood cells, affecting the oxygen transport process from the lungs to all body tissues, which causes the development of anemia in childhood and its persistence throughout life. Hemoglobin, which is composed of two proteins (alpha globin and beta globin), plays a crucial role in red blood cells for transporting oxygen to all body tissues. When the body is unable to produce one of these proteins, red blood cells cannot produce normal hemoglobin, rendering them unable to carry oxygen from the lungs to the tissues [2].

Prolidase is an enzyme classified under the number (EC 3.4.13.9) that catalyzes the hydrolysis of the substrate, leading to the breakdown of dipeptides that contain proline or hydroxyproline derivatives at the terminal end of the alpha carbon, resulting in free proline. Prolidase plays a significant role in the final stages of the degradation of proteins rich in amino acids, such as the breakdown of collagen. It contributes to cell growth by regulating growth factors and transcription, and it has important roles in various physiological aspects, pathological processes, and cell growth and differentiation [3]. The substrate that prolidase acts upon is imidodipeptides resulting from the degradation of collagen, which contain proline or

hydroxyproline at their ends. It exhibits activity in plasma and various organs such as the brain, heart, uterus, and red blood cells, but shows abnormal activity under conditions like liver disorders and osteoporosis [4]. Prolidase depends on manganese as a cofactor, associated with the vicinity of the active site, to carry out the activation process in the cleavage of dipeptides, breaking the bond in the (glycine–proline) compound to produce two compounds: glycine and L-proline. Therefore, Prolidase requires specific conditions with a temperature range of 35-55 °C and a pH range of 6-8, along with a narrow specificity regarding the substrate to hydrolyze only a few terminal peptide bonds, thereby regulating vital processes and promoting the breakdown of proteins into smaller peptides and amino acids. Its importance lies in the release and recycling of proline in the protein systems of bacterial enzymes [5].

These are a group of elements, complex compounds, enzymes, and vitamins that possess an important biological property for the bodies of many living organisms. They have the ability to inhibit the activity and formation of free radicals, which helps prevent or slow down the oxidation process by continuously removing active forms of oxygen and nitrogen from the body, rendering them unable to damage cells [6].

Glutathione (GSH) is considered a non-enzymatic, water-soluble endogenous antioxidant, existing in the form of a tripeptide composed of three amino acids (Glutamic Acid, Glycine, Cysteine). It plays a role in building and repairing tissues, combating free radicals, preventing and delaying cell damage, and detoxifying chemical substances within the liver. It has a low molecular weight and exists in both oxidized form (GSSG) and reduced form GSH [7]. GSH is regarded as a reducing agent capable of donating a hydrogen atom found in high oxygen-consuming and energy-producing organs such as the brain, kidneys, and liver, helping to maintain cell membranes from oxidation and oxidative damage. Therefore, there is an inverse relationship between GSH concentration in tissues and oxidative stress levels [8].

Malondialdehyde (MDA) is considered an antioxidant that results from the oxidative degradation of lipids that occurs spontaneously in the body's cells. MDA is one of the most important indicators used to investigate oxidative stress in body tissues, serving as a clear marker for lipid peroxidation. It is significant for monitoring oxidative damage caused by reactive oxygen species and estimating the end products of lipid oxidative degradation. MDA is produced through the oxidation of unsaturated fats, particularly those containing two or three double bonds, being a secondary product of the oxidation of polyunsaturated fatty acids after degradation by reactive oxygen species. Fatty acids are more susceptible to oxidative stress when they interact with free radicals in the process of lipid peroxidation, which primarily leads to MDA production [9], [10].

Catalase (CAT) is one of the oxidoreductase enzymes (1.11.1.6 E.C) that plays a crucial role in protecting cells from the toxic effects of hydrogen peroxide, as it catalyzes the decomposition of peroxide into O2 and H2O. Therefore, the levels of catalase enzyme in red blood cells of patients with mild thalassemia respond to oxidative threats, while in patients with severe thalassemia, the levels of antioxidant enzymes return to their normal levels due to the presence of normal red blood cells resulting from multiple blood transfusions [11].

Vitamin D is considered an essential nutrient for maintaining bone health and calcium balance, and for the alignment of the skeletal structure during periods of rapid growth from early childhood until puberty. It is obtained from external sources through the diet (such as fatty fish), and then it is transported to the liver, where it is converted by parathyroid hormone to 25-hydroxyvitamin D3. It then transforms in the kidneys into dihydroxyvitamin D3 (1,25-), with 25OHD being the circulating form of vitamin D in the serum. This vitamin plays a crucial role in regulating calcium absorption in the intestines, helping to maintain serum calcium concentration. Therefore, when vitamin D levels drop, conditions such as rickets and osteoporosis can occur [12], [13].

Research objectives:

1) To estimate the effectiveness of prolidase in patients with thalassemia and compare it with a control group.
2) To assess the levels of antioxidants by examining (VitD, GSH, MDA, CAT) in patients with thalassemia and comparing them with a control group.

2 MATERIALS AND METHODS

A case study was designed for thalassemia patients and a control group, totaling 90 samples from both patients and healthy individuals. Blood samples were collected from clinically diagnosed patients, comprising 60 samples: 30 from males and 30 from

females, aged between 5 and 20 years, at the Thalassemia Unit of Al-Ramadi Teaching Hospital. The control group consisted of 30 healthy samples, with 15 from males and 15 from females. A total of 5 mL of venous blood was drawn from thalassemia patients and healthy individuals using a single-use plastic syringe and was divided based on the type of examination. Then, 2 mL of blood was placed in test tubes containing an anticoagulant (EDTA) for measuring hematological variables, while the remaining 3 mL was placed in plastic test tubes without anticoagulant and left at room temperature until clotting occurred. The blood samples were centrifuged for 10 minutes at a speed of 3000 revolutions per minute to separate the serum, which was then aspirated using a micropipette and distributed into three numbered tubes to avoid repeated freezing and thawing that could affect enzyme activity. The samples were stored at a temperature of -20 °C until analysis, and the required biochemical tests were conducted later.

2.1 Estimation of Prolidase in Serum

The activity of prolidase was estimated by its catalytic action on its substrate, the dipeptide Glycine-Proline (Gly-L-Pro), to hydrolyze it, releasing the free amino acids glycine and proline. The amount of released proline was measured colorimetrically after it reacted with ninhydrin at a wavelength of 515 nm, using proline as a standard substance [14].

Serum was diluted by adding 250 µL of a dilution solution to every 50 µL of serum. The mixture was then incubated for 24 hours at 37 °C to prepare the diluted serum for addition. Following this, 100 µL was added to the control samples.

The activity of the prolidase enzyme in the blood samples under study was calculated based on the following (1) [15].

$$\textit{Prolidase Activity} = (\textit{Abs of test} - \textit{Abs control}) / (\textit{Abs of standard}) \times 2.4 \times [S]. \quad (1)$$

2.2 Determination of Glutathione Level

The modified method was used to determine the level of reduced GSH in the serum of thalassemia patients, which relies on the use of Ellman's reagent containing DTNB. This reagent reacts strongly with glutathione, reducing the thiol (SH) group to produce a yellow-colored complex that has a maximum absorbance at a wavelength of 412 nm. The concentration of the resulting product depends on the concentration of glutathione present in the serum [16].

A solution of sulfosalicylic acid was prepared by dissolving 2 g of the acid in 50 mL of distilled water and stored in the refrigerator until use. The phosphate buffer solution was prepared by mixing 30 mL of a sodium phosphate dibasic solution (Na_2HPO_4) at a concentration of 0.08 M, which was prepared by dissolving 0.284 g of Na_2HPO_4 in 50 mL of distilled water, with 15 mL of a KH_2PO_4 solution at a concentration of 0.6 M, which was prepared by dissolving 4.08 g of KH_2PO_4 in 25 mL of distilled water and then completing the volume to 50 mL. The Ellman's reagent solution was prepared at a concentration of 0.01 mmol by dissolving 0.004 g of DTNB in 100 mL of the phosphate buffer solution (pH=7.4) and stored in the refrigerator until use. Then, 150 µL of serum was added to a test tube containing 150 µL of a 4% sulfosalicylic acid solution and left for 5 minutes. The solution was then separated by centrifugation at 2000 rpm for 5 minutes. Following that, 4.5 mL of Ellman's reagent was added, and after 5 minutes, the absorbance was measured at 412 nm using a spectrophotometer.

The concentration of reduced GSH in the blood serum of patients and healthy individuals was calculated based on the following (2) [17]:

$$\textit{Conc. of GSH } (\mu mol/L) = (A \text{ at } 412 \text{ nm}) / (E \times L) \times 10^6 \quad (2)$$

2.3 Determination of VitD3 Concentration

Vitamin D labeled as biotin (OH-25) was estimated in the laboratory using an enzyme-linked immunosorbent assay (ELISA) kit. The color intensity, measured by a spectrophotometer at 450 nm, is proportional to the VitD3 concentration in the sample. The sample result is calculated directly from the standard curve by comparing the optical density (OD) of the samples and standard solutions [18].

The modified TBA-thiobarbituric acid reaction method was used to estimate the concentration of MDA, which represents one of the main products of lipid oxidation. The measurement depends on the reaction between malondialdehyde and TBA with lipid peroxides in an acidic medium to form a pink (TBA-MDA) complex, and its absorbance is measured at a wavelength of 532 nm [19].

Thiobarbituric acid (TBA) solution was prepared by dissolving 0.67 g of TBA with 20 g of trichloroacetic acid and 2 mL of glacial acetic acid, then completing the volume to 100 mL with distilled water. The solution was prepared immediately

before use. Trichloroacetic acid (TCA) solution was prepared in two concentrations: the first concentration of 17.5% was prepared by dissolving 17.5 g of TCA in 100 mL of distilled water, and the second concentration of 70% was prepared by dissolving 70 g of TCA in 100 mL of distilled water. Both solutions were kept in the refrigerator until use. Then, 150 µL of blood serum was added to a test tube, and 1 mL of 17.5% TCA solution was added to it, followed by 1 mL of TBA solution. The mixture was mixed well and then incubated in a boiling water bath at 100 °C for 30 minutes. The tubes were then cooled, and 1 mL of 70% TCA solution was added to them. The mixture was left at 37 °C for 20 minutes, and the supernatant was separated by centrifugation at 2000 rpm for 10 minutes. After that, the absorbance was measured at a wavelength of 532 nm using a spectrophotometer after the appearance of the pink color due to the formation of the colored complex.

The concentration of MDA in the blood serum of patients and healthy individuals was calculated based on the following (3) [20]:

$$Conc.\ of\ MDA\ (\mu mol / L) = (A\_test - A\_blank) / (E^{\circ} \times L) \times D \times 10^{6} . \quad (3)$$

2.4 Determination of Catalase Concentration

The activity of the catalase enzyme was estimated by measuring the decrease in absorbance resulting from the consumption of the substrate (hydrogen peroxide). Hydrogen peroxide reacts with ammonium metavanadate under acidic conditions to reduce vanadium to vanadium (III). Hydrogen peroxide is a strong oxidizing agent that forms a red-orange peroxovanadium complex that absorbs at 452 nm [21].

A buffered phosphate solution with a concentration of 50 mM and a pH of 7 was prepared. A hydrogen peroxide solution with a concentration of 10 mM was prepared using the buffered phosphate solution, and a vanadium reagent solution was prepared at a concentration of 0.01 M of ammonium metavanadate in 0.5 M sulfuric acid.

The activity of the catalase enzyme is calculated based on the following equation (4):

$$Catalase\ Activity\ of\ test\ (kU) = 2{,}303/t * \log(A1/A2) . \quad (4)$$

3 STATISTICS

Statistical analysis was conducted using the SPSS statistical package version (21). Data were evaluated using the arithmetic mean, and the means were compared using Duncan's multiple range test at a significance level of ($P \leq 0.05$). Analysis of Variance (ANOVA) was employed to find significant differences between the patient group and the healthy group, while Excel version (2010) was used to create graphical representations.

4 RESULTS AND DISCUSSION

The Prolidase activity is measured in serum blood.The activity of the prolidase enzyme was evaluated in the blood of patients with thalassemia and the control group. The results showed an increase in the activity of the enzyme in the serum of thalassemia patients. When a statistical comparison was made between the activity of the enzyme in the serum of thalassemia patients and the activity of the enzyme in the serum of apparently healthy individuals (the control group), it was found that there was a significant increase in the activity of the enzyme in individuals with thalassemia, as shown in Table 1 and Figure 1.

The results of the current study align with the findings of researchers (Cakmak) and (Belli) and their group .The increase in the activity of the prolidase is attributed to a dysfunction in collagen turnover, which leads to various disease conditions and disease progression. The prolidase may play a role in metabolic disorders in individuals with thalassemia, as collagen is a major extracellular component present in various tissues, including red blood cells. prolidase (Iminodipeptidase) is a homogenous enzyme that releases carboxyproline and hydroxyproline from prolidase , facilitating collagen turnover, cell reorganization, and growth in the body. The activity of the prolidase is a determining factor in the bioregulation of collagen, and the breakdown of tissue barriers is stimulated by proteolytic enzymes. Therefore, inhibiting the enzyme's activity may be considered a therapeutic target for patients with thalassemia. Although collagenases initiate the degradation of the peptide bonds in collagen, the final step of the breakdown is stimulated by the prolidase , where a significant decrease in collagen quantity has been observed in patients, which may be associated with an increase in prolidase activity in the tissues [22], [23].

Table 1: The activity of the prolidase in the blood serum of patients and the control group.

Parameters	Mean ± SD Groups					
	Male Patients	Female Patients	Total Patients	Male Control	Female Control	Total Control
Prolidase (IU/L)l	800.71 ± 5.78	796.06 ± 9.88	798.385 ±7.83	239.05 ± 25.11	229.07 ±3.42	234.06 ±14.26

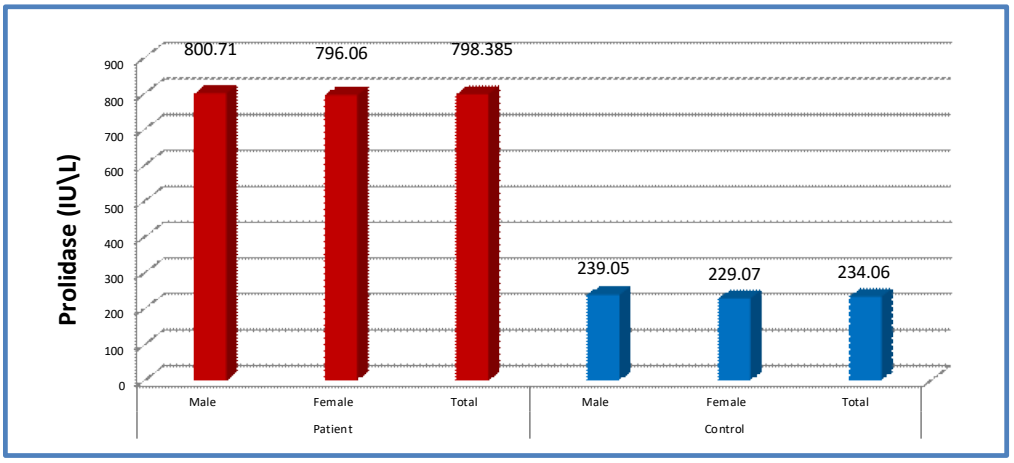

Figure 1: Comparison between different groups in the level of the prolidase.

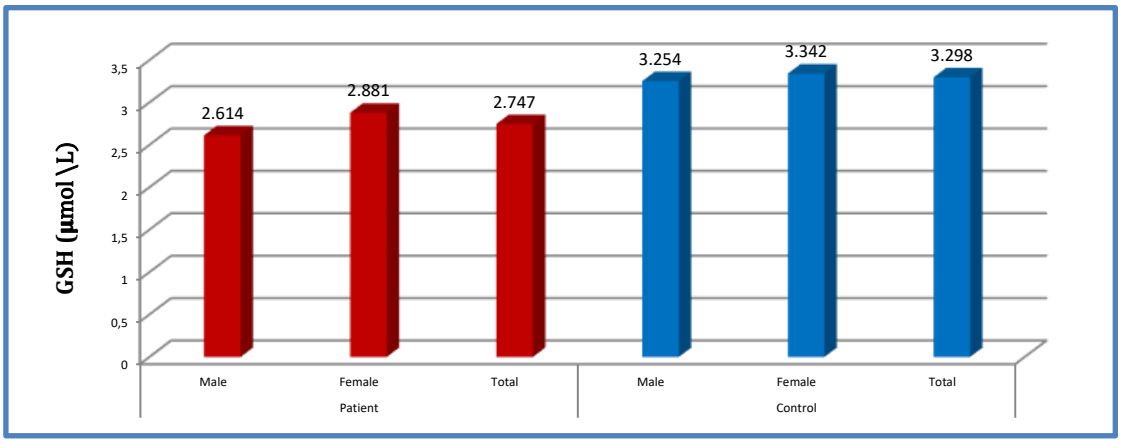

Figure 2: Comparison between different groups in GSH level.

Table 2: The effectiveness of antioxidants in the blood serum of the sick and healthy groups.

Parameter (Units)	Male Patients	Female Patients	Total Patients	Male Control	Female Control	Total Control
GSH (µmol/L)	2.614 ±1.11	2.88 ±1.01	2.747 ±1.01	3.254 ±1.72	3.342 ±1.50	3.298 ±1.35
MDA (µmol/L)	3.678 ±3.78	3.627 ±2.05	3.774 ±2.05	2.591 ±1.47	2.166 ±1.16	2.378 ±1.37
Vit D3 (ng/mL)	7.664 ±4.472	6.943 ±5.19	7.330 ±5.19	9.166 ±7.139	7.833 ±4.16	8.498 ±2.07
CAT (K/mL)	0.832 ±0.21	0.688 ±0.21	0.760 ±0.19	1.023 ±0.21	0.129 ±0.24	1.086 ±0.52

3.1 Estimation of Antioxidant Levels

The levels of antioxidants (CAT, D3, MAD, GSH) were estimated in Table 2 for thalassemia patients and control groups according to gender. Comparisons between patient groups and control groups showed a significant increase in the level of (MAD, CAT) and a significant decrease in the level of (GSH, D3) at the probability level ($P \leq 0.05$) in the serum of thalassemia patients compared to (control groups).

3.2 Estimation of GSH Concentration Levels

The level of GSH was estimated in the blood of patients with thalassemia and the control group. The results showed a decrease in the level of GSH in the serum of thalassemia patients. When a statistical comparison was made between the activity of GSH in the serum of thalassemia patients and the activity of GSH in the serum of apparently healthy individuals (the control group), it was found that there was a significant decrease in the activity of GSH in people with thalassemia compared to the healthy group, as shown in Table 2 and Figure 2.

The results of the current study, as shown in Table 2, indicated that the level of GSH in the serum of thalassemia patients is lower than that of healthy individuals (control group). These findings are consistent with those of the researcher Hadeer Hayder [24]. Furthermore, these results align with the findings of researchers Salih and Khalid and their group [25], who reported a significant decrease in the concentration of glutathione GSH in the serum of thalassemia patients compared to the glutathione levels in the serum of healthy individuals (control group). The results of this study are also in agreement with the study conducted by Attia and his team [26], where they found a reduction in the concentration of glutathione GSH due to excessive production of hydrogen peroxide. Glutathione GSH serves as a key reductant within cells, making it highly sensitive to oxidative stress, and it has several important functions, such as protecting against oxidative stress, regulating gene expression, and reducing the activation of programmed cell death. Glutathione GSH is one of the essential antioxidants for recycling vitamins E and C, and it is very effective in helping the body combat free radicals. Thus, glutathione GSH participates in the cellular defense system against oxidative stress by detoxifying free radicals and reactive oxygen species. Therefore, the decreased level of glutathione GSH in thalassemia patients leads to increased cellular sensitivity to oxidative stress .

3.3 MDA Concentration Levels

MDA level was estimated in the blood of patients with thalassemia and the control group. The results showed an increase in the level of MDA in the serum of thalassemia patients. When a statistical comparison was made between the activity of MDA in the serum of thalassemia patients and the activity of MDA in the serum of apparently healthy individuals (the control group), it was found that there was a significant increase in the activity of MDA in people with thalassemia compared to the healthy group, as shown in Table 2 and Figure 3.

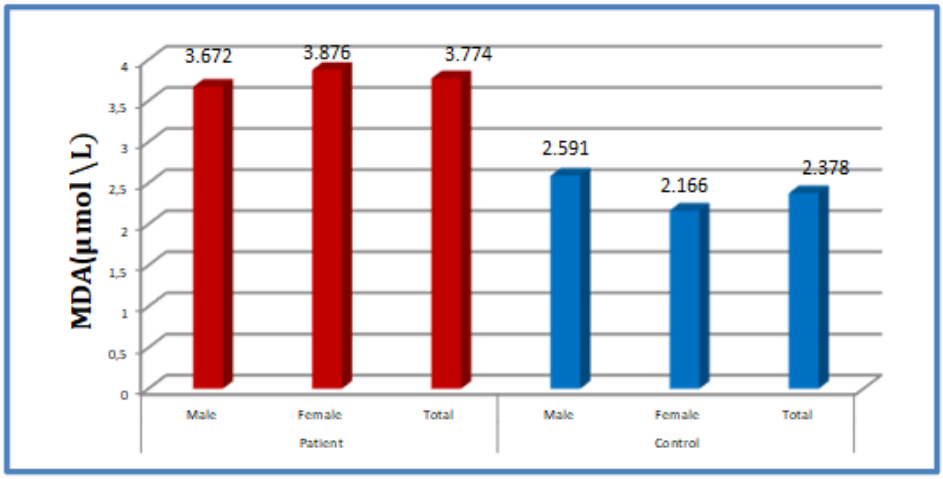

Figure 3: Comparison between different groups in MDA level.

The results of the current study, as shown in Table 2, indicated that the level of MDA in the serum of thalassemia patients is higher than the level of MDA in healthy individuals (control group). These findings are consistent with those reached by the researcher Lubis and his group [27], They investigated the levels of MDA in thalassemia patients dependent on blood transfusions, revealing that the MDA levels in these patients are significantly higher than those in healthy individuals due to the increased amount of iron in the patients, which enhances oxidative stress and leads to elevated MDA levels. Therefore, iron overload is a serious issue in transfusion-dependent thalassemia. Additionally, the current study results align with those of researcher ALAYUNT and his team [28], who found that MDA values are higher in thalassemia patients compared to the control group. It is believed that the reduced oxygen utilization capacity in thalassemia patients may play a role in the elevated levels of MDA. The oxidant and antioxidant properties were investigated in childhood thalassemia patients receiving regular blood transfusions and chelation therapy, allowing the researcher to find higher levels of MDA in thalassemia patients compared to the control group. The elevated levels of malondialdehyde MDA are considered a good indicator of oxidative damage in the blood of patients with thalassemia, as it is the end product of the oxidation of polyunsaturated fatty acids, which is commonly used as a marker for lipid peroxide levels. Therefore, the iron overload in thalassemia patients leads to tissue oxidative injury and an increase in MDA concentration. Thalassemia patients are primarily exposed to oxidative stress due to the accumulation of iron in their bodies. Thus, maintaining antioxidant systems could be beneficial in protecting thalassemia patients from more severe complications of the disease. Anemia in patients with thalassemia leads to hemolysis in the peripheral circulation and ineffective erythropoiesis, causing accelerated red blood cell death. The increase in iron resulting from continuous blood transfusions or oxidative stress in thalassemia patients leads to a significant consumption of antioxidants. Hence, the main goal of blood transfusion is to address anemia, suppress ineffective erythropoiesis, and inhibit the increased absorption of iron from the gastrointestinal tract, as iron accumulation can lead to toxicity and damage to the tissues where iron is deposited [29].

3.4 Estimating Vitamin D3 Levels

Vitamin D3 levels were estimated in the blood of patients with thalassemia and the control group. The results showed a decrease in the level of Vitamin D3 in the serum of thalassemia patients. When a statistical comparison was made between the effectiveness of Vitamin D3 in the serum of thalassemia patients and the effectiveness of Vitamin D3 in the serum of healthy individuals (the control group), it was found that there was a significant decrease in the effectiveness of Vitamin D3 in people with thalassemia compared to the healthy group, as shown in Table 2 and Figure 4.

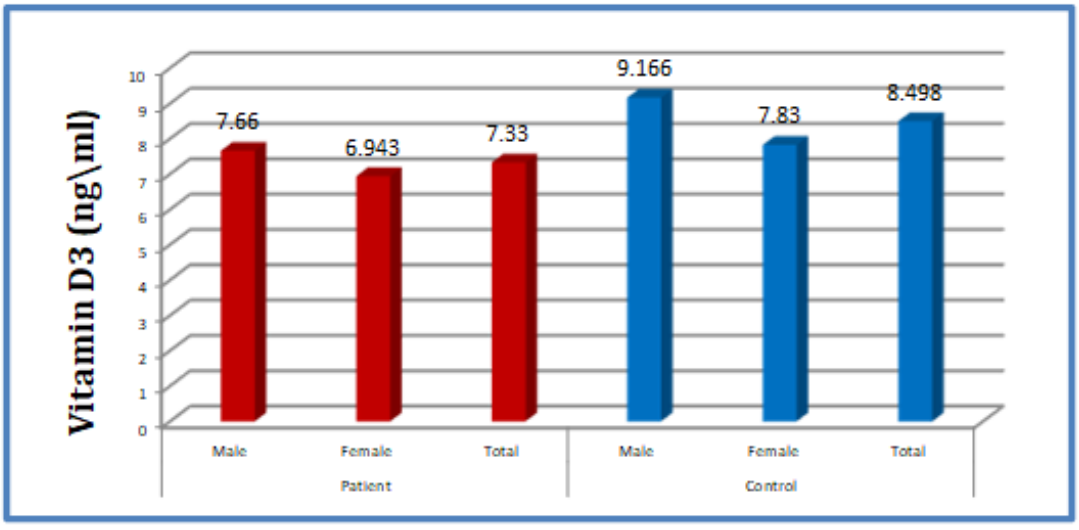

Figure 4: Comparison between different groups in terms of Vitamin D3 level.

The results of the current study, presented in Table 2, indicated that the level of Vitamin D3 in the serum of thalassemia patients is lower than the level of Vitamin D3 in healthy individuals (control group). These findings are consistent with those reported by researcher Alzubaidi and his team , who found a decrease in Vitamin D3 levels in thalassemia patients compared to the control group due to iron deposition in the liver and skin, which disrupts the process of converting Vitamin D from one form to another, leading to an increased risk of fractures and osteoporosis due to the regulatory effect of Vitamin D on bone cells. The results of the current study also align with those of researcher Al-Rubae and his team , indicating that thalassemia patients are at greater risk of Vitamin D deficiency due to limited sun exposure, low bone mass, impaired calcium metabolism, and increased iron absorption, which reduces calcium absorption since high ferritin levels interfere with the production of 25-hydroxy Vitamin D in the liver due to increased iron in the liver, negatively affecting bone metabolism who found that children with thalassemia receive regular blood transfusions at specified time intervals, which may lead to iron deposition in the liver, resulting in a decrease in the synthesis of Vitamin D-25OH [30], [31]. Anemia resulting from iron deficiency significantly impacts the intestinal absorption of fat-soluble vitamins such as Vitamin D. Consequently, patients with thalassemia show a notable deficiency in Vitamin D3 levels due to poor vitamin absorption and inadequate dietary intake necessary to maintain normal Vitamin D levels. This leads to liver disorders that hinder the liver's ability to convert Vitamin D into its active form, due to iron overload in the liver or improper functioning of endocrine tissues, resulting in the progression of clinical manifestations of Vitamin D deficiency. Vitamin D3 is considered an antioxidant that protects biological membranes and other cellular components from oxidative damage, which contributes to its depletion and reduced effectiveness in thalassemia patients due to iron deposition in the liver and their inability to convert Vitamin D3 into its active form. Moreover, low levels of Vitamin D3 in patients can significantly contribute to decreased bone mass and the onset of osteoporosis due to increased pressure on the outer membrane containing pain fibers, leading to generalized bone pain. It has been observed that elevated ferritin levels in patients correlate with reduced Vitamin D levels, which affects heart function in these patients [32].

3.5 Catalase Enzyme Concentration Levels

The level of CAT was estimated in the blood of patients with thalassemia and the control group. The results showed an increase in the level of CAT in the serum of thalassemia patients. When a statistical comparison was made between the activity of CAT in the serum of thalassemia patients and the activity of CAT in the serum of apparently healthy individuals (the control group), it was found that there was a significant increase in the activity of CAT in people with thalassemia compared to the healthy group, as shown in Table 2 and Figure 5.

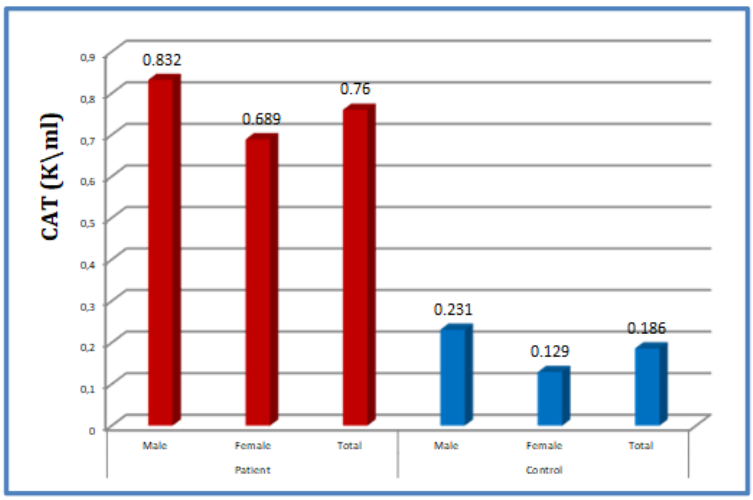

Figure 5: Comparison between different groups at the CAT level.

The results of the current study in Table 2 show that the level of CAT in thalassemia patients is higher than the level of CAT in healthy individuals (control group). These results are consistent with the findings of researcher Melo and his team [33], who observed an increase in catalase levels in patients compared to healthy individuals (control group) due to an increase in oxidative factors such as H2O2, causing the oxidation of components in red blood cells. The enzyme catalase is responsible for detoxifying cells and protects hemoglobin from oxidative damage. The elevated activity of catalase in red blood cells leads to increased levels of oxidative factors like H2O2, thus causing oxidation of cell components. An increase in levels of antioxidant enzymes such as CAT and glutathione peroxidase (GPX) in red blood cells of patients with mild thalassemia responds to the rising threat of oxidation, whereas in patients with severe beta thalassemia, the levels of antioxidant enzymes return to their normal levels due to the presence of normal red blood cells from multiple blood transfusions. It has been found that catalase occupies a place among the relevant biocatalysts due to its exceptional catalytic rate and thermal stability in destroying harmful hydrogen peroxide and producing water and oxygen in living systems by reducing free radical damage, making it an important biomarker. It has the highest turnover rate among enzymes, enhancing antioxidant defense systems in cells [34].

5 CONCLUSIONS

The findings of this study confirm that the flower bud extracts of *Rosa damascena*, particularly the ethanolic and aqueous forms, exhibit significant antibacterial activity against a range of clinically important Gram-positive and Gram-negative bacterial strains. Among the two, the ethanolic extract demonstrated superior inhibitory effects, notably against *Staphylococcus aureus*, *Serratia*, and *Pseudomonas aeruginosa*, suggesting that ethanol may be more efficient in extracting key antimicrobial phytochemicals.

The measurement of prolidase levels can be used as an initial indicator to identify individuals with thalassemia, and the elevated levels of prolidase in the serum of thalassemia patients contribute to iron balance. There is oxidative stress in the serum of thalassemia patients compared to healthy individuals, indicated by higher levels of MDA and lower levels of GSH, while CAT levels increased in thalassemia patients compared to the control group. Additionally, the levels of vitamin D3 decreased in thalassemia patients compared to healthy individuals. We can conclude that the significant increase in the levels of the studied biochemical indicators may be due to certain diseases, such as kidney, heart, and liver diseases, which are also characteristic features of beta thalassemia patients.

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