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Measurements the Level of Lipid Peroxidation and Some Antioxidants in Blood Serum of Thalassemia's Patients

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Abstract: Repeated blood transfusion in beta thalassemia patients may lead to peroxidative tissue injury by secondary iron overload. In the present study, (43) patients with beta thalassemia. We have evaluated hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), iron (Fe), ferriten, uric acid, glutathion (GSH), malondialdehyde (MDA), Vitamins C and E and electrolytes as sodium (Na), potassium (K), and chloride (Cl). The findings were compared with (25) age matched healthy individuals were included in this study as a control group. A significant increase in the levels of WBC, Fe, ferritin, MDA and Vit C ($P < 0.001$), whereas significant decrease in the levels of Hb, PCV, RBC, GSH and Vit E ($P < 0.001$) was observed. Uric acid, Na and K were significant increase ($P < 0.05$) in the patients when compared with controls, while there was a non-significant increase in mean value of Cl. These results were suggesting that oxidative stress and reduced antioxidant defense mechanism play an important role in pathogenesis of beta thalassemia major. We can conclude that defective membrane transport is responsible for observed changes of lipid peroxidation and some antioxidants. These results may help to understand the altered electrolyte homeostasis in thalassemia but there is still need of many future studies to clarify their mechanism of generation and pathological significance.

Keywords: Antioxidant, Beta thalassemia, Hemoglobin, Glutathion, Malondialdehyde.

Introduction

Thalassemia is an inherited blood disorders characterized by abnormal hemoglobin production. Symptoms depend on the type and can vary from none too severe. Regularly there is mellow to serious frailty (low red platelets) which can bring about inclination drained and fair skin. There may likewise be bone issues, an expanded spleen, yellowish skin, dull pee, and among kids' moderate development (Rund, 2016; Hashim et. al., 2020).

There are two main types of thalassemia, beta thalassemia, which includes the subtypes major, intermediate. It occurs when the body cannot produce beta globin. Alpha thalassemia occurs when the body cannot make alpha globin. This type of thalassemia also has two serious types hemoglobin H disease and hydrops fetalis. Thalassemia minor, people with this case do not usually have any symptoms. Both α- and β-thalassemia are

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often inherited in an autosomal recessive manner. For the autosomal recessive forms of the disease, both parents must be carriers for a child to be affected. If both parents carry a hemoglobinopathy trait, the risk is 25% for each pregnancy for an affected child (Kingchaiyaphum et. al., 2020).

Thalassemia is a hereditary blood issue that causes hemoglobin inadequacy and extreme weakness, keeping organs from oxygen, which represses their capacity to work appropriately. The survival of patients with thalassemia has progressively improved with advances in therapy; however, rehashed blood transfusion in beta thalassemia noteworthy patients may provoke peroxidative tissue harm by assistant iron over-load (Antonio et. al., 2019).

Oxidative damage incited by free globin chains has been involved in the pathogenesis of the film varieties from the standard saw in β thalassemia. We decided if thalassemia could represent anomalous cation transport framework. Thalassemia is an innate hemolytic issue caused by a fractional or finish insufficiency of alpha-or beta-globin chain blend. Homozygous bearers of beta-globin quality imperfections experience the ill effects of extreme iron deficiency and different genuine entanglements from adolescence (Srinoun et. al., 2019). The significant irregularity inside red blood cell (RBC) of patient with thalassemia results from the precipitation of unstable hemoglobin chain, which is available in overabundance. The RBC film is to a great degree hurt by the wealth empowering hemoglobin chains. Relatively every part of the thalassemic blood cell layer is changed: lipids, proteins, sialoglycoproteins and glycolipids. This layer harm speaks to a vital instrument prompting pallor in thalassemia (Al- Janabi et. al., 2021).

Method

Patients were enrolled in the present study to the Hematology center in Diyala. Samples of (43) patients were included in this study (22 males and 21 females) ranging in their age between (2-37) years, were collected during the period June to November 2017. They are diagnosed clinically based on serious paleness and hemoglobin electrophoresis. Every one of the patients were analyzed frequently on more than one occasion per month by clinicians. They were consistently accepting erythrocytes transfusions (stuffed RBC) consistently.

The control group include Twenty- five age matched healthy individuals were included in this study (15 males and 10 females) as a control group. None of these group had history of anemia, abnormal complete blood counts and abnormal hemoglobin electrophoresis results.

Hematology measurements were applied by Horiba Medical technology which will provide a significant improvement in differentiating blood cells.

Materials

- The GSH was determined by using modify method of (Sedlak and Lindsay, 1968)
- Serum lipid peroxide was measured by Kei Satoh (Wysocka et. al., 1995).
- Vit C was determined according to (Omaye et. al., 1979) method.
- Serum Vit E was resolute by (Varley et. al., 1979).
- Uric acid level was estimated by Enzymatic methods of (Tietz, 1999).
- For estimation of Iron, serum was deproteinized by Ramsay's Dipyridyl Method (Varley, 1967).
- Ferritin was dictated by using Elecsys technology with cobas instrument (Tietz, 1999).
- The estimation of serum Na and K by using (Annino and Giese, 1976).
- The colorimetric method was used to determine the Cl by (Snell, 1981).

Statistical Analysis

The information was performed utilizing the Statistical bundle for the sociology (SPSS) program. Results were communicated as mean ± standard deviation (SD). The t-test used to analyze the noteworthiness of the mean contrasts between two gatherings. The distinctions were viewed as noteworthy if the acquired p esteem was less or equivalent to 0.05 (Hinton, 2004).

Results and Discussion

In individuals with beta thalassemia, low levels of hemoglobin prompt an absence of oxygen in numerous of parts of the body. Thalassemia upsets the typical creation of hemoglobin and sound red platelets. This causes weakness and, the blood doesn't have enough red platelets to convey oxygen to tissues leaving the exhausted patients (Psatha et. al., 2018). Thalassemia disrupts the normal production of hemoglobin and healthy red blood cells. This causes anemia and, the blood doesn't have enough red blood cells to carry oxygen to tissues leaving the fatigued patients (Wafaa Al-Mosawy, 2017).

Significant lower mean levels of the red cell PCV values in patients were observed ($P < 0.001$), as compared to control group. This might be produced because of low mean corpuscular hemoglobin fixation (MCHC), on the grounds that the absence of typical amounts of intracellular hemoglobin, which fills in as a substrate for the powerful oxygen radicals and could result in an abundance of free radicals which oxidize different film segments prompt layer harm in thalassemia RBCs (Anselmo et. al., 2020) as shown in table (1).

Table 1. The Hb, PCV, RBC and WBC concentration in serum of thalassemia patients and control group.

	Mean ± SD	
Parameters	Control n= 25	Patients n= 43
Hb (g \ L)	14.64 ± 0.42	8.18 ± 1.14***
PCV %	41.07 ± 1.72	19.27 ± 3.14***
RBC $* 10^9$ \ L	4.86 ± 0.17	2.66 ± 0.09***
WBC $* 10^9$ \ L	6.41± 0.52	45.41 ± 7.05***

*** Significant difference between controls at ($P < 0.001$)

A significant increase in serum WBC ($P < 0.001$) of thalassemia patients when compared with control group. Leukocytosis is very common in acutely ill patients. It occurs in response to a wide variety of conditions, including viral, bacterial, fungal, or parasitic infection, hemorrhage, and exposure to certain medications or chemicals including steroids. Leukocytosis can be a reaction to various infectious, inflammatory, and, in certain instances, physiologic processes as stress or exercise (Chen et. al., 2020).

Table 1. The GSH, MDA, Uric acid, Iron and Ferritin concentration in serum of thalassemia patients and control group.

	Mean ± SD	
Parameters	Control n = 25	Patients n= 43
GSH $* 10^{-5}$ (µmole /L)	7.44 ± 0.22	1.56 ± 0.43***
MDA (µmole/L)	0.85 ± 0.13	1.51 ± 0.08***
Uric acid (mg /dl)	389.42 ± 16.81	395.11 ± 17.64**
Iron (µg /dl)	83.97 ± 7.38	379.80 ± 49.96***
Ferritin (ng /ml)	71.73 ± 7.33	3484.21 ± 447.29***

** Significant difference between controls at ($P < 0.01$)
*** Significant difference between controls at ($P < 0.001$)

The GSH level was decreased highly statistically significant, as shown in table (2), The present investigation announced an inadequacy in levels of decreased glutathione, which is 34.4% lower than in controls. These outcomes recommended that GSH is a noteworthy intracellular decreasing specialist, which is extremely touchy to oxidative pressure and has a few essential capacities (Abo-Shanap et. al., 2020).

Marked changes in the antioxidant pattern were also observed in thalassemia patients, the results in table (2) showed that there was a huge increment ($p < 0.001$) in the level of serum MDA in patients with beta thalassemia when contrasted with controls. The strongest predictors of elevated MDA were liver iron concentration. Our outcomes propose that the estimation of peroxidation items, mustached with assessment of cancer prevention agents, might be a basic proportion of iron harmfulness in thalassemia, in addition to the conventional indices of iron status. In thalassemia there is overabundance generation of receptive oxygen intermediates, these occasions prompt oxidative pressure. This oxidative pressure and a conceivable considerable quickened apoptosis may add to abbreviated life expectancy of erythrocytes. MDA a result of lipid peroxidation is produced in abundance sums in supporting the way that huge measure of layer bound iron is available in thalassemic erythrocytes. (Choudhary et. al., 2017).

The results in table (2) showed a significant increase in uric acid concentration. Uric acid formation occurs only in tissues that contain the enzyme xanthine oxidase. The increased body burden of uric acid is a result of increased *de novo* purine synthesis, increased purine nucleotide degradation diminished renal excretion of urate, or a combination of these defects. The increased of uric acid is due to that the uric acid is one of the antioxidant which present in plasma in high concentration. The leakage of substances from damaged cells into the plasma may have increased plasma antioxidants concentrations making changes difficult to detect (Kadiiska et. al., 2015).

A significant increase in serum iron was observed in beta thalassemia major when compared with controls as shown in table (2). Absence of beta globin chains lead to accumulation of unpaired alpha globin chains. Excess presence of the alpha globin chains is a primary reason for the cellular oxidative damage and also iron overload. As a result of high serum iron in beta thalassemia, there is an enhanced generation of reactive oxygen species ROS (Motta et. al., 2020).

Also, there was a significant increase in serum ferritin concentration in thalassemia patients when compared with control group as shown in table (2). Blood transfusion burden is an important measure of total body iron balance. Ferritin is a relatively inexpensive and widely-available measure, useful in monitoring chelation therapy. Liver iron concentration reflects total body iron stores, but incompletely stratifies the risks of iron overload complications (Khoshfetrat et. al., 2014). Ferritin is the iron storage protein serves to store iron in a non-toxic form, to deposit it in a safe form, and to transport it to areas where it is required. A significantly high level of ferritin is found in patients with iron overload and this may help differentiate thalassemia patients from those with iron deficiency, both of which will have a low red blood cell count (Sobhani et al., 2019).

In this study, the results in table (3) showed that there is a significant increase in vitamin C in serum of patients with thalassemia compared with the control group. The increased amount of vitamin C might be to the accumulation of iron in tissues could increase oxidative stress. Until now, some studies have been shown that ascorbic acid acts as an antioxidant in the presence of high level of iron and traps free radicals, and prevents the diffusion of these components to membrane of red blood cells and low density lipoprotein particles (Mohammad et. al., 2014).

Table 3. The Vitamins C and E concentration in serum of thalassemia patients and control group.

	Mean ± SD	
Parameters	Control n= 25	Patients n= 43
Vit C (µmole/L)	27.65 ± 0.87	30.34 ± 1.08***
Vit E (µmole/L)	17.83 ± 0.78	15.68 ± 4. 65***

*** Significant difference between controls at ($P < 0.001$)

Highly significant depletion ($p<0.001$) in serum vitamin E was observed in present study as shown in table (3). Vitamin E plays a key role in protecting cells against oxidative damage. The antioxidant role of Vitamin E is attributed to its ability in quenching highly reactive lipid peroxide intermediate by donating hydrogen and this prevents extraction of hydrogen. This assists in restricting self-perpetuated lipid peroxidation chain reaction (Sandro et. al., 2020).

Table 4. The Na, K and Cl concentration in serum of thalassemia patients and control group.

	Mean ± SD	
Parameters	Control n= 25	Patients n= 43
Na mmol/dl	136.45 ± 12.93	144.25 ± 15.93*
K mmol /dl	4.07 ± 0.47	6.01 ± 0.92*
Cl mmol/dl	95.9 ± 18.07	98.44 ± 19.12 (NS)

* Significant difference between controls at ($P < 0.05$)

There is a significant increase in the mean values of Na and K for patients, more than control. The mean value of Cl concentration in patients were non-significantly raised as compared to control as shown in table (4). The Na concentration in thalassemic patients increased may be due to reduced pump activity (Sirirat et. al., 2019). Impairment of anion and cation transport in thalassemia is very obvious. Thalassemia RBCs particularly from splenectomized patients' loss K because of an increase in selective permeability of the membrane to K, which results in shrinkage of RBCs and increased cellular rigidity. These results may help to understand the altered electrolyte homeostasis in thalassemia but there is still need of many future studies to clarify their mechanism of generation and pathological significance the K-loss in β-thalassemia by increasing the activity of K-Cl cotransport (Bayejid et. al., 2015).

Unusual layer work assumes a significant part in the modification of film cation transport as saw in thalassemia RBCs. Changes in the levels of serum sodium and potassium mirrors the inadequate film transport of the cations in the red cell layer of thalassemia. These outcomes give an affirmation that strange cation homeostasis may add to the pathogenesis of thalassemia (Pinto et. al., 2019).

Conclusion

We can conclude that defective membrane transport is responsible for observed changes of lipid peroxidation and some antioxidants. These results may help to understand the altered electrolyte homeostasis in thalassemia but there is still need of many future studies to clarify their mechanism of generation and pathological significance.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

References

Al- Janabi, G. , Al-Fahham, A. & Mohammed, R. (2021). Pathophysiology of sickle cell anemia: review article. *Indian Journal of Forensic Medicine and Toxicology, 15*(2), 2037-2043.

Piga, A., Perrotta, S., Gamberini, M. R., Voskaridou, E., Melpignano, A., Filosa, A., ... & Attie, K. M. (2019). Luspatercept improves hemoglobin levels and blood transfusion requirements in a study of patients with β-thalassemia. *Blood, The Journal of the American Society of Hematology*, *133*(12), 1279-1289.

Annino, J. S. & Giese, R. W. (1976). *Clinical the chemistry: principles and procedures* (4th ed.). Little Brown and Co.

Abo-Shanab, A. M., Kholoussi, N., Habib, D., Helwa, I. A., Hussin, G., Omar, N., ... & Fahmy, A. (2020). Use of echocardiography and glutathione S-transferase to detect heart complications in β-thalassemic patients. *Egyptian Pharmaceutical Journal*, *19*(3), 244.

Kingchaiyaphum, B., Sanchaisuriya, K., Fucharoen, G., Chaibunruang, A., Hess, S. Y., Hinnouho, G. M., ... & Fucharoen, S. (2020). Hemoglobins F, A2, and E levels in Laotian children aged 6- 23 months with Hb E disorders: Effect of age, sex, and thalassemia types. *International Journal of Laboratory Hematology*, *42*(3), 277-283.

Rund, D. (2016). Thalassemia 2016: modern medicine battles an ancient disease. *American Journal of Hematology*, *91*(1), 15-21.

Anselmo, F. C., Ferreira, N. S., Mota, A. J. D., Gonçalves, M. D. S., Albuquerque, S. R. L., Fraiji, N. A., ... & Moura Neto, J. P. D. (2020). Deletional alpha-thalassemia alleles in amazon blood donors. *Advances in Hematology*, *2020*, 1-6. https://doi.org/10.1155/2020/4170259.

Hinton, P. R. (2004). *Statistical explained* (2nd Edition). Rontledge.

Motta, I., Bou-Fakhredin, R., Taher, A. T., & Cappellini, M. D. (2020). Beta thalassemia: new therapeutic options beyond transfusion and iron chelation. *Drugs*, *80*(11), 1053-1063.

Kadiiska, M. B., Peddada, S., Herbert, R. A., Basu, S., Hensley, K., Jones, D. P., ... & Mason, R. P. (2015). Biomarkers of oxidative stress study VI. Endogenous plasma antioxidants fail as useful biomarkers of endotoxin-induced oxidative stress. *Free Radical Biology and Medicine*, *81*, 100-106.

Srinoun, K., Sathirapongsasuti, N., Paiboonsukwong, K., Sretrirutchai, S., Wongchanchailert, M., & Fucharoen, S. (2019). miR-144 regulates oxidative stress tolerance of thalassemic erythroid cell via targeting NRF2. *Annals of hematology*, *98*(9), 2045-2052.

Sirirat, K., Sriwantana, T., Kaewchuchuen, J., Paiboonsukwong, K., Fucharoen, S., Ritthidej, G., ... & Sibmooh, N. (2019). Pharmacokinetics and pharmacodynamics of single dose of inhaled nebulized sodium nitrite in healthy and hemoglobin E/β-thalassemia subjects. *Nitric Oxide*, *93*, 6-14.

Choudhary, M., Vyas, R. K., & Lahoti, A. (2017). Correlation of oxidative stress with serum antioxidant enzymes level in thalassemia in a tertiary level hospital of western Rajasthan. *International Journal of Biotechnology and Biochemistry*, *13*(2), 155-65.

Khoshfetrat, M. R., Mortazavi, S., Neyestani, T., Mahmoodi, M. R., Zerafati-Shoae, N., & Mohammadi-Nasrabadi, F. (2014). Iron and vitamin C Co-Supplementation increased serum vitamin C without adverse effect on zinc level in iron deficient female youth. *International Journal of Preventive Medicine*, *5*(8), 1037.

Hashim, N. A., Abdullah, Y. J., & Ibadi, H. A. (2020). Evolution of some biochemical and hematological parameters of thalassemia patients in Maysan Governorate, Iraq. *Annals of Tropical Medicine and Public Health*, *23*, 231-238.

Psatha, N., Reik, A., Phelps, S., Zhou, Y., Dalas, D., Yannaki, E., ... & Papayannopoulou, T. (2018). Disruption of the BCL11A erythroid enhancer reactivates fetal hemoglobin in erythroid cells of patients with β-thalassemia major. *Molecular Therapy-Methods & Clinical Development*, *10*, 313-326.

Sandro, A., Naidu, M., Philipp, S., Timm, S., Michaela, A., Axel, W., Katarzyna, O., Lothar, H., Manuela, S., Camilla, L., Cornelia, K., Josef, M., Dirk, J., Frauke, N., Birgit, R., Martin, H., Christian, B., Katja, M., Marcus, C., Tomas, G., Manfred, K., Martina, U. & Georg, W. (2020). Glutathione peroxidase 4 and vitamin E control reticulocyte maturation, stress erythropoiesis and iron homeostasis. *Haematologica*,*105*(4), 937–950.

Sedlak, J. & Lindsay, R. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical biochemistry*, *25*, 192-205. https://doi.org/10.1016/0003-2697(68)90092-4.

Sobhani, S., Rahmani, F., Rahmani, M., Askari, M., & Kompani, F. (2019). Serum ferritin levels and irregular use of iron chelators predict liver iron load in patients with major beta thalassemia: a cross-sectional study. *Croatian Medical Journal*, *60*(5), 405-413. https://doi.org/10,3325/cmj.2019.60.405

Omaye, S.T., Turnbull, J.D., & Sauberlich, H.E. (1979). Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. *Methods in enzymology, 62*, 3-11.

Tietz, N.W. (1999). *Text Book of Clinical Chemistry* (3rd Ed.). Burtis C.A. and Ashwood.

Pinto, V. M., Poggi, M., Russo, R., Giusti, A., & Forni, G. L. (2019). Management of the aging beta-thalassemia transfusion-dependent population–The Italian experience. *Blood reviews*, *38*, 100594.

Varley, H. (1967). Ramasays Dipyridyl method for total ironbinding capacity. In *Practical Clinical Biochemistry*, (pp. 475).

Varley, H., Gowenlock, A. & Bell, M., (1979). *Practical clinical biochemistry: hormones, vitamins, drugs and poisons* (5th ed.). Williams Heinemann Medical Books Ltd.

Al-Mosawy, W. F. (2017). The beta-thalassemia. *Scientific Journal of Medical Research, 1*(1),24-30.

Wierusz-Wysocka, B., Wysocki, H., Byks, H., Zozulińska, D., Wykrętowicz, A., & Kaźmierczak, M. (1995). Metabolic control quality and free radical activity in diabetic patients. *Diabetes Research and Clinical Practice*, *27*(3), 193-197.

Chen, Y., Cai, N., Lai, Y., Xu, W., Li, J., Huang, L., ... & Chen, J. (2020). Thalidomide for the Treatment of Thrombocytopenia and Hypersplenism in Patients With Cirrhosis or Thalassemia. *Frontiers in Pharmacology*, *11*, 1137. https://doi.org/10.3389/fphar.2020.01137

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