Study of Biochemical Parameters in Major Thalassemia Patients

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Abstract:

Thalassemia is a genetic disorder that affects the production of hemoglobin, the protein that carries oxygen in red blood cells. This disorder alters some blood and biochemical parameters, leading to anemia and other complications. We conducted a study on 120 thalassemia patients from the Thalassemia Center at Baghdad Hospital in Iraq, from April 2022 to March 2023. The patients had an average age of 13 ± 3.5 years. We measured their hematological and biochemical parameters and compared them with healthy controls. We found that the patients had lower levels of red blood cells (RBC), hemoglobin (Hb), and packed cell volume (PCV) than the controls, and higher levels of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), alkaline phosphatase, and urea. The differences were statistically significant ($P < 0.05$). Our study shows the impact of thalassemia on blood and biochemical parameters and highlights the need for effective treatment and management of this disorder.

Keywords: Thalassemia, Alkaline Phosphatase, Hemoglobin, GOT, GPT

1. Introduction:

Thalassemia (Mediterranean anemia) is a hereditary sickness that is transmitted from parents to youngsters through genes and influences the capacity to supply hemoglobin in the human body, which ends up in excessive anemia (Olivieri, 1999). Thalassemia is the maximum common sickness in the world, specifically in the Middle East and Southeast Asia (Najdecki et al., 1998). Thalassemia is typically identified within the first six months of the new child, and it can be fatal if the patient no longer receives the right remedy (Weatherall, 1965). Children with thalassemia need a blood transfusion every three weeks which allows them to live and stay an everyday lifestyle. This sickness was identified for the first time using the physician Cooley in 1925.

Thalassemia may be divided into:

- Thalassemia minor is whilst the character consists of one copy of the thalassemia beta gene. This reasons slight signs and symptoms of anemia, and the individual can transmit the disease to his or her children (Borgna-Pignatti et al., 2004).
- Thalassemia predominant is when the man or woman has the disease and suggests clear signs of the sickness

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from an early age. This takes place when individuals who deliver the thalassemia beta gene decide to marry and feature youngsters (Vichinsky et al., 2005).

The signs of thalassemia appear within the affected person at some stage in the primary year of age because of the breakdown of blood cells and display severe signs and symptoms of anemia as follows (Abd-Ali and Abd-Reza, 2019):

a) Pale pores and skin with yellowing every so often.
b) Delayed increase.
c) Loss of urge for food.
d) Frequent infections.

As anemia persists, different symptoms seem inclusive of changes inside the shape of the bones, specifically the facial bones and cheeks. The facial functions end up one-of-a-kind of this disorder. Also, there may be an expansion of the spleen and liver and behind-schedule growth of the child. In slight instances (among providers of the disorder), there can be moderate anemia that is not substantive to the patient, and she or he lives a completely everyday life and does not want any remedy. These cases can be detected using chance (Vichinsky et al., 2005).

Thalassemia fundamental does now not have an effect on the fetus within the mom's womb because the fetus has a unique sort of hemoglobin known as fetal hemoglobin, which is different from adult hemoglobin. When the fetus is born, most of the hemoglobin in its frame is of the kind HB of the fetus inside the first six months of age, but the problem of thalassemia lies in the incapability of children to produce enough quantities of adult hemoglobin, so the symptoms of thalassemia principal start to seem in youngsters in the first year of age (Abd-Ali and Abd-Reza, 2019). The most effective very last solution for thalassemia is to carry out a bone marrow transplant, however, the issues of matching blood between the donor and the affected person do now not permit a large number of patients to go through this operation. The nice option to dispose of this ailment is to take away its causes (Saltman, 1989). The lifespan of red blood cells within the bodies of sufferers may be very brief. These cells progressively die, inflicting extreme anemia.

Therefore, thalassemia patients go through periodic blood transfusions to maintain the red blood mobile count number near the desired degree, which improves the affected person's condition and guarantees the shipping of oxygen to the tissues and ordinary growth of the body. Anemia worsens, and the kid's increase is impaired, as a result, the bone marrow expands and becomes unable to produce sufficient red blood cells, and as a result, the shape of the kid's forehead and bones modifications (Zurlo et al., 1989). Blood transfusion every three weeks to preserve ordinary hemoglobin. Daily consumption of the drug "Desferal" below the skin to put off extra iron in the frame earlier than it seeps into exclusive components of the body. In case of excessive splenomegaly, its miles are removed and folic acid is given to produce red blood cells. Bone marrow transplantation. The future treatment for thalassemia is gene therapy (Hong et al., 2013). The danger of neglecting treatment results in (Shah et al., 2013):

a) Severe and chronic anemia.
b) Delay in bodily and mental boom and puberty.
c) Future deformities inside the bones, especially the cranium bones, and this leads to osteoporosis.
d) Enlargement of the liver and spleen, which causes common swelling in the abdomen.
e) Weak immunity.

2. Material and Method:

We are used the following devices and materials in the project:

Table 1 The device and materials used in the project

	Instruments or material	Type or company
1	Plane tubes	AFCO-DISPO
2	Spectrophotometer	Shimadzu -Japan
3	Centrifuge	ROTOFIX 32A
4	Vortex	Hook, USA
5	Water bath	Memmert-Italy
6	Automatic pipette	Oxford - UK
7	Electrical Balance	METTLER TOLEDO
8	Kit for Alkaline Phosphatase	bioMerieux -France
9	Kit for Urea	Thermo Fisher Scientific - American
10	Kit for GOT	Thermo Fisher Scientific - American
11	Kit for GpT	Thermo Fisher Scientific - American
12	Double Distilled Water	Organic Lab.

10.61704/jpr.v24i2.pp54-60

2.1 Blood samples

Blood serum samples were taken from sufferers with thalassemia during their go to Baghdad Hospital for Maternity and Children, Thalassemia Centre, for treatment and care. (120) The samples have been decided on from these sufferers throughout the research period. Their ages ranged from 6 years to twenty years (15 ± 5.2). All those patients had their clinical history taken and blood exams done on them in the blood laboratory. For the period from 8/10/2022 to 8/3/2023, 5ml of blood was drawn from them in the morning. Blood assessments (RBC, Hb, PCV) were executed on the samples within the blood laboratory in the sanatorium. The samples was acquired by putting them in clean and dry test tubes after which putting them in a centrifuge at 4000 rpm for five minutes, wherein the serum became separated from the clot through a micropipette. The serum was changed and positioned in new plastic looked at tubes and saved at -20C until the analysis turned into accomplished. Blood samples were taken from wholesome human beings (fifty-four) for evaluation purposes, considering that their serum is the manipulated sample.

2.2 Methods of measuring some biochemical components and some enzymes

- Method of measuring alkaline phosphatase enzyme:

The basis of this method relies upon the activity of this enzyme inside the alkaline medium consistent with the subsequent equation:

$$Phenylphosphate \xrightarrow[PH\ 10]{Alkaline\ Phosphatase} Phenol + Phosphate$$

The released phenol is measured by using the presence of the reagent four-amino antipyrine and the substance potassium ferricyanide. The presence of sodium arsenate is to inhibit enzyme activity. Then the solution is measured at a wavelength of 510nm. The quantity of absorption is proportional to the amount of released phenol, which depends on the enzyme activity.

- Method of measuring GOT enzyme:

The activity of this enzyme is measured by means of colorimetric techniques consistent with the subsequent reaction equation:

$$Aspartate + \alpha - Ketaglutarate \leftrightarrow Oxaloacetate + glutamate$$

The oxaloacetate shape is measured with the aid of colorimetric methods by reacting it with the reagent 2, four-dinitrophenylhydrazine to shape the hydrazone spinoff. This by-product is measured at a wavelength of 505nm. GOT stands for glutamic-oxaloacetic transaminase, which is also referred to as aspartate aminotransferase (AST). It is an enzyme that catalyzes the switch of an amino group from aspartate to α ketoglutarate, forming oxaloacetate and glutamate. GOT is discovered in numerous tissues, specifically in the liver and coronary heart, and its stage in the blood can indicate tissue damage or disease.

- Method of measuring urea:

The urea was measured using S180 Kit Urea and the premise of this enzymatic method can estimate the attention of urea in each urine and serum. The urease enzyme hydrolyzes urea to ammonia in line with the subsequent equation:

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

In an the alkaline medium, the ammonium ion with salicylate and hypochlorite forms the indophenol reagent (2, 2-dicarboxyindophenol) and which exhibits green color. This reaction is inspired via the presence of the reagent sodium nitroprusside in line as show in the following equation:

$$NH_4 + Salicylate + Hypochlorite \rightarrow Indophenol$$

This reagent can be measured by means of a spectrophotometer at a wavelength of 580nm. The depth of the coloration is proportional to the quantity of urea within the blood serum. Urea is the primary give up made from nitrogen metabolism in people and most mammals. It is synthesized in the liver from ammonia and carbon dioxide, and then transported to the kidneys for excretion in urine.

10.61704/jpr.v24i2.pp54-60

– Method of measuring GPT enzyme:

The activity of this enzyme is measured with the aid of colorimetric techniques through the following response:

$$Alanine + \alpha - Ketoglutarate\ Pyruvate \xrightarrow{GPT} Glutamate$$

The hydrazone derivative is formed with the aid of reacting with 2, four-dinitrophenylhydrazine. This reagent is measured with the aid of a spectrophotometer at a wavelength of 505nm. The absorbance is proportional to the interest of this enzyme. GPT stands for glutamic-pyruvic transaminase, which is also called alanine aminotransferase (ALT). It is an enzyme that catalyzes the switch of an amino institution from alanine to α-ketoglutarate, forming pyruvate and glutamate. GPT is in particular determined in the liver and its degree within the blood can indicate liver harm or sickness.

3. Statistical Analysis:

The outcomes were analyzed the usage of the SPSS 18 statistical software program to determine the implied and preferred deviation, in addition to different variables. The differences were considered sizeable when $p < 0.05$.

4. Result and Discussion:

Table 2 represents the values of blood tests for all patients with thalassemia and healthy people.

Table 2 The values of blood tests

Variable		n	Mean ± SD	Range		SE	95%C.I.		*P* value
				Min	Max		lower	upper	
RBC (10^{12}/L) Patients	♂	100	3.52±0.97	2.0	5.60	0.18	3.15	3.91	p<0.05
	♀	15	3.58±0.85	2.9	4.8	0.19	3.15	3.97	
RBC (10^{12}/L) controls	♂	15	4.64±0.24	4.22	5.10	.06	4.50	4.74	p<0.05
	♀	12	4.54±0.15	4.4	4.85	0.04	4.49	4.65	
Hb for Patients (g/dl)	♂	100	8.87±2.6	6.0	15.5	0.46	7.70	9.65	p<0.05
	♀	15	8.54±2.5	5.80	12.70	0.55	7.25	9.50	
Hb for controls (g/dl)	♂	20	13.25±0.5	12	14	0.13	12.8	13.50	p<0.005
	♀	15	12.70±0.44	12.10	13.30	0.12	12.35	12.85	
PCV for Patients	♂	100	28. 71±8. 21	17.0	49	1.47	25.70	31.72	p<0.005
	♀	15	28.35±7.55	16.0	42	1.68	24.80	31.85	
PCV for controls	♂	15	43.77±1.45	42.0	46.0	0.37	43.85	43.45	p<0.005
	♀	15	42.23±1.43	41	45	0.41	41.30	44.10	

From Table 2, which indicates the blood tests for thalassemia patients in comparison with healthful people, it is clear that there are vast differences among the values of the sufferers and the values of the healthy human beings in phrases of PVC, RBC, and Hb. The value of p becomes less than 0.05 for all the variables. The RBC values for the patients, each male and female, were (3.53 ± 98) and (3.59 ± 0.8) million cells consistent with milliliter, respectively, while the RBC values for the healthy people, each woman, and man, were (5.14 ± 0.55) and (5.25 ± 0.65) million cells in step with milliliter, respectively.

As for the hemoglobin values, they were (8.68 ± 2.5) g/100 ml and (8.42 ± 2.4) g/100 ml for the patients, each ladies, and men, respectively, whilst they were (13.26 ± 0.6) g/a hundred ml and (12.69 ± 0.42) g/100 ml for the healthful humans, both males, and females, respectively.

As for the packed mobile quantity (PCV) values, while evaluating those values, there have been different substantial differences. The PCV values for the patients, each woman, and man, have been (28.7 ± 8) g/a hundred ml and (28.35 ± 5.7) g/100 ml, respectively, whilst they were (43.66 ± 1.44) g/a hundred ml and (42.1 ± 1.25) g/one hundred ml for the wholesome human beings, each male and females.

The blood tests that are finished to detect on thalassemia include a complete blood count (CBC), blood smear, iron

10.61704/jpr.v24i2.pp54-60

studies, and hemoglobin electrophoresis. These tests determine the variety, size, shape, and type of pink blood cells and hemoglobin, in addition to the iron stages and usage in the frame. Thalassemia sufferers usually have low RBC, hemoglobin, and PCV values, as well as strange crimson blood cell morphology and hemoglobin sorts (Shah et al., 2o13).

The remedy of thalassemia relies upon the severity of the condition. Mild forms of thalassemia might not require any remedy, whilst mild to extreme forms may additionally need common blood transfusions, iron chelation remedies, and stem mobile transplants. Blood transfusions help increase the hemoglobin and oxygen levels within the blood, however, additionally, they cause iron overload, which could damage the organs. Iron chelation therapy is a treatment that removes extra iron from the body. A stem cell transplant is a procedure that includes replacing faulty bone marrow cells with healthy ones got from a compatible donor (Neufeld, 2010).

Table 3 Represents some biochemical variables of serum blood for thalassemia patients and healthy people.

Variable	n	Mean ± SD	Range		SE	95% C.I		*p value*
			Min	Max		lower	upper	
GOT (U/ml)								
Patients	100	13. 5± 8.55	5.0	46.0	0.78	12.0	15.5	*p>0.05*
Controls	50	37.6±4.48				23.5	53.4	
GPT(U/ml)								
Patients	120	18.70±3.95	5.0	154	1.7	15.0	22.5	*p>0.05*
Controls	55	111.7±13	26	165	22	66	156	
Alkp U/L								
Patients	70	170±30	72	252	12.60	145	195	*p>0.05*
Controls	25	83±25	50	132	5.55	70	94	
Urea mmol/l								
Patients	75	5.15±2.25	2.50	9.10	0.25	4.65	5.55	
Controls	35	3.3±0.63	2.20	4.60	0.11	3.10	3.50	

From Table 3, which represents a few biochemical variables inside the serum of thalassemia sufferers, we are found that there are significant variations in both enzymes GPT and GOT as compared to healthy human beings. The suggested values of the enzyme GOT have been (8.59 ± 13.8 U/ml) for the sufferers and (4.4 ± 38.6 U/ml) for the wholesome human beings as shown in figure 1, and the value of p became less than zero.05. As for the suggested values of the enzyme GPT, they were (18.77 ± 3.9 U/ml) for the sufferers and (111.7 ± 13 U/ml) for the wholesome humans as shown in figure 2, and the value of p was much less than 0.05. These enzymes are distributed in lots of tissues of the human body, and the enzyme GOT is greater active than the enzyme GPT and the first enzyme is more abundant within the heart, liver, skeletal muscles, and kidney tissues, even as the liver includes massive quantities of the enzyme GPT and other tissues together with the kidney, heart and skeletal muscle mass incorporate considerable amounts of this enzyme.

The causes of the lower of these two enzymes beneath the normal stage for healthy people can be because of the quantities of iron present inside the serum of the sufferers, which can be deposited in those organs, resulting in the breakdown of fat of some cells of these organs.

As for the alkaline phosphatase enzyme, the effects of the studies showed a huge boom inside the pastime of this enzyme in comparison to the interest of the enzyme inside the serum of wholesome humans. The suggested cost of this enzyme inside the serum of thalassemia patients was (171 ± 29 U/ml), at the same time as the activity of this enzyme in the serum of healthy people turned into (83 ± 25 U/ml) as shown in figure 3, and therefore there has been a sizeable distinction as the value of p was less than 0.05. The motive for this may be due to the reality that the maximum of the activity of this enzyme comes from the bone tissue and because thalassemia sufferers suffer from the breakdown of this tissue, this results in the leakage of this enzyme into the bloodstream and that reason

10.61704/jpr.v24i2.pp54-60

growth in the interest of this enzyme.

As for urea values, the study showed that there is a significant difference between the average urea values for patients compared with healthy people, the value was $p < 0.05$. The average urea values in the serum of thalassemia patients were l/mmol (24.2 ± 14.5), while the average urea values in The serum of healthy people were l/mmol (60.0 ± 33.3) as shown in figure 4. The reason for this increase in the amount of urea may be due to due to the presence of large amounts of iron in the serum of these patients, this will lead to the deposition of amounts of iron in the kidney tissue, which leads to the loss of the most important function of the kidney, which is renal filtration, where waste products come from metabolic processes through the blood to the renal glomeruli and then filtered there through small tubules. There may be a reason through which these results can be explained, which is the state of oxidative stress. Which happens to these patients.

The presence of large amounts of iron in thalassemia patients will lead to the formation of a hydroacyl radical through A well-known reaction is the reaction fenton reaction.

$$Fe^{+2} + H_2O_2 \rightarrow OH^- + OH + Fe^{+3}$$

This free radical is considered one of the strongest free radicals in the cell, so we see it attacking all biomolecules, it seems from fat that Proteins and DNA, which leads to cell death, in addition to other effects (Bazvand et al., 2011). This presented research, includes the study of the enzyme ALP (phosphatase Alkaline in thalassemia patients. Which is one of the hereditary blood diseases characterized by a low percentage of blood within the hemoglobin of red blood cells, where the ALP enzyme is found in the liver and bone marrow, and the upper part of the basophosphate enzyme in the blood serum comes from These two tissues (bone marrow-liver) are secreted into the circulatory system, so the high activity of this enzyme is often what is due to the pathological conditions of these two tissues or perhaps the reason is due to the effects of iron on liver cells, perhaps Increased iron triose may destroy liver cells, which leads to the transfer of this enzyme in large quantities to the bloodstream and thus To increase the effectiveness of this enzyme compared to healthy people, as the rate of activity of this enzyme for sick people (171.64) L / (U) with the values of healthy people, whose activity rate of this enzyme was 83.07 (L / U) and this The results were consistent with previous Literature conducted on Thalassemia patients, where the researchers found the enzyme Alkaline phosphate has a high moral elevation of $p < 0.001$ in infected children and for both sexes if its value reaches to 150 ± 1366 L / U at the Anamnesis of people with the disease vs. L \ U. 30.60 at healthy males (Munir et al., 2013).

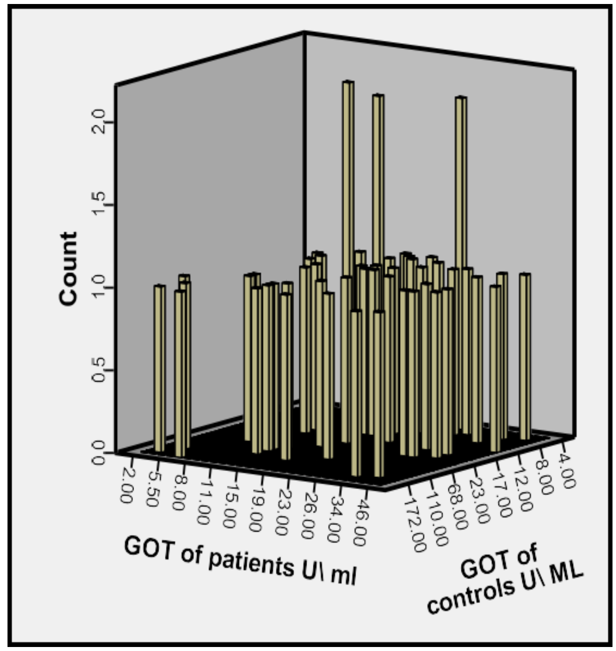

Fig. 1. The GOT enzyme values of patient's vs. controls

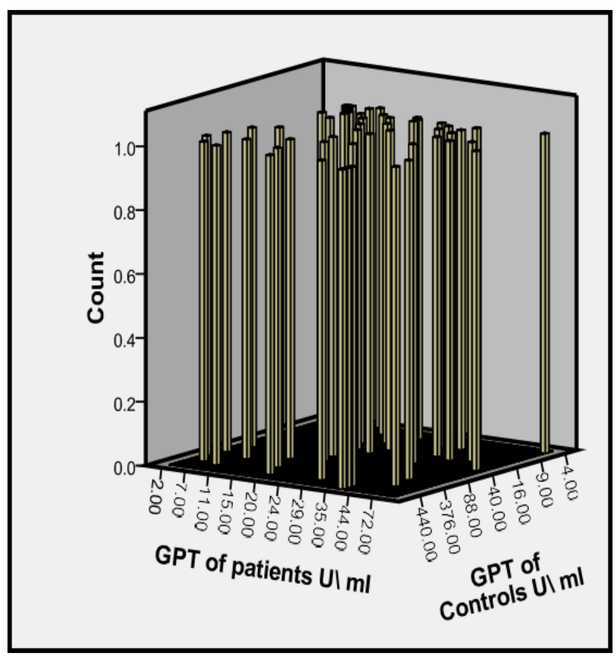

Fig. 2. The GPT enzyme of patient's vs. controls

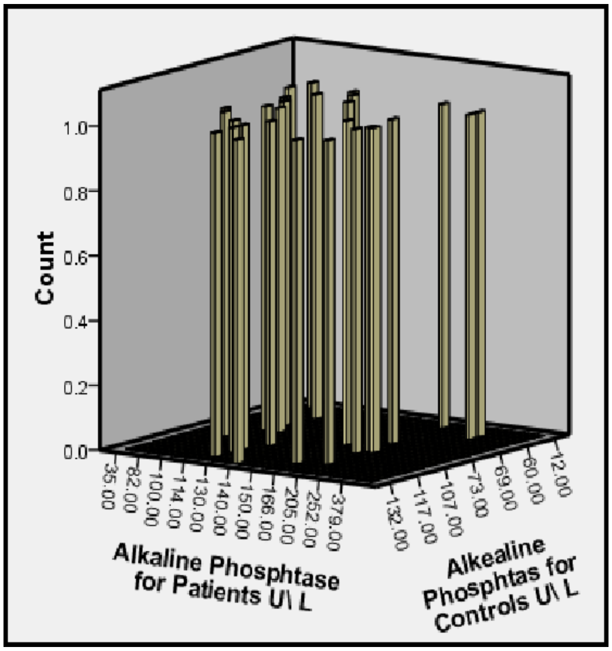

Fig. 3. The alkaline phosphatase values of patient's vs. controls

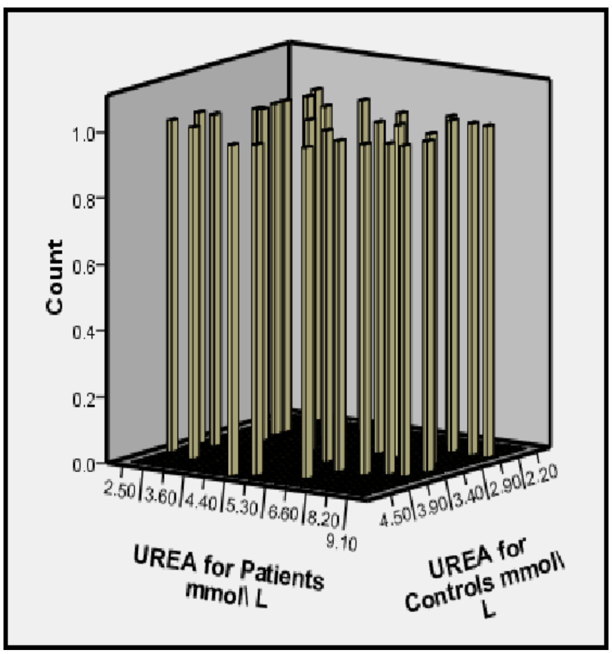

Fig. 4. The urea values of patient's vs. controls

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