

Level of Serum Melatonin & Sialic Acid in Patients with Beta-thalassemia versus Normal Individuals

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Symptoms of thalassemia minor may begin to manifest as early as infancy or later in life. In addition to mild to severe anemia, patients with this disorder may also present with developmental delays and skeletal abnormalities. The main objective of the subsequent investigation was to assess and compare blood levels of melatonin and sialic acid in patients with Beta-thalassemia and healthy individuals. This case-control study compared the serum levels of healthy individuals with those of Beta-thalassemia patients. The total sample size of the study was 104. Out of these 52 individuals were grouped under the category of control group which had healthy individuals. The other group also consisted of 52 individuals who were diagnosed with Beta-thalassemia. These results indicated that there was a significant decrease in serum melatonin as in the controlled group it was 12.07 pg/ml and in the case group it was found as 7 pg/ml, $p < 0.01$. The value of Ceruloplasmin was also seen significantly different, as the level of Ceruloplasmin increased in the case group (34.13 mg/ml) as compared to the healthy individuals (51.08 mg/ml). Additionally, the other major findings were the fall of Hb in the case group which was 7 g/dL as compared to the control group where the means was 12.56 g/dL. In conclusion melatonin and sialic acid could be added to the list of measured parameters in patient with beta-thalassemia and might be served as a marker for diagnosis and prognosis.

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Beta-thalassemia is a disorder where the production of hemoglobin is reduced.¹ All cells in the body receive oxygen through hemoglobin, a protein found in red blood cells, that helps deliver oxygen throughout human body. But, unfortunately, for those who suffer from -thalassemia, their hemoglobin levels may be dangerously low. This means that their body does not receive enough oxygen, which can lead to a whole host of health problems.^{2,3} In addition, affected individuals have anemia, which can have more serious effects such as pale complexion, weakness, and fatigue. There are three types of beta thalassemia, which are categorized based on the severity of the symptoms they cause. Thalassemia major, also known as Cooley's anemia, is the more severe of the two types, and can lead to life-threatening complications. Meanwhile, thalassemia intermedia and thalassemia minor are less severe, but still require proper management and care.⁴

During the first two years of life, the main signs and symptoms of thalassemia begin to manifest themselves.⁵ Anemia in children can be fatal. They fail to thrive because they cannot gain weight and grow as fast as normal, as well as may develop yellowish discoloration of the sclera and the skin. Affected individuals may have misaligned bones and enlarged spleens, livers, and hearts. Adolescents with thalassemia major may have delayed onset of puberty.^{6,7} Many thalassemia major patients endure severe pain and often require blood transfusions to replenish red blood cells.⁸ Long-term blood transfusions can cause iron to build up in the body, negatively affecting the liver, heart, and hormones.

The last carbohydrate residue of many glycoproteins is Sialic acid. The shape of glycoproteins is maintained by negatively charged polar molecules, which are essential for the function and durability of the protein.^{9,10} Desialylation is like the villain who initiates the catabolism of glycans by removing the terminal sialic acid residues on glycans. This process is a pivotal part of sialic acid metabolism and has a significant impact on the structure and functions of glycans, including glycoprotein.

Desialylation of proteins results in the loss of sub-terminal galactose residues, which leads to the removal of the protein from circulation by galactose receptors in the liver, leading to early termination.¹¹ Compared to thalassemia major,

thalassemia intermedia is less severe. Symptoms of mild thalassemia may first appear in infancy or later in life. Affected individuals may have developmental delays and bone abnormalities in addition to mild to moderate anemia.¹² In a recent investigation, blood samples from 18 patients with thalassemia major and 10 controls were examined to determine the sialic acid content of red blood cell membranes.¹³ Among patients who underwent splenectomy several years ago, only four had a lower-than-average salivary acid to protein weight ratio. In the 14 patients who were still alive but did not have their spleen removed, the salivary acid/protein ratio was 50% higher than usual.^{14,1} The primary purpose of the following study is to evaluate the level of serum sialic acid and melatonin of the patients with Beta-thalassemia and compare it with the levels of normal individuals.

PATIENTS AND METHODS

The study is a case-control research comparing the blood serum levels of Serum Melatonin and Sialic Acid in normal individuals and compared to blood serum levels in the patients with Beta-thalassemia.

In total, 104 subjects were enrolled, with half of these being healthy controls, and the rest being individuals who suffered from Beta-thalassemia.

Laboratory analysis was performed on blood drawn in the morning after a 10-hour fast. Informed consent signed and collected from all participants, blood withdrawn and serum collected, frozen at -20°C until analysis. Serum melatonin levels were quantified by enzyme-linked immunosorbent assay (ELISA) using a kit. Haematological parameters were obtained with an automated hematology analyzer (BC-300 PLUS - Mindray, China). Serum total sialic acids was measured by colorimetric method using commercial kit (Elabsience, USA).

The evaluated parameters included Melatonin(pg/ml), Ceruloplasmin [mg/ml], Copper [μmol/L] and Selenium μg/L. The study further evaluated PCV (%), WBC (x10⁹/L), Monocytes(x10⁹/L), Hb (g/dL), Lymphocytes (x10⁹/L), and Neutrophils (x10⁹/L).

All statistical analyses were performed using SPSS. From the observed parameters, the continuous variables were expressed as mean ± SD and compared by one-way analysis of variance (ANOVA) and post hoc test in Turkey. The significance level was adjusted to P < 0.05 and confidence interval 95%.

Table 1 Serum measured parameters between studied groups

Parameters	Serum		p value
	Group A(Control)	Group B	
Melatonin (pg/ml)	12.07 ± 4.74	7 ± 3.52	0.01
Sialic acid (mmol/L)	1.61±0.13	3.1±0.51	0.001
Ceruloplasmin (mg/ml)	34.13±2.74	51.08±4.97	0.001
Copper (µmol/L)	13±2.98	25±2.24	0.001
Selenium (µg/L)	51. ± 19	39.87±21.2	0.001

RESULTS

The serum levels of the measured parameters are presented in [Table 1](#). There was a significant difference between the controls and the patients with Beta-Thalassaemia.

The haematological parameters are presented in [Table 2](#). Again, these was a significant difference between the two groups.

There was a significant decrease in serum melatonin (controls: 12.07 pg/ml vs case group: 7.0 pg/ml, $p < 0.01$). The levels of sialic acid and ceruloplasmin were significantly different, as the level of total sialic acid and ceruloplasmin increased in the case group (3.1mmol/L, 51.08mg/ml, respectively) as compared to the healthy individuals (1.61mmol/L, 34.13mg/ml, respectively). As expected, there was a drop in haemoglobin levels in the case group as compared to the control group (control: 12.56 g/dL vs case group: 7 g/dL).

DISCUSSION

This study showed that the melatonin levels in the blood serum significantly decreased in the patients with Beta-thalassemia. Such findings

have been discussed in multiple studies previously. It has been found that red blood cells (RBCs) or erythrocytes are particularly susceptible to oxidative stress due to the lack of a nucleus and mitochondria, the presence of iron-containing hemoglobin, and the presence of large amounts of fatty acids in their specially designed lipid bilayer membranes.^{16,17} The main function of RBCs is to deliver oxygen to the tissues.¹⁸ Therefore, red blood cells must flow through the microvasculature, which requires high structural deformability and elasticity of red blood cells. The deformability of erythrocytes depends on the availability of a healthy cytoskeletal framework and fluid membrane.¹⁹ The structural and functional composition of erythrocyte homeostasis may be disturbed by various conditions. One of them is ROS, which causes hemolytic anemia in thalassemia patients and leads to oxidative damage of red blood cells. Melatonin is a potent free radical scavenger and antioxidant that successfully protects red blood cells from oxidative damage.²⁰ Melatonin also enhances the gene expression of antioxidant enzymes in red blood cells and chelates free iron. Erythrocytes produce and store large amounts of

Table 2 Haematological finding between studied groups

Parameters	Serum		p
	Group A(Control)	Group B	
PCV (%)	37.87±3.76	23.1± 4.79	0.001
Hb (g/dL)	12.65 ± 1.3	7± 1.4	0.001
WBC (x 10 ⁹ /L)	5.6±2	13.2± 7.98	0.001
Monocytes (x 10 ⁹ /L)	0.86±0.3	1.77±1.3	0.001
Lymphocytes (x 10 ⁹ /L)	2.84 ± 0.68	5.86 ± 2.01	0.01
Neutrophils (x 10 ⁹ /L)	2.43±0.76	5.97±0.67	0.01

melatonin, which has a local protective effect. All this suggests that melatonin is a significant factor in safeguarding the structural and functional integrity of red blood cells.²¹

The findings also showed that haemoglobin levels was markedly reduced in the patients of Beta-thalassemia. This is not unexpected, since beta-thalassemia is known to have such an effect. Beta-thalassemia patients have low levels of hemoglobin, resulting in oxygen shortages in several body regions.²² In addition to this, low hemoglobin levels prevents sufficient oxygen from reaching tissues. Recent studies indicate that patients with beta-thalassemia are more prone to developing atypical blood clots, which can be detrimental to their overall health.²⁴

Finally, a fascinating discovery was made during research. Scientists found that thalassemic erythrocyte membranes have approximately 25% less glycolic acid compared to normal erythrocyte

membranes. Through the process of density centrifugation, they also observed that glycoconjugate from aged thalassemic erythrocytes had only half the level of sialic acid compared to that of young thalassemic erythrocytes. These findings shed light on the unique characteristics of thalassemic erythrocytes and offer new insights into the nature of glycoconjugate in aging cells.²⁵ Thalassemic erythrocytes' plasma and erythrocyte membranes were examined for potential sialic acid activity. In contrast to plasma from healthy donors, there was no evidence of enhanced sialic acid activity in patient plasma. The enhanced sialic acid hydrolysis in erythrocytes with thallasemia should thus be explained by additional locations of sialic acid activity or other scenarios.²⁶

CONCLUSION

The study has shown that the level of melatonin of the patients with Beta-thalassemia is reduced, whereas the levels of sialic acid is increased.

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