ON BETA THALASSEMIA MUTATIONS, LABORATORY FINDINGS AND TREATMENT OF ALBANIAN PATIENTS

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ABSTRACT

The β-thalassemia syndromes (major and intermedia) are caused by mutations that result in the reduced or non-production of beta- globin chains. More than 300 disease-causing mutations have been identified so far. Here, the chronic anemia prevails among all the of thalassemia syndromes. It is estimated that 1,5 % of the world's population has β-thalassemia, while in Albania this prevalence goes to approximately 7 %, (ranging from 7-11% in particular areas). The treatment protocol of patients has significantly improved in the last decades. In the present study there are 65 patients involved to investigate their molecular diagnosis of beta -thalassemia and to correlate these data with their follow up. The IVS-I-110 mutation is found in 37% of the patients. The same data are reported for the East Mediterranean region. Cod 39, a severe mutation responsible for severe clinical outcomes in patients, especially with homozygote manifestation, is found in 18% of the patients. So, patients with this mutation-s type have mean hb level 8,1 compare to mean hemoglobin level in total group which is 8,5 g/dl. This is statistically significant. (t= 1,954, p=0,05). The mean number of PRBC-s / month is 3,8 units of PRBC compared to the total of patients' needs which is 3,1 units of PRBC. A meticulous follow up of these patients, taking into account their molecular presentation at the beginning, is an important predictor for the future outcome.

***Keywords*:** β - thalassemia syndromes, allele, mutation, pure red blood cells lactate dehydrogenases, Ca 15-3, indirect bilirubin

1. INTRODUCTION

Haemoglobinopathies are very serious clinical conditions caused by genetic mutations. They represent the commonest monogenic diseases in the world.

The recombinant DNA techniques have been used for the for a better understanding of thalassemia syndromes (including beta thalassemia and sickle cell disease), the first diagnosed diseases from the intrauterine life. Consequently, the treatment protocol of patients has significantly improved.

There 530 patients with haemoglobinopathies registered in Albania.

Continuous monitoring of biochemical indexes is important for the follow up of the patients with haemoglobinopathies.

Haemoglobinopathies (β thalassemia and sickle cell disease) refer to a heterogeneous inherited group of hemoglobin, characterized by a quality or quantity deficit of hemoglobin synthesis.

Haemoglobinopathies are part of autosomal recessive diseases, the most prevalent genetic blood diseases in the world (Ther *et al.*, 2006). As people affected from these types of diseases suffer from a chronic hemolytic anemia throughout their life, permanent regular blood transfusion therapy is a crucial to them.

The Thalassemia International Federation stated that there are approximately 500 000 patients with beta thalassemia registered in treatment and follow-up. There are more than 100 000 patients suffering from sickle cell disease worldwide (data are mainly collected from USA database) (Kountouris *et al.*, 2014).

It is estimated that nearly 7,1 % of the population is a carrier of any type of haemoglobinopathies in Albania. Currently, there are at least 530 patients (children and adults) diagnosed and in treatment in the country. The majority of our patients are diagnosed major and intermediate with beta thalassemia (84,5%), and the reminder (15,5%) diagnosed with the sickle cell disease. It is not a gender-based disease. There are approximately 300 genetic mutations of β thalassemia. There are 20 most mutations manifested among the Mediterranean population. 12 out of 20 mutations appear to decrease the beta globin chain synthesis, while 8 mutations seem to be responsible for the absence of beta globin chain **(**Antoniou and Grosveld 1999**).**

Wonke (2001) said that there are a lot of clinical manifestations which vary from mild to severe anemia, and could be fatal if systematically untreated. Patients with anemia during their treatment with blood intake, manifest high ferritin levels in blood, developing iron overload in major organs not only due to frequent transfusions, but also to the increased

intestinal absorption. Iron overload causes cardiac, hepatic, endocrine dysfunction. In addition, inappropriate treatment might be fatal.

Kountouris *et al*., (2014) stated that appropriate treatment improves significantly peoples living conditions**.** Today, their longevity goes to 60 years. Consequently, frequent monitoring and assessment of laboratory data are important.

Hemolysis, the destruction of red blood cells and the release of their contents (cytoplasm) into surrounding fluid (e.g. blood plasma), is a typical phenomenon of the desease. Here, indirect bilirubin, LDH and tumor marker Ca 15-3 are of crucial importance (Taher *et al.*, 2010).

The present study aims to give some insights on the molecular diagnosis of the beta thalassemia mutations, laboratory findings and treatment of 65 randomly selected Albanian patients aged between 2 - 18 years, and to correlate these data with their follow up. The consent for data publication has been given.

2. MATERIALS AND METHODS

In the present study 65 patients with haemoglobinopathies are randomly selected. They are followed up only at our clinic and their consent for data publication has been given. Other pathologies are excluded in this group of patients who are aged between 2 - 18 years.

Data on beta thalassemia mutations are here reported, and blood samples for Ca15-3. LDH, Indirect Bilirubin and pre transfusion level of haemoglobine collected. Laboratory investigation is routinely carried out at the laboratory of the University Hospital Center ‘Mother Teresa’ and Laboratory of National Center of Blood Transfusion in Tirana, Albania.

3. RESULTS AND DISCUSSIONS

The chart 1 plots demographic distribution of the patients showing that the majority of the patients comes from western Albania probably due to plasmodium falciparum here present in the past which confirms the hypothesis of this co-existence.

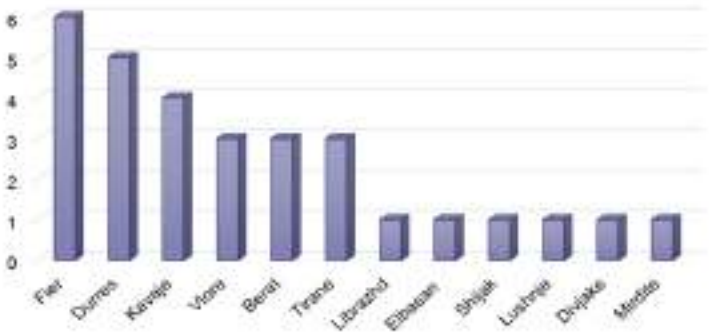


Chart 1. Demographic distribution of the patients.

Table 1. Types of beta-thalassemia mutations among the patients

MILD MUTATIONS ß ++	MODERATE MUTATIONS ß +	SEVERE MUTATIONS ß °
-101 -87 -88 -28 -IVSI-106	**IVS I-110 (36,4%)** **IVS I-6 (18,3%)**	**IVS I-1 (9,1%)** **Cod 39 (18,3%)** **Cod 44 (13,6%)** **IVS 1-116 (4,5)**

Table 1 informs about the types of beta-thalassemia mutations among the patients. It could be noted that the IVS-I-110 mutation is the most frequent mutation among the patients by counting 37% of patients.

Table 2. The mean value of pre -transfusion hemoglobin level

	Gender	Number of patients	Mean value	S.D.
Hb	Male	37	**7,64**	1,00
	Female	28	**7,65**	1,07

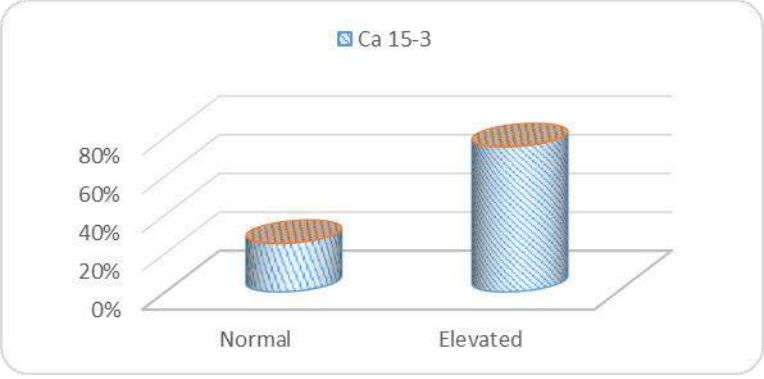
Table 2 reports the mean value of pre -transfusion hemoglobin level. As it could be noted both males and females patients manifest very low pre-transfusion hemoglobin levels due to severe hemolysis caused by the mutation, lack of blood donation, especially in summer, and rare antigenic structures of rhesus blood groups.

Table 3. Codon 39 mutation, hemoglobin level and quantity of blood needed

	Patients with Cd 39 mutation	Number of patients	t	p
Hb (g/dl)	8.1 (0.9)	8.5(0.7)	1.9	0.03
PRBC (n/month)	3.8 (1.1)	3.1(1.1)	2.3	0.02

**PRBC- Pure Red Blood Cells*

Patients with genotype Cd39, have low hemoglobin level compared to total number of patients which is statistically significant (independent sample t test statistic =1.954 p= 0.03). Therefore, they need more blood units (PRBC) in order to cope with a qualitative life (independent sample t test statistic = 2.33 p= 0.02).



Graph.2 Ca 15-3 indicator in patients with haemoglobinopathies.

Ca 15-3 is present in 75% of the patients, compared to 25% of patients who have normal values.

Table 4. The mean value of Ca 15-3 in patients with haemoglobinopathies.

	Gender	Number of patients	Mean	S.D.
Ca 15-3	Male	37	**49,08**	17,6
	Female	28	**48,20**	17,2

Males exhibit slightly higher mean value of Ca 15-3 than to females (49,08 vs. 48,20).

Table 5. The mean value of LDH in patients with haemoglobinopathies

	Gender	Number of patients	Mean	S.D.
LDH	Male	37	**696,7**	414,4
	Female	28	**879,6**	494,4

Table 5 shows a higher LDH value in females compared to males.

The majority of patients manifest double and triple LDH level. The number of patients with normal values is 15 patients, there are 12 patients with double LDH value and there are 16 patients with triple LDH value.

Table 6. The mean indirect bilirubin level in patients with haemoglobinopathies

	Gender	Number of patients	Mean value	S.D.
Indirect Bilirubin	Male	37	**1,95**	0,87
	Female	28	**2,96**	2,32

A student test has been made for two separated variables. It is estimated that there is no significant statistical difference between patients (male/female) for all variables studied in the confidence interval 95%.

Table 7. Student test used to compare hemolytic indicators

	t value	p
Ca 15-3	0,127	0,99
LDH	-1,428	0,19
Indirect Bilirubin	-1,988	0,05
Hemoglobin	-0,034	0,99

There is a significant correlation between high levels of Ca 15-3 and high levels of LDH, indirect bilirubin and the low levels of pre-transfusion hemoglobin ($p<0,05$).

Patients who do not undergo transfusion based on their needs have high levels of tumor marker Ca 15-3.

There is a good correlation between the high values of Ca 15-3 and the high values of LDH, indirect bilirubin and low hemoglobin level ($p<0,05$).

Patients with haemoglobinopathies have high levels of Ca 15-3, probably related to ineffective erythropoiesis at bone marrow level due to the accelerated turnover of erythroblasts. The latter is closely related to the high levels of other hemolytic biological parameters like: LDH and mostly indirect

bilirubin. Ca 15-3 is found at higher levels in 75% of the patients, while 25% of the patients have normal levels of the Ca 15-3.

Albania reports a high prevalence of haemoglobinopathies alike in other Mediterranean countries. The guidelines provided by the Thalassemia International Organization are followed for the prevention and treatment of blood disorders. So, the most common treatments for this disease are blood transfusion plus iron chelation (BTIC) therapy and bone marrow transplantation (BMT). Consequently, patients using these procedures experience different health-related quality of life (HRQoL).

4. CONCLUSIONS

The haemoglobinopathic data here reported reflect our clinical and laboratory practices.

The hemolytic data of the 65 patients are here evaluated and compared to other neighboring Balkan and Mediterranean countries. Here we could mention the frequencies of the most common β-thalassemia alleles (IVS-I-110, Codon 39, IVS-I-6) **(**Kohler et al., 2021**).** Babameto-Laku *et al*., (2011) and Aessopos *et al*., (2014) stated that the IVS-I-110 mutation has its highest frequency in the east Mediterranean region (Cyprus, Lebanon, Greece, Republic of Macedonia, Turkey), whereas the codon 39 mutation is found mainly in the west (Sardinia, Sicily, Spain).

The correlation between type of genetic mutation and some laboratory data are a means to address appropriate management of beta thalassemia syndromes. The genotype Codon 39 is a severe mutation (the third as per percentage among our patients) People with this genotype (Codon 39) manifest lower pre transfusiion hemoglobin level compare to total group of patients, and this is statistically significant (t= 1,954, p=0,05)

These patients need more blood compared to total of patients and this is statistically significant: t=2,33 and p=0,02.

The mean number of pure red blood cells per month is 3,8 units compare of the total of patients' need. All these results are similar with those of literature, especially data reported from Mediterranean countries **(**Cappellini *et al*., 2019**).**

The Ca 15-3, LDH, indirect bilirubin and level of pre transfusion hemoglobin are in the present study evaluated and compared with (Antoniou and Grosveld 1999; Wonke 2001; Taher *et. al*., 2010) where it is stated that hemolysis is always present in our patients.

The tumor marker Ca 15-3 is found at higher levels in 75% of the patients, while 25% have normal levels of the tumor marker Ca 15-3.

The majority of patients showed higher LDH level (70%).

The mean hemoglobin values was 7,64 in males and 7,65 in females.

There is a good correlation between the high values of Ca 15-3 and the high values of LDH, indirect bilirubin and low hemoglobin level ($p<0,05$).

Kreka (2017) stated that patients with hemoglobinopathies manifest higher levels of Ca 15-3, which might be due to ineffective erythropoiesis of bone marrow. This marker is induced by an increased erythroblasts apoptosis. Cappellini *et al.*, (2019) stated that hemolysis always present is supported scientifically by the high values of LDH, indirect bilirubin, and low levels of hematocrit and hemoglobin.

The treatment protocols and guidelines followed have been updated across the years. Consequently, their longevity goes to 60 years.

5. RECCOMENDATIONS

Blood supply remains the most efficient therapeutic treatment. Blood donation is a continuous need.

Iron chelation therapy has significantly improved their quality of life and patients longevity (removing iron overload from patient's organs).

On the other hand, the improvement of personalized treatment using genetic modifiers still in trials and other specific drugs seem to be of a great benefit for all patients with haemoglobinopathies (Stephanou *et al.*, 2019).

Collaboration among specialists is of irreplaceable importance

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