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Comparison of biochemical parameters of prevalent hemoglobinopathies with healthy individuals

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Abstract

Thalassemia is the most frequently seen monogenetic disorders around the world that is inherited as a recessive single-gene disease, resulting from mutations in α-or β-globin gene clusters. In this study Beta Thalassemia Intermedia, Beta Thalassemia Minor, Sickle Cell Disease patients and the healthy control individuals both incidence and serum parameters were examined and analyzed retrospectively between 2015-2017 from Public Health information system. Beta Thalassemia Intermedia, Beta Thalassemia Major, Beta Thalassemia Minor and Sickle Cell Disease patient groups incidences were evaluated on the basis of their HPLC analysis. Patients and control groups serum parameters levels were analyzed from their autoanalyzer results. These serum biochemical tests datas were controlled and taken from Siirt Public Health Centre information system. In each year, there were recorded approximately 12 Beta Talassemia patients, 2 Beta Thallassemia Major patients, 340 Beta Thalassemia Minor patients and 33 Sickle Cell Disease patients.Beta Thalassemia Intermedia patients serum parameters levels were compared with Beta Thalassemia Minor Patients serum parameters and control group serum levels. There were seen statisticaly significant difference between Beta Thalassemia Intermedia patient's serum ast, albumin, creatine, amylase, phosphor, iron, iron binding capacity, total protein, sodium, bilirubin direct, bilirubin total, LDH level and control serum parameters levels, $p<0.05$. But there were not seen statistically significant differences between Beta Thalassemia Minor patients serum levels and control serum levels. Studies like this should be done nationwide, in each city to determine the current state of abnormal hemoglobinopathies in Turkey. Thus, a broader, comprehensive national screening program is needed.

Keywords: Hemoglobinopathy, beta thalassemia ıntermedia, anemia, beta thalassemia minor

Introduction

β-Thalassemia (BT) is an hereditary blood disease caused by more than 200 gene mutations, mainly noticed by antenatal detection and screening. BT is a crucial common health issue in Mediterranean territories and in Turkey. As stated by World Health Organization (WHO) evaluation, the incidence of BT carriers is 5.1% global and change across countries as well as province within countries. The recognition of BT is frequently set by clinical and laboratory detections [1]. An inherited haemoglobinopathy, Sickle Cell Disease (SCD) is, described by haemolysis and microvascular vaso-occlusion which lead to a serious clinical status with complicated pathophysiology and a marked phenotypic variability. The latest indicates the interaction of many factors which involve leukocyte dysfunction, platelet interactions with endothelial cells, increased levels of circulating pro-inflammatory cytokines, oxidative stress, endothelial damage, reduce effectiveness of nitric oxide, activation of haemostasis and amplify microvascular thrombosis [2]. BT is a single gene disorder that is most often caused by mutations in the genetically transmitted α or β globin gene cluster. Approximately 7% of the world's population is a carrier of a globin gene mutation. It places great responsibilities on families and society. A fetus with β-Thalassemi major (BTM) does not live. It dies in the uterus or shortly following birth, endangering the mother's health. BTM leading to a severe progressive anemia, usually beginning 3-6 months after birth. The majority of BTM die by the age of 5 if they are not treated with a orderly transfusion program and chelation cure [3]. Mutations of BT are classified as serious, light and quite and may give rise to variable clinical and hematological phenotypes. Hematological phenotypes range

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from symptomless carrier to heavy transfusion-dependent type. According to increasing severity, three clinical and hematological conditions; BT carrier, β-Thalassemia intermedia (BTI) and BTM have been identified [4]. Iron overload is a major challenge in the treatment of BT, hemachromatosis and sideroblastic anemia. It continues even after treatment of BTM with bone marrow transplantation. Iron overload is the result of increased iron in plasma and interstitial fluids due to increased iron absorption and repeated transfusions. Storage of iron in tissues such as the heart, liver, endocrine glands and others leads to tissue damage and organ failure. Chelation therapy and phlebotomy have treatment difficulties, side effects and contraindications in iron overload [5]. Based on the clinical and genetic background of patients, BT can be categorized as BTM, BTI or BT minor (carriers). Patients with BTM usually need continuous blood transfusions starting before the age of 2 and die in the first or second decade of life if not treated [6]. BTI is the expresson used to designate individuals presenting clinical cases among light form (BT minor) and serious form (BTM). Red cell production in patients with BTI may be sufficent to keep up hemoglobin levels greater than 7 g/dL without red blood cell transfusion and supply some patients asymptomatic up to adulthood. Fall in hemoglobin amount mightoccur with progressive age, through contagious period, gestation, surgery, and with the progress of hypersplenism, forming transfusion therapy unavoidable [7]. Homozygous and compound heterozygous forms of BT have several phenotypes, traditionally defined as BTM and BTI, and recently described as transfusion-dependent and non-transfusion-dependent BT [8]. When both parents are carriers of BT, the probability of having a child with BTM or BTI per pregnancy may be 25% (1 in 4). Therefore, carrier screening for BT is very important for BT control in the reproductive age population. Since both the α and BT carriers are classified according to microcytosis and hypochromia, the average corpuscular volume (MCV) and average corpuscular hemoglobin (MCH) are the two most important clinical indicators for BT carrier screening [9]. BT consists of a group of genetical situations related with inactive red blood cell production and persistent rupture of red blood cells. Within BT's, heterozygous BT (often mentioned as BT minor, BT carrier state or BT trait) is evaluated by the World Health Organization to act on 1 to 5% of the world inhabitants. It is mostly confront among the Mediterranean region, Middle and Eastern, central and south Eastern Asia [10].

In this study, we aimed to investigate the incidence of BTI, BT minor and SCD diseases in the province of Siirt. In addition, it is aimed to determine the incidence of different hemoglobinopathy diseases and to investigate the biochemical findings of the patients.

Materials and Methods

Prior to the start of the study, preliminary permits for the feasibility of the study were obtained from the Public Health Directorate. This study was approved ethically by the decision of Siirt University Non-Interventional Clinical Research Ethics Committee dated 24/ 05/ 2017 and number 02.03. In this study, the files of patients admitted to Siirt Public Health Unit between 2015-2017 and diagnosed as hemoglobinopathy were examined. BTI and BT minor patients diagnosed as the experimental group, the control group consisted of healthy individuals who came to routine control. Fasting blood sugar, urea, uric acid, bun, creatinine, AST, ALT, bilurubin, LDH, albumin, total protein, P, Ca, Mg, Na, K, CI, iron, iron binding capacity, triglycerides, cholesterol, HDL, LDL, CRP, alkaline phosphatase parameters were analyzed retrospectively from patient files.

In addition, patients with SCD were included in the study and control group in healthy subjects. The biochemical parameters of SCD patients were also evaluated retrospectively. In the Siirt Public Health biochemistry laboratory, biochemical parameters were studied using Siemens Dimension RxL Max Integrated Chemistry System.

Statistical Analysis

Data were expressed as mean±standard deviation (SD). The comparisons of BT, BTI and control gropu parameters were performed using One Way Anova test. Tamhane test was used to determine statistically significant differences between the three groups because of the heterogenity of data values. A p value <0.05 was accepted as significant. Independent T test was employed for SCD patients and conrol group data comparison. Data were analyzed using the SPSS for Windows computing program (Version 22.0).

Results

A total of 22455 people applied to the Siirt Public Health Unit in 3 years between 2015 and 2017. In 2015; 420 people in 2016; 424 people, in 2017; 360 people were diagnosed with hemoglobinopathy. An average of 2 patients diagnosed with BTM, 12 patients with BTI, and 340 patients with BT minor were recorded in Siirt province in each year. In the relevant years, 99 people were diagnosed with SCD. Approximately 33 new SCD patients were identified each year. In 2015; Among 420 patients diagnosed with hemoglobinopathy, 30 were diagnosed as SCD. Of the 424 patients diagnosed with hemoglobinopathy, 41 were diagnosed as SCD in 2016. In 2017; among 360 patients diagnosed with hemoglobinopathy, 28 were diagnosed as SCD.

Serum AST, albumin, creatine, amylase, phosphorus, iron, iron binding capacity, total protein, sodium, bilirubin direct, bilirubin total, alkaline phosphatase and LDH levels of BTI diagnosed patients were statistically significant different compared to the control group's serum levels ($p < 0.05$) table 1.

However, no significant difference was found between the serum levels of patients with BT Minor and the serum levels of the control group.

Biochemical values of patients with SCD such as serum AST, ALT, albumin, sodium, bilirubin direct, bilirubin total and cholesterol were statistically significant different compared to the control group ($p < 0.05$) table 2.

Table 1. The BTI, BT minor and control group statistics.

	BTI			BT Minor			Control Group			
	N	Mean	Std. Deviation	N	Mean	Std. Deviation	N	Mean	Std. Deviation	P value
Age	48	12.29	6.070	48	32.83	15.713	48	42.96	16.973	.000
Glucose mg/dl	46	96.87	16.631	47	98.93	25.226	48	109.33	42.858	.106
Creatinine mg/dl	44	.59	.128	48	.69	.210	46	.75	.199	.000
AST U/L	47	40.50	23.422	48	25.99	17.505	47	17.87	6.392	.000
ALT U/L	47	39.54	42.687	47	34.43	14.432	47	35.06	10.555	.601
Iron µg/dl	15	152.87	100.090	43	88.02	47.249	44	82.66	39.986	.000
Iron B.C. µg/dl	13	235.31	109.986	42	345.69	70.618	45	369.67	72.630	.000
Amylase U/L	40	48.45	20.051	36	67.03	21.056	38	57.87	18.205	.000
LDH U/L	43	315.86	164.342	38	203.82	74.442	38	192.05	40.083	.000
Biluribin D. mg/dl	46	.51	.243	46	.15	.101	44	.13	.049	.000
Sodium mmol/L	44	138.35	2.960	47	139.86	3.113	40	137.88	2.409	.004
Potassium mmol/L	46	4.17	.439	47	4.33	.448	42	4.23	.404	.208
Albumin g/dl	39	4.59	.542	46	4.06	.375	40	4.10	.310	.000
Bilirubin T. mg/dl	48	2.19	1.659	47	.92	1.530	45	.54	.263	.000
Phosphor mg/dl	25	4.39	.686	39	3.75	.789	39	3.46	.499	.000
Calcium mg/dl	43	9.40	.511	44	9.25	.579	44	9.16	.347	.067
Magnesium mg/dl	36	1.91	.318	38	2.00	.173	38	1.98	.186	.250
Uric Acid mg/dl	36	3.65	1.347	38	3.89	1.127	39	4.01	1.149	.432
Urea mg/dl	44	28.13	9.631	45	25.28	8.416	48	25.68	8.140	.254
Chlore mmol/L	42	101.38	3.746	44	103.84	7.214	41	102.46	2.589	.076
Total Protein µg/dl	39	7.05	.870	42	7.43	.626	40	7.51	.449	.006
CRP µg/dl	42	5.10	5.321	40	3.82	3.916	38	6.08	18.682	.671
Alkaline Phosphatase (ACP) U/L	39	192.90	93.196	31	138.35	241.180	36	88.03	59.369	.010

Table 2. The Sicle Cell Disease and control group statistics

	SCD			Control Group			
	N	Mean	Std. Deviation	N	Mean	Std. Deviation	P value
Age	41	29.32	13.63	43	41.60	15.73	.000
Glucose mg/dl	37	97.85	26.884	43	96.42	11.537	.765
Creatinine mg/dl	39	.78	.198	41	.77	.191	.780
AST U/L	38	22.69	7.932	41	18.66	7.144	.020
ALT U/L	38	25.08	12.667	41	35.41	12.174	.000
Iron µg/dl	28	70.13	39.906	37	88.78	43.172	.080
Iron B.C. µg/dl	23	350.04	85.619	35	363.69	67.154	.501
Amylase U/L	20	69.35	26.404	27	66.59	25.045	.717
LDH U/L	22	206.50	48.897	27	188.89	43.587	.189
Biluribin D. mg/dl	25	.19	.136	35	.13	.043	.026
Sodium mmol/L	35	140.09	2.877	28	137.50	1.856	.000
Potassium mmol/L	36	4.22	.357	30	4.18	.422	.661
Albumin g/dl	27	4.36	.377	31	4.08	.343	.005
Bilirubin T. mg/dl	26	.82	.540	37	.58	.332	.050
Phosphor mg/dl	24	3.55	.785	29	3.40	.574	.460
Calcium mg/dl	34	9.44	.455	37	9.26	.315	.058
Magnesium mg/dl	25	2.03	.495	31	1.94	.154	.373
Uric Acid mg/dl	19	5.69	7.874	33	3.73	1.069	.294
Urea mg/dl	35	23.40	5.862	42	24.97	8.196	.346
Chlore mmol/L	27	102.52	2.400	29	102.07	2.344	.476
Total Protein µg/dl	25	7.50	.612	29	7.59	.372	.516
CRP mg/dl	24	3.62	2.065	32	3.34	3.730	.726
Alkaline Phosphatase (ACP) U/L	20	84.85	52.725	27	79.11	22.121	.651
Triglyseride mg/dl	25	128.04	89.113	41	141.27	90.129	.563
Cholesterol mg/dl	24	146.33	43.373	41	178.98	33.283	.001
LdlCholesterol mg/dl	18	86.92	26.418	35	102.64	29.346	.062

Discussion

In this study, the incidence and blood serum biochemistry levels of the hemoglobinopathy diseases such as BTM, BTI, BT Minor and SCD between 2015-2017 were investigated. In Siirt province, where consanguineous marriages are very frequent and the number of children is high, 2 patients diagnosed with BTM, 12 patients with BTI and 340 patients with BT Minor were determined each year. In this study, the number of patients diagnosed with SCD is 99, and approximately 33 new SCD patients were identified each year. The frequency of hemoglobinopathy was found to be BTminor 4.54%, BTI 0.18%, SCD disease 0.44%.

BT is also described "Mediterranean Anemia" due to its popularity in the Mediterranean basin. It affects roughly 4.4% of every 10,000 live births throughout the world. One of the Mediterranean basin countries Palestine is, in which BT illness is common. In the Gaza Lane, the average ratio of BT trait is 3.0%–4.5% [11]. The prevalence of BT disease is 2.1% in Turkey, and it influences substantially large populations in other Mediterranean countries. One of the treatment options of these patients to survive is continual red blood cell (RBC) transfusions. Because of these transfusions, iron accumulates in the body, and chelation therapy is used to prevent the toxic effects of iron [12]. Sirirat et al. measured the serum levels of healthy subjects and BT patients, they found statistical difference between two groups in iron, ferritin, direct biluribin, ast, alt and creatinin level [13]. Uric acid and LDH serum levels in BT patients and control groups were observed statistically different and important [2]. Serum glucose, ferritin and hs CRP were not found different between the two groups, whereas serum creatinine levels were found lower in the BT group. The mean hemoglobin concentration was significantly lower in the BT group compared to controls [10]. Both BT and SCD are crucial public health issues in southern Turkey. Whole estimated frequency of BT trait is 2% in Turkey. On the other hand Sickle cell disease is not distributed homogeneously in Turkey. According to the studies accomplished in southern Turkey, the incidence rates vary from 1.4 to 10.8% for BT trait and from 0.5 to 44.2% for sickle cell trait [14]. In a study, when BT patiens were divided into three groups according to their ferritin levels, blood serum AST and ALT levels were found to be statistically higher than control group levels [15]. There were no significant differences between of serum trigliserid and total colesterol levels of BT subjects compared to controls while HDL-C and LDL-C levels were found significantly lower [16]. In another study, the serum levels of CRP were found importantly higher in patients compared to healthy person, in any case of remedy (hydroxyurea or splenectomy), the result of a chronic underlying infection of BTI patients [17]. Patients have significantly higher AST, ALT, phosphorous level while they have significantly lower alkaline phosphatase levels compared to controls [18]. In a retrospective study conducted in Hatay, the frequency of BT and hemoglobinopathy was investigated. The data were obtained from 70226 individuals who applied to Hatay Antakya State Hospital Hemoglobinopathy Center between January 2006 and October 2012 for pre-marital anemia research. The frequency of hemoglobinopathy was determined as BT carriage 6%, SCD carrier 6.3%, α-BT carrier 12.9% and other abnormal hemoglobinopathy variants 4.2%. Researchers have found that frequency of BT trait and other hemoglobinopathies in Hatay higher than the other cities in Turkey [19]. In another study, β -BT features and frequency of mutation were determined in Van Lake region. In the study, 1014 healthy students studying in boarding schools between the ages of 12-18 were examined. Complete blood count was performed in all donors. Frequency of BT trait was determined as 0.78% [20]. Education programs, screening programs (school, marriage, early pregnancy, newborn), genetic counseling, PIGT (Preimplantation genetic diagnosis), prenatal diagnosis can be done to prevent BT. With this study, the frequency of BTM, BTI and BT minor diseases in Siirt province between 2015-2017 was investigated. In Siirt province, where consanguineous marriages are frequent and the number of children is high, 2 patients diagnosed with BTM, 12 patients with BTI and 340 patients with BT minor were identified.

This study we carried out in the province of Siirt is a local study. Such studies should be done nationwide to determine the current state of abnormal hemoglobinopathies. Therefore, a broader, comprehensive national screening program is needed. Our study will contribute to public health protection efforts in the province of Siirt. This research we conducted is of great importance in determining individuals/carriers of hemoglobinopathy, informing the diagnosed individuals about their disease, raising their awareness and organizing their treatment, and taking preventive measures against the disease. Since the incidence of hemoglobinopathy species is determined for the province with the data obtained, it will be a source for future studies. In these diseases, information and preliminary diagnosis can be made more effectively. As a result, training, screening and follow-up programs importantly should be carried out throughout the country and regionally for hereditary diseases such as BT and SCD whic are shorten the individual's average life expectancy and result in death in our country.

Conclusion

Whit this study, it should be crucial to recommended to take precautions for such hereditary diseases that are closely related to consanguineous marriages that shorten the average life expectancy and life quality in our country, and to implement these measures in each city by making them a health policy. Both the role of family health physicians and the effective use of public health laboratories are very important in determining and implementing treatment protocols for various genetic transition diseases that require follow up.

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Conflict of interests
The authors declare that they have no conflict of interest.

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Ethical approval
This study was approved ethically by the decision of Siirt University Non-Interventional Clinical Research Ethics Committee dated 24/05/2017 and number 02.03.

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