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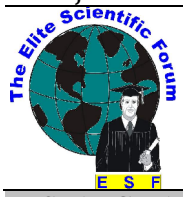

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RESEARCH ARTICLE

Hematological and Biochemical Changes in Male Patients with Thalassemia Major

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ABSTRACT

The present study reports the hematological and biochemical parameters in β-thalassemia patients with transfusional iron overload and chelation therapy. Fifty β-thalassemia major male patients and fifty healthy male subjects were selected form Punjab, Pakistan. The t-value of studied hematological parameters were as follows: red blood cell count (127.9, P<0.00), platelet count (52.02, P<0.00), mean corpuscular volume (28.19, P<0.001), mean corpuscular hemoglobin (58.42, P<0.00), neutrophils (4.05, P<0.001), mean corpuscular hemoglobin concentration (44.86, P<0.00), hemoglobin (79.8, P<0.001), monocytes (294.5, P<0.00), lymphocytes (32.28, P<0.001), hematocrit value (120.3, P<0.00), total leukocyte count (11.03, P<0.001), eosinophils (21.34, P<0.000) and erythrocyte sedimentation rate (286.8, P<0.00). The biochemical parameters were as follows: alanine aminotransferase (88.22, P<0.00), aspartate aminotransferase (86.95, P<0.00), urea (20.82, P<0.00), total protein (31.17, P<0.001), albumin (11.31,P<0.00), globulin (20.06, P<0.001), triglyceride (72.21, P<0.001), Cholesterol (55.78,P<0.00), high density lipoproteins (46.28, P>0.00), low density lipoproteins (71.03, P<0.00) and ferritin (934.7, P<0.000). Statistically highly significant difference was detected for these parameters. The lower level of RBCs, Hb, HCT, MCHC, MCV, RDW, MCH, Creatinine and HDL were found in beta thalassemic male patients and higher level of liver enzymes (ALT, AST), ferritin, TG, cholesterol, LDL and urea were observed in thalassemic patients than healthy volunteers. The hematological and biochemical profiles were significantly altered in thalassemic patients as compared to healthy individuals. All these disturbances are linked with severe anemia, β-globin chain lysis, blood transfusions, accumulation of α-chains and elevated free iron contents. Based upon findings of the present study, it can be suggested that blood biochemistry parameters should be considered necessary for better therapeutic protocol to recommend proper blood transfusion with supplementation of antioxidant vitamins, dose and frequency of chelating drug for patients of beta thalassemia.

INTRODUCTION

β-thalassemia major is an inherited disease resulting from reduction or total lack of β-globin chains described by microcytic hypochromic anemia (Cao and Galanello, 2010: Pirinccioglu et al., 2011). Thalassemias are classified basically into α and β types because of affected globin chain. In β-thalassemia major rigorously impaired synthesis of β-globulin and unpaired alpha chains of hemoglobin form α-tetramers, extremely insoluble that precipitates within erythrocyte (Patel et al., 2012). So erythrocyte becomes fragile and its rapid breakdown takes place resulting in ineffective erythropoeisis (Alavi et al., 2013; Kuldeep et al., 2011). β-thalassemia is accompanied with metabolic irregularities, storage of excessive iron in different organs of body, chronic hypoxia and cellular injury (Saha and Tamrakar, 2011). Proper transfusion courses

sustain a least level of Hb 9-12 g/dL up to 12 years in β-thalassemic major patients (Old et al*.,* 2001). A main complication of this treatment is iron overload which accumulates in body and leads to organ dysfunctioning (Youssef et al*.,* 2013). Iron initially stored in bone marrow, then in liver and heart may be a key causative factor in ailments such as heart failure, cancer, renal and nervous disorders (Widad et al*.,* 2003, Sarkar et al*.,* 2012). Cardiac problems due to iron overload are still the primary reason of mortality in beta thalassemia patients (Mansi et al*.,* 2013). Thalassemia was originated in Mediterranean regions, as a result of hemoglobin's modification against malarial parasite *Plasmodium flaciparum* (Galanello and Origa, 2010). Many developing countries of the world are facing threat of thalassemia. Treatment approaches due to access of latest techniques and facilities in developed and developing countries are also practiced differently including bone marrow transplantation and gene therapy in former but not available in later respectively. (Ain et al*.,* 2011; Ahmed et al*.,* 1996). Many β-thalassemic patients expire during their childhood and adolescence due to non-availability of safe transfusions and chelation medication (Alwan and Modell, 2003). Estimation of carrier rate is 5-7% that represents the carrier frequency of 9.8 million in Pakistani population (Lodhi, 2003). However there is no accessibility of registration record of Pakistani patients. The Inquiry of hematological and biochemical parameters is significant in the assessment of the corporeal condition of patients (Munir et al*.,* 2013). In our study, we investigated the biochemical indices of kidney, liver and cardiac functioning. The findings might help in prevention of serious damage to liver, kidney and heart. We discuss here trends of hematological and biochemical parameters in healthy male and thalassemic male patients from different areas of Punjab for the improvement in transfusion plans and chelation strategies to make better and longer life of β-thalassemic patient.

MATERIALS AND METHODS

Fifty healthy male individuals from Dera Ghazi khan and fifty β-thalassemic male patients registered in Sundas foundation Faisalabad and Lahore with D.G. Khan origin, having 3-15 years of age range were considered. Blood samples were collected in heparinzed test tube from the healthy and patient male volunteers after fasting of twelve hours, according to ethical principles laid down in the declaration of Helsinki (WHO, 1996). The research was conducted in the Department of Chemistry and Biochemistry, University of Agriculture Faisalabad, Pakistan. All subjects were investigated for any kind of abnormalities in their medical history followed by physical examination (age, weight, height, body temperature and blood pressure). Single blank blood sample (4 mL) was drawn from both healthy and β-thalassemic male patients and distributed in 3 divisions. (A) Hematological parameters were recorded through hematology analyzer (Beckman Coulter AcT Diff II), (B) ESR was determined as the rate at which red blood cells sediment in a period of one hour using westergreen tube (C) biochemical parameters were determined with the help of Clinical Chemistry analyzer, Micro lab. 200, Merk, (Germany)

Statistical analysis

SPSS package (SPSS version 19) was used to analyze the data statistically. Comparison of hematological and biochemical parameters in healthy and β-thalassemic patients was done with the help of t-test. The values with $P<0.005$ were considered statistically significant (Steel et al*.,* 1997).

RESULTS AND DISCUSSION

Every category of thalassemia can be identified approximately by Hb level and further hematological investigations (Munir et al*.,* 2013). Fifty healthy males (mean age 10.71, range 4.8-15 years; mean body weight 34.80, range 19-50 kg; height 54.8, range 37-68 cm; body temperature 98.2, range 97-99 ºF; blood pressure systolic 106, range 90-120 and diastolic 70, range 60-80 mm Hg respectively) and thalassemic male subjects (mean age 10.2, range 4-15 years; mean body weight 25.7, range 9-48 kg; height 42.3, range 28-55.5 cm; body temperature 97.4, range 95-98.6 ºF; blood pressure systolic 108, range 90-120 and diastolic 74.3, range 60-80 mm Hg respectively) were included in the study.

The mean values of hematological parameters are given in Table 1. The parameters were significantly different in thalassemic patients ($P<0.001$) when compared with healthy volunteers (Table 1). Decreased levels of erythrocytes were found in thalassemic patients. Lower levels of RBC's might be due to continuous breakdown of globin chains that led to erythrocyte rupture before maturation (Erslev, 1995: Galanello and Origa, 2010). Hemoglobin levels were lass in thalassemic patients (9±0.20) as compared to the healthy volunteers (12.1±0.19 g/dL). Thalassemic male patients might have abnormalities associated with lower Hb level, thus suffer from anemia resulting in less oxygen contents in blood, leading to tissue damage and many other pathological problems. Saha and Tamrakar (2011) reported 50% decreased level of Hb in Iraqi beta thalassemic patients when compared with healthy volunteers. Chakraborty and Bhattacharyya (2001) also reported decreased Hb level up to 50% in β-thalassemic patients as compared to that of healthy normal volunteers, these findings assist our results (Attia et al., 2001). A decreased level of red blood cells distribution

width was observed in beta thalassemic (10.3±0.14) male patients as compared to healthy volunteers (44.6±0.97) as an outcome of anemia. All β-thalassemic patients showed significantly (P<0.001) lower levels of red blood cell indices (MCHC, MCH, MCV, HCT) when compared with healthy volunteers, due to anemic condition (mild to severe) of the patients. In accordance with our findings Chakraborty and Bhattacharyya (2001) also reported lower red cell indices in beta thalassemic patient than healthy volunteers. Data obtained after clinical analysis (Pavlova et al., 2007) showed a constant drop in hemoglobin level due to less erythrocytes number and decreased values of RBC indexes (MCV, MCH, MCHC, HCT). Elevated levels of both platelets and ESR were noted in beta thalassemic patients' representing the affected population at greater risk of heart stroke and systemic ailments.

The levels of white blood cells were near normal in both beta thalassemic patients (7±0.57) and in healthy volunteers (7.92±0.15 x 10^3/µL). Elevated levels of platelet counts in β-thalassemic patients were recorded that might be due to continuing anemia accompanied by hypercellular beta thalassemia major (Al-Harbi et al., 2011). It may explain the significant negative correlation among hemoglobin concentration and platelet count (r = -0.326 where P<0.05). Neutrophils were slightly higher in patients, whereas lymphocytes and monocytes were significantly in elevated in healthy volunteers might be due to viral infections. Lower levels of eiosinophils were found in beta thalassemic patients than in healthy volunteers.

The mean values of biochemical parameters are reported in Table 2. ALT and AST were in elevated levels in thalassemic patients than healthy volunteers representing liver and heart damage due to iron, oxidative stress caused by iron burden (Attia et al., 2011). Raised levels of ALT in majority of beta thalassemic males might be due to liver infection. While increased levels of triglycerides, cholesterol, LDL, in thalassemic patients indicating heart problems might be due to oxidative stress and iron burden. Increased urea levels were observed in thalassemic patients due to muscle wasting. Total protein, creatinine, globulin and HDL levels were decreased in patients as compared to normal due to lower muscle tissue which is in accordance with Hamed and ElMelegy, (2010) was reported significantly (P<0.001) higher level in patients. Lower level of lipid profile in β-thalassemic patients was reported by Arica et al. (2012). Ferritin level is the main test to check iron status in thalassemic patients. It was found highly significant (P<0.001) in beta thalassemic patients as compared to healthy volunteers and in accordance with our experimental findings significantly (P<0.001) elevated level of ferritin was also found by Nadeem et al. (2004) and Simsek et al. (2005) in thalassemic patients on comparison with control group.

Table 1: Hematological parameters of healthy and β-thalassemic male patients

Parameters	Groups	
	Healthy	β-Thalassemic patients
Erythrocyte indices		
RBCs (10^6/µL)	4.81±0.06	2.7±0.10
Hemoglobin (g/dL)	12.1±0.19	9±0.20
RDW (%)	44.6±0.97	10. 3±0.14
HCT (%)	38.±0.50	25.6±0.53
MCHC (g/dL)	34.4±0.15	32.1±0.33
MCH (pg)	25.9±0.18	22.2±0.41
MCV (fL)	77.84±0.50	73.7±0.91
Platelets (10^3/µL)	254.4±9.08	342±7.7
ESR (mm/1^{st}.hr)	7.72±0.38	49.6±0.96
Leukocyte indices		
TLC (10^3/µL)	7.92±0.15	7±0. 57
Monocytes (%)	7.64±0.39	25.9±0.20
Eosinophils (%)	1.34±0.16	0.8±0.08
Neutrophils (%)	46.1±1.05	47.1±1.39
Lympocytes (%)	36.8±1.06	45±1.45

RBCs: Red blood cells, RDW= red blood cell distribution width, TLC= total leukocyte count, HCT: Hematocrit, MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean corpuscular hemoglobin, MCV: Mean corpuscular hemoglobin volume, ESR: Erythrocyte sedimentation rate. All the values differ significantly (P<0.001).

Table 2: Biochemical parameters of healthy and β-thalassemic male patients

Biochemical Parameters	Groups	
	Healthy	β-Thalassemic patients
ALT (unit/L)	26.16±4.8	159±10.4
AST (unit/L)	26.4±0.79	80.1±4.3
Triglycerides (mg/dL)	113.1±5.1	175±3.4
Cholesterol (mg/dL)	142±3.65	183±3.7
LDL (mg/dL)	50±1.41	80.6±2.7
HDL (mg/dL)	43.2±0.85	34.1±1.1
Urea (mg/dL)	24.6±0.48	27.1± 0.7
Creatinine (mg/dL)	1.13±0.05	0.85±0.09
Total protein (g/dL)	6.66±0.18	5.73±0.11
Albumin (g/dL)	4.17±0.12	3.92± 0.1
Globulin (g/dL)	2.486±0.21	1.82±0.1
Ferritin (µg/dL)	105±2.77	5155±263

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HDL: High density lipoprotein, LDL: Low density lipoprotein. All the values differ significantly (P<0.001).

In beta-thalassaemia major patients, the main cause of mortality and morbidity is organ failure due to deposits of iron (Saha and Tamrakar, 2011). Highly raised iron level was observed in our beta-thalassemia patients may be due to erythrocyte hyperhemolysis and chronic blood transfusion. From our experimental data it was concluded that blood transfusion leads to iron burden in extracellular and intracellular space resulting in oxidative stress, heart problem and dysfunctioning of liver and kidney. Thus this study would be useful for alleviating complains of thalassemic patients providing them healthy and longer life.

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