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Hematological and Biochemical status of Beta thalassemia in Pakistani and Afghani patients of Quetta city, Pakistan

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Abstract

Thalassemia is a genetic blood disorder in which body is unable to synthesis hemoglobin characterized by chronic anemia. Improper erythropoiesis is the major problem in thalassemia. 50 Pakistani and 50 Afghani patients (male and female) from various public sector hospitals of Quetta city a year, were include in this study. Patients were divided into four groups (N=25 each group): Group-I: Included male and female Pakistani control individuals, Group-II Included male and female Pakistani thalassemia patients, Group-III included male and female Afghan control individuals, Group-IV included male and female Afghan thalassemia patients. BMI (Kg/m^2) was recorded. 3-5 ml of blood was collected, serum was isolated and biochemical analysis for hematological parameters (Hb, MCV, MCH, MCHC, and PCV), renal function test (urea, creatinine) and liver function test (AST, ALT) were done. A significant reduction in the BMI ($P<0.0001$) was reported both in Pakistani and Afghani thalassemia patients as compared to normal individuals. Significant decrease was found for hematological parameters in thalassemia patients in both populations and in both genders. Serum AST, ALT, creatinine and urea was significant increase in both Pakistani and Afghani thalassemia patients as compare to control. The prevalence of thalassemia is more severe in Afghani patients as compare to Pakistani patients since in Afghanistan health facilities are very poor, inter tribe marriages are very common and lack of knowledge.

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Introduction

Thalassemia is a group of inherited blood syndromes caused by disruption in hemoglobin synthesis due to defect in α and β-globin chain (Karim *et al.*, 2016). The three clinical conditions related to severity of thalassemia are recognized in correlation to unbalance between α globin and β globin chains are: thalassemia minor, thalassemia intermediate and thalassemia major (Risoluti *et al.,* 2018). The prevalence of thalassemia is highest in Southeastern Europe, Mediterranean countries, Arab and Asian countries (Cebrian *et al.,* 2016). This disease is characterized by deregulation of β-globulin chain, red blood cells destruction leads to severe anemia, iron over loading in vital organs, cardiomyopathy and eventually cellular death (Theodorou *et al.,* 2016; Thein, 2018). Pale skin, hormone imbalance, renal abnormalities, growth depression, frequent diarrhea, liver and abdominal enlargement are the most common clinical signs observed in thalassemia babies at the age of 6-24 months (Galanello and Origa, 2010; Karim *et al.,* 2016. The β-thalassemia major is caused by mutation on β-globlin chain (Galanello *et al.,* 2011), more than 300 mutations has been recognized on β-globin ((Rujito and Sasongko, 2018) most of them are very rare and about 20 common alleles constitute 80% of the known thalassemia globally (Nasr *et al.,* 2012).

Iron overloading due to abnormal erythropoiesis is one of the common problem in thalassemia (Bekhit *et al.,* 2017), thus proper iron chelation therapy is needed to protect the vital body organs from effects of iron overload (Hagag *et al.,* 2014; Yuksel *et al.,* 2016). Iron metabolism is unidirectional in human and unable to eliminate from the body, therefore, additional iron is start to deposit in the vital organs like heart, liver, spleen and endocrine organs etc and cause certain complications in thalassemia patients (Rund and Rachmilewitz, 2005; Taher *et al.,* 2006).

In Pakistan the high prevalence rate of thalassemia is mainly associated with inter-family marriages, lack of pre marriage thalassemia test, increase birth rate, poor health facilities and huge population (Maheen *et al.,* 2015). In the present study we selected Pakistani population of Quetta city verse Afghan refugees as a considerable number of Afghan refugees are living in Quetta city, the data between these two populations are lacking as far as the Quetta city is concern and inter marriages between these two social groups are very common.

Materials and methods

Experimental deign

A total of100 healthy individuals and 100 β thalassemia patients were enrolled in the present study (50 Pakistani and Afghani) from various public hospitals of the Quetta city during the period of one year (August 2018 to August 2019). Complete history of blood transfusion per week and hyper-transfusion complications along with iron chelation therapy were collected for each participant. Patients suffering with hepatic, renal and cardiac problem were excluded from the study. Patients taking any hormonal therapy, suffering from AIDS, hepatitis B and C or any other genetic disorder were also excluded. Body mass index (BMI) of all candidate was calculated (Kg/m^2).

The individuals were divided into following groups:

Group-1: This group contain normal male and female Pakistani individuals (N=25 each).

Group-II: This group included thalassemia male and female Pakistani patients (N=25 each).

Group-III: This group included normal male and female Afghan refugees of Quetta city (N=25 each).

Group-IV: This group contain thalassemia male and female Afghani patients (N=25 each).

Blood collection and biochemical analysis

Under well sterilized conditions 3-5 ml blood was collected through vein puncture, serum were collected and stored at -20°C till further bio-chemical analysis. Liver function test (AST, ALT) and renal function test (Urea, creatinine) was performed by using commercially available kits (Randox, UK).

Hematological parameters (Hb, MCV, MCH, MCHC and PCV) were done according to the laboratory protocol.

Statistical analysis

Results were presented as Mean ± S.E.M. Two way ANOVA followed by Tukey's post hoc test was performed by using Graph Padprisim (version 6). Differences were considered significant at P< 0.05.

Results and discussion

The β-thalassemia characterized by clinical heterogeneity and numerous genes are associate with this genetic syndrome (Pilon *et al.,* 2006). β-thalassemia is health threating genetic blood disease unable the patient to produce sufficient healthy erythrocytes and due to deficiency of hemoglobin the patients totally dependent on regular blood transfusion (Ferdaus *et al.,* 2010). β- thalassemia major is most widespread as it is common in certain populations and tribes. Certain genes and underlying mechanisms are involved to compensate the excess α-globin chain, for instance, individuals with the β-thalassemia have slightly abnormal red blood cells but no significant anemia found in these individuals.

On the other hand, other individuals who have heterozygotes or homozygotes for β-thalassemia facing mild to severe forms of anemia and other thalassemia related complications (Lai *et al.,* 2006).

Table 1. Serum hematological and biochemical parameters (Hb, PCV, MCHV, MCH & MCHC) in normal and thalassemia individuals of Pakistani and Afghani male & female of Quetta city.

Parameters	Male				Female			
	Group-1	Group-II	Group-III	Group-IV	Group-1	Group-II	Group-III	Group-IV
Hb (g/dl)	15.83±0.21	6.94±0.13***	16.05±0.18	6.55±0.20***	13.00±0.23	6.64±0.19***	13.71±0.24	5.98±0.11***
PCV (%)	42.27±0.34	35.3±1.46*	42.57±0.32	28.53±1.08**	37.20±0.51	10.64±2.20***	39.27±0.58	17.52±1.37***
MCV (fL)	87.34±1.03	68.80±1.97**	89.24±0.69	70.50±1.49**	85.04±1.23	70.23±1.88**	86.46±1.14	74.76±3.33***
MCH (pg)	29.75±0.39	21.26±0.46*	29.57±0.29	19.5±0.47*	28.34±+0.39	16.74±0.46**	29.84±0.35	13.93±0.47***
MCHC (g/dl)	34.22±0.15	36.93±1.51	33.85±0.37	37.22+1.36	33.18±0.31	26.33±1.87*	33.52±0.38	34.75±0.75
AST (U/L)	27.08±1.75	67.08±5.21**	27.16±1.68	72.4±5.36**	22.72±1.69	70.72±5.68***	31.00±1.70	78.04±5.29***
ALT(U/L)	37±2.23	118.96±7.85**	38..6±1.91	132.76±8.51***	34.88±2.22	138.8±10.84***	41.08±2.00	138.04±13.54***
Creatinine(mg/dl)	0.79±0.06	1.80±0.10*	0.88±0.04	2.44±0.11**	0.89±0.03	1.88±0.09*	0.84±0.04	2.44±0.13**
Urea(mg/dl)	15.6±0.82	55.84±5.31**	14.64±0.84	42.96±4.73**	16.2±0.65	49.48±3.63***	16.8±0.87	30.16±1.76***

Data is represented as Mean ± S.E.M. * presents P<0.05, ** presents P<0.001 and *** presents P < 0.0001.

Growth retardation is the most common feature in thalassemia as also showed in our study. We measured a body mass index (BMI) and found a significant decrease in the BMI (Kg/m²) for both genders of Pakistani (P<0.0001) and Afghani (P<0.0001) patients in comparison with healthy individuals (Fig. 1). However, gender-wise no significant difference was recorded among the two nationalities. Growth retardation is very clearly noticed on the early onset of puberty (Ayyash and Sirdah, 2018). Nutritional deficiency, severe anemia, insufficient growth hormone, improper treatment, hypoxia and certain other factors contributed to bone abnormalities, skeleton deformities, muscular weakness, osteoporosis and growth retardation (Delvecchio and Cavallo, 2010; Noetzli *et al.,* 2012). However, if Hb level is maintained up to 9.5-10.5 g/dl through regular blood transfusion the growth rate, muscular and bone development may be improved at significant level (Galanello and Origa, 2010).

Many studies revealed that screening of hematological parameters are associated with proper diagnosis of anemia in the patients of β-thalassemia and other microcytic anemia's (Schoorl *et al.,* 2015). Chronic anemia is the characteristic feature on thalassemia (Chutvanichkul *et al.,* 2018; Risoluti *et al.,* 2018) as also cleared from our results. A significant reduction was observed for the hematological parameter i.e., Hb (P<0.0001 both for

Pakistani and Afghani patients), PCV (P<0.05 in Pakistani male, P<0.001 for Pakistani female and P<0.0001 for male and female Afghani patients), MCV (P<0.001 for male and female Pakistani patients, P<0.001 for male Afghani and P< 0.0001 for female Afghani patients), MCH (P<0.05 for Pakistani, P<0.001 male Afghani and P<0.0001 for female Afghani patients) and MCHC showed significant results only in male Afghani patients (P<0.05) than normal individuals (Table 1). However, the difference between Pakistani and Afghani patients was found to be non-significant. We found that Afghani patients were facing worst health condition and severe anemia as compared to Pakistani patients. Reduced Hb level (<7 g/dl), MCV (> 50 < 70 fl) and MCH (> 12< 20 pg) is associated with β- thalassemia major (Greene *et al.,* 2015).

Redundant erythropoiesis and hemolysis causes anemia in thalassemia (Chutvanichkul *et al.,* 2018).

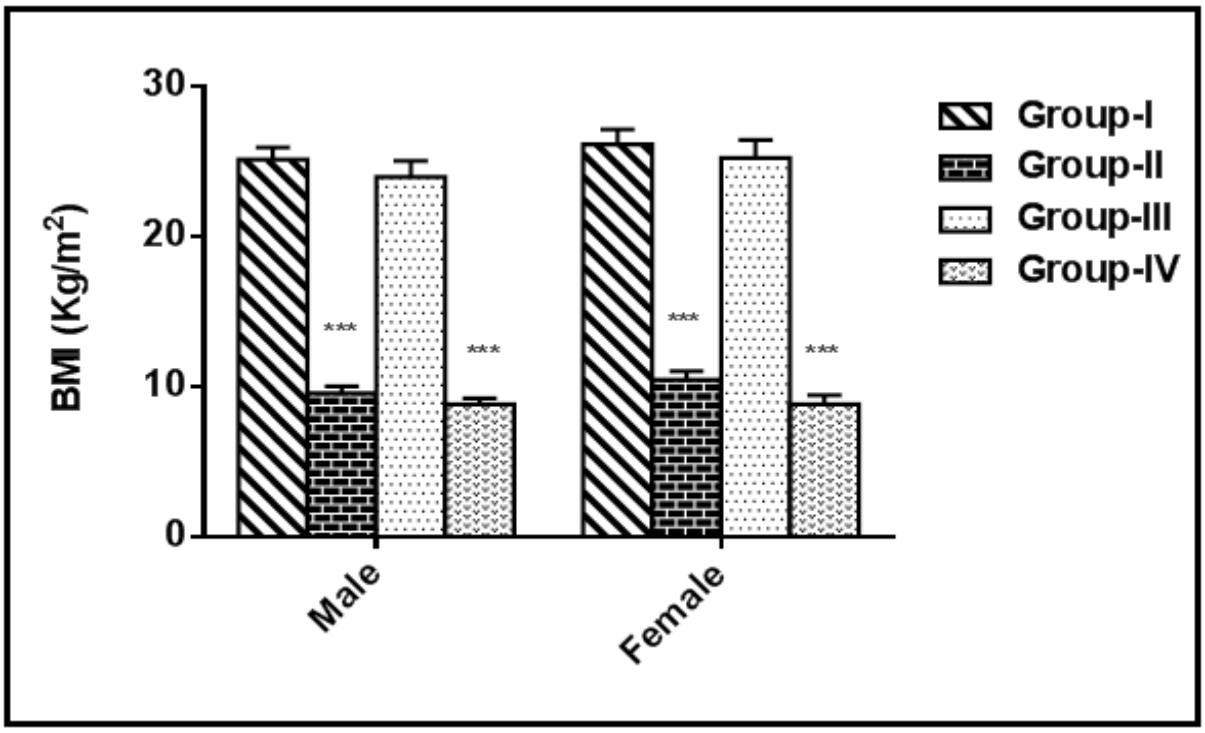

Fig. 1. Body mass index Kg/m² (BMI) in normal and thalassemia individuals of Pakistani and Afghani male & female of Quetta city. *** represent a significant difference between Group-I, Group-II, Group-III & Group-IV.

Renal and hepatic failure is the most frequent occurring events in thalassemia. In case of serum ALT a significant increase (P<0.0001) was found both in Pakistani and Afghani patients. For AST a significant increase (P<0.001) was observed in Pakistani and Afghani (P<0.0001) patients, however no significant difference was recorded between the genders (Table 1). The results are in agreement with other researchers (Karim *et al.,* 2016; Ayyash and Sirdah, 2018).This abnormal secretion of hepatic enzymes in thalassemia clearly indicates the liver damage (Hosen *et al.,* 2015) and deregulation in metabolic activities particularly in muscles (Salama *et al.,* 2015). Therefore, screening of hepatic enzymes is of great significant value to evaluate the liver damage in thalassemia patients (Saral *et al.,* 2015). The mortality rate due to liver diseases increased in patients of β-thalassemia (Faruqi, 2014).

A significant increase for urea (P<0.001) and creatinine (P<0.0001) was found in all groups of thalassemia patients. Furthermore Afghan thalassemia patients were more commonly effected with hepatic and renal failure than Pakistani patients. Urea and creatinine level is of significant importance to determine the renal function (Mansi *et al.,* 2013).

Chronic anemia, hypoxia, iron overloading, and toxic effects of iron chelation therapy are the major reasons of renal dysfunction in thalassemia patients (Koliakos *et al.,* 2003). Creatinine clearance in thalassemia patients is a matter of serious concern due to regular blood transfusion (Quinn *et al.,* 2011). Elevated creatinine level in blood leads to improper glomerular filtration and hyper calciuria (Maleknejad *et al.,* 2009).

Conclusion

Thalassemia is a matter of great attention by the Government. We found a high prevalence rate of thalassemia in Quetta city and this disease was severe in Afghan refugees living in Quetta city.

This high prevalence is attributed due to low literacy rate, inter family marriages, poor health facilities, no proper screening before blood transfusion.

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