ORIGINAL ARTICLE

CORRELATION OF SERUM FERRITIN WITH HAEMOGLOBIN A2 LEVEL IN BETA THALASSEMIA TRAITS

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Background: Haemoglobin-A2 is considered as a paramount diagnostic parameter for the detection of beta-thalassemia trait which may vary with the fluctuation of body iron stores. The current study aims to evaluate the correlation of serum ferritin as a parameter of body iron stores with haemoglobin-A2 level in beta-thalassemia traits. **Methods:** This cross-sectional study was conducted on total 134 known beta-thalassemia traits in Rehman Medical Institute-Peshawar, Pakistan from October 2018 to June 2019. Blood samples from the contributors were drawn in EDTA and plain tubes for complete blood counts, haemoglobin-A2 and serum ferritin estimation. Participants were categorized into 3 groups on the basis of iron status; beta-thalassemia traits with low ferritin (Group A), normal ferritin (Group B) and high ferritin levels (Group C). Pearson correlation was applied to analyse the correlation between the variables. **Results:** Out of total 134 known beta-thalassemia traits, 73 (54.5 %) were males and 61 (45.5%) were females. Participants of group A with low ferritin were 22 (16.4%), group B with normal ferritin were 96 (71.6%) and group C with high ferritin were 16 (11.9%). Group A shows lowest mean haemoglobin-A2 level comparatively to Group B and Group C, with some effect of serum ferritin on haemoglobin-A2 level. **Conclusion:** Haemoglobin-A2 value decreases when there is a decrease in serum ferritin and show slightly increase with high ferritin level as compared to normal ferritin level or body iron stores in beta-thalassemia traits. However, this correlation is not significant enough to mask the actual diagnosis of the disease.

Keywords: Beta Thalassemia Trait; Haemoglobin A2 level; Serum Ferritin

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INTRODUCTION

Beta thalassemia is the common inherited hemoglobinopathy with autosomal recessive inheritance pattern.[1] As reported by World Health Organization (WHO), the carrier rate of beta thalassemia is about 5% of world's population.[2] In Pakistan carrier rate varies from 5–8 percent and nearly 5000 children are diagnosed with beta thalassemia major annually, which makes it one of the portentous health issue in Pakistan.[3,4] The main factor behind high prevalence in Pakistan is consanguinity. About 37.1% cases are reported as first cousin marriages and 50.3% as total consanguineous marriages.[5] Accurate detection of beta thalassemia trait is important for identifying couples with their offspring at risk of thalassemia major.[5]

Beta thalassemia trait (BTT) which is an asymptomatic form of haemoglobinopathy, caused by an abnormality in one of the two HBB genes, leads to reduction in beta chain production, the degree of which is identified by mutation status at beta globin gene.[6] In carrier the alpha chains production remains unaffected.[7] These alpha chains pair up with the available beta chains decreasing the levels of haemoglobin A while the haemoglobin A2 level (normally <3.5%) increases by the combine transcriptional and post-translational effect (alpha-delta dimer formation).[8]

Increased haemoglobin A2 level (HbA2) is considered as an integral and reliable haematological discovery for the determination of thalassemia traits.[9,10] However, it is difficult to identify some traits if the level of haemoglobin A2 is not in the typical carrier range, i.e., 3.8–6.0%. These atypical traits/carriers have haemoglobin A2 levels between normal and thalassemia carrier range, i.e., borderline 3.3–3.8%.[11] Such borderline haemoglobin A2 levels are usually associated with decreased red blood cell indices and are generally due to mild thalassemia mutations, co-inheritance of α-thalassemia or coincident iron deficiency anemia.[12,13] Therefore, it is necessary to precisely and accurately specify the percentage of HbA2 level to make correct diagnosis of beta thalassemia trait.

Iron has a very important role in haemoglobin synthesis. In humans, red blood cells contain the majority of iron (2–2.5 g out of total body iron 3–4 g) in the form of haemoglobin.[14] Serum ferritin is a useful marker reflecting body iron stores.[15] Iron level in beta thalassemia traits could be normal, elevated or even reduced if associated with concomitant iron deficiency anaemia. Iron deficiency is one of the major issue interfering with the

diagnosis of beta thalassemia trait as reported by few previous studies showing low haemoglobin A2 levels in iron deficiency anemia.[16,17]

Heme concentration which depends on ferritin level in the body is also known as transcriptional regulator of globin chain synthesis. Decrease in heme level will activate heme regulated inhibitor (HRI) which will directly decrease the synthesis of globin chains (Haemoglobin A2), although the effects of high heme concentration on globin chain synthesis is not clear.[18] The purpose of this research is to evaluate the association of serum ferritin levels indicating body iron status or heme concentration and HbA2 levels in individuals with beta thalassemia trait.

MATERIAL AND METHODS

Comparative cross-sectional study was conducted at Diagnostic Laboratory of Pathology Department, Rehman Medical Institute-Peshawar, Pakistan after the approval of Research and Ethical Committee of the Institute. Informed written consent was obtained from the individuals or guardian and confidentiality was maintained at every step.

The time span of the current study was from October 2018 to June 2019. A total of 134 (n=134) known beta thalassemia trait individuals (61 females and 73 males), were recruited in the study. After taking detailed clinical history, participants with any infective/inflammatory disorder, on iron supplementation and any condition leading to false increase in serum ferritin level (which is an acute phase reactant) were excluded from the study. Also, those with other hemoglobinopathies, raised C-reactive protein, erythrocyte sedimentation rate or increase total leukocyte count were excluded from the study. These entrants were graded into 3 groups: BTT with low serum ferritin level (Group A), BTT with normal serum ferritin level (Group B) and BTT with high serum ferritin level (Group C).

A sample of 3ml venous blood was drawn in purple top K3- EDTA vacutainer (BD, Malaysia), from each subject for complete blood count and analysed on an automated haematology analyser (Sysmex XN-1000, Japan). Haemoglobin A2 level was determined in BTT individuals by automated capillary electrophoresis analyser (Sebia, Capillary 2, France). Three millilitres of blood was also collected in plain non-additive vacutainer (BD, Malaysia) for estimation of serum ferritin level from all individuals. Serum ferritin level was ascertained by an automated chemical analyser (Architect ci8200, Abbot, USA) based on chemiluminescent microparticle immunoassay technology (CMIA).

The collected data was entered in Excel sheet and analysed through SPSS statistical software (version 22). All analysed data was presented in the form of graphs and tables. Descriptive statistics were analysed for all variables and Pearson correlation was applied to evaluate the relationship between serum ferritin and haemoglobin A2 level.

RESULTS

Out of total 134 (n=134) known beta thalassemia trait individuals, 73 (54.5%) were males and 61 (45.5%) were females with mean ages of 15.9±18.4 years. Their mean haemoglobin level was 9.9±1.40 g/dl, mean haemoglobin A2 level was 5.388±0.90% and mean serum ferritin level was 154.4±406.4 ng/ml (Table-1). These individuals with beta thalassemia traits were divided into three groups based on their iron status (Table-2). Group A includes 22 participants with iron deficiency having low ferritin levels (16.4%), Group B comprises of 96 (71.6%) and Group C incorporated 16 (11.9%) participants with normal and high serum ferritin levels respectively.

Consolidated results of haemoglobin (Hb), total red blood cell counts (TRBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), Haemoglobin A2 level (HbA2) and serum ferritin level of all groups are shown in Table-3.

After interpreting the mean values of haemoglobin, total red blood cell count and disparate red blood cell indices, no statistically significant analytic differences were seen among the participants of low, normal and high serum ferritin groups, while variation in mean±SD of haemoglobinA2 levels were found in all groups (Table-3). Mean haemoglobin A2 level was low (4.7±0.9) in group A participants with iron deficient stores indicated by low serum ferritin (10.4±4.8); however, it was found to be still in the typical diagnostic range (>3.5%) set for beta thalassemia traits. Mean haemoglobin A2 level was slightly higher (5.6±0.6) in group C participants having high serum ferritin (881.1±95.2) as compared to group B individuals (HbA2 =5.4±0.8) with normal iron stores (71.6±41.36). Only 3 patients showed iron overload with serum ferritin >1000 ng/ml and were included in group C. Pearson correlation was applied and scatter plots were drawn to see the relation between serum ferritin and haemoglobin A2 levels in all three groups with low, normal and high serum ferritin levels (Figures 1, 2 and 3). Though serum ferritin and HbA2 showed some correlation in group A (r =0.18; p=0.40) and group C (r = -0.24; p = 0.380) yet it was not significant enough to cause hindrance in HbA2 based diagnostic determination of beta thalassemia traits.

Table-1: Age and haematological parameters of beta thalassemia traits (n= 134)

Parameters	Age (years)	Hb (g/dl)	TRBC (10^6/µl)	PCV (l/ l)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW-SD (fl)	RDW-CV (%)	HbA2 (%)	Serum ferritin (ng/ml)
Mean±SD	15.9±18.4	9.9±1.4	5.6±0.7	0.32±0.04	59.1±6.3	18.2±3.7	30.4±1.6	33.2±3.2	18.4±2.7	5.38±0.9	154.4±406.4

Hb = haemoglobin, TRBC = total red blood cell count, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, RDW = red cell distribution width, SD = standard deviation, CV = coefficient of variance, HbA2 = haemoglobin A2 level

Table-2: Description of beta thalassemia traits (n = 134) as per iron status based on serum ferritin levels

Group A- Participants with low serum ferritin (males: < 17 ng/ml; females: <14 ng/ml)	22
Group B- Participants with normal serum ferritin (males:17–230 ng/ml; females:14–150ng/ml)	96
Group C- Participants with high serum ferritin (males: >230 ng/ml; females: >150 ng/ml)	16

Table-3: Consolidated results of haematological parameters of different groups based on serum ferritin levels (n= 134)

Parameter	Group A- Low (n=22)	Group B- Normal (n=96)	Group C- High (n=16)	*p*-value
Age (years)	7.8 ±11.8	13.8 ±16.9	40.0 ±18.0	.002
Hb (g/dl)	9.3 ± 2.1	10.0± 1.1	9.7 ± 1.4	.292
TRBC (10^6/µl)	5.4 ± 0.5	5.7 ± 0.7	5.3 ± 0.8	.854
PCV (l /l)	0.32± 0.05	0.33 ± 0.03	0.31 ± 0.04	.412
MCV (fl)	60.2 ± 8.8	58.4 ± 5.8	61.1 ± 4.3	.205
MCH (pg)	17.5 ± 3.6	18.1 ± 2.5	18.6 ± 1.3	.789
MCHC (g/dl)	28.8 ± 2.4	30.7 ± 1.2	30.5 ± 1.2	.367
RDW-SD (fl)	35.3 ± 5.3	33.1 ± 3.2	36.1 ± 5.3	.405
RDW-CV (%)	18.6 ± 2.9	18.2 ± 2.5	19.4 ± 2.7	.259
HbA2 (%)	4.7 ± 0.9	5.4 ± 0.8	5.6 ± 0.6	.695
Serum ferritin (ng/ml)	10.4 ± 4.8	71.6 ± 41.36	881.1 ± 95.2	.904

Data is shown as mean ± SD, Hb = haemoglobin, TRBC = total red blood cell count, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, RDW = red cell distribution width, SD = standard deviation, CV = coefficient of variance, HbA2 = haemoglobin A2 level

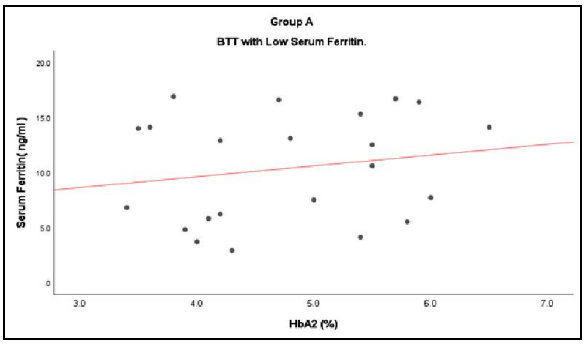

Figure-1: Scatter plot of serum ferritin and HbA2 in 22 cases with low serum ferritin levels. Pearson Correlation coefficient = 0.18; *p*=0.40.

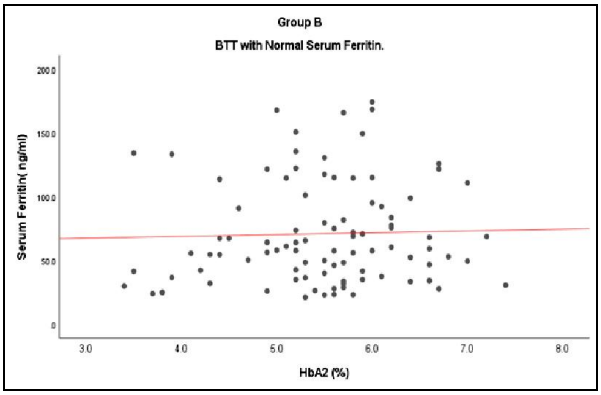

Figure-2: Scatter plot of serum ferritin and HbA2 in 96 cases with normal serum ferritin levels. Pearson Correlation coefficient = 0.31; *p*=0.76.

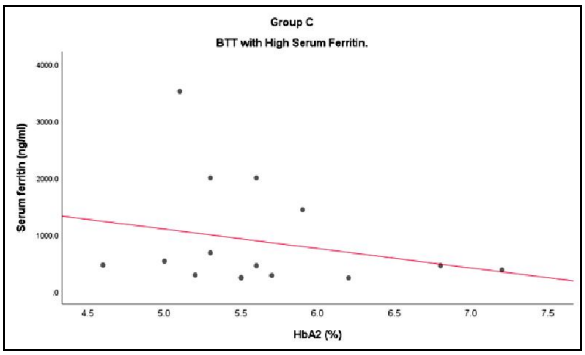

Figure-3: Scatter plot of serum ferritin and HbA2 in 16 cases with high serum ferritin levels. Pearson Correlation coefficient = -0.24; *p*=0.38.

DISCUSSION

Haemoglobin A2 (HbA2) determination is considered the most pivotal and decisive tool in the identification of beta thalassemia trait as long-established by several research studies. The normal level of HbA2 falls in a very narrow range due to which a slight variation in HbA2 value could alter the diagnosis of beta thalassemia trait to normal. Therefore, a careful interpretation of HbA2 level is imperative to identify beta thalassemia trait.[19]

The affiliation between serum ferritin and HbA2 levels in beta thalassemia traits has been the topic of interest for researchers from many decades. Iron status in beta thalassemia traits was reported to be low, normal or high as compared to reference

range.[20] There are many studies showing association of serum ferritin and HbA2 levels in beta thalassemia with coexisting iron deficiency anaemia; however no study was found from this region indicating the correlation of these two parameters in beta thalassemia traits with normal or high body iron stores. This current study implies the correlation of serum ferritin and HbA2 in beta thalassemia traits grouped into 3 categories as per iron status based on serum ferritin levels.

The findings of our study reveal that 22 (16.4%) out of total 134 participants were reported with iron deficiency having low serum ferritin levels, 96 (71.6%) with normal serum ferritin while 16 (11.9%) participants had high ferritin levels. Analysis of iron status especially iron deficiency is vital as it interferes with the identification of beta thalassemia trait. The impact of low serum ferritin on HbA2 level in beta thalassemia traits has been studied in many research articles with different results. Some authors found iron deficiency as a source of diagnostic impediments by lowering HbA2 level from typical reference range found in beta thalassemia trait. Study conducted by Sarika Verma *et al*. in 2014, reported lower HbA2 levels in beta thalassemia traits with low serum ferritin and significant improvements in levels after iron supplementation.[21] Another study from Pakistan by Usman *et al*. demonstrated similar findings of low HbA2 in beta thalassemia trait with concurrent iron deficiency causing diagnostic problems.[22] However, conflicting data was noted by other authors like Cristina Passarello *et al*. in 2012, Sharma P *et al*. and Kamal AA *et al*. in 2015, showing no significant impact of iron deficiency on HbA2 in such patients. They found striking elevation of HbA2 level in beta thalassemia trait even in presence of concomitant iron deficiency. Although HbA2 level may fall in iron deficiency yet it still remains high in diagnostic range of beta thalassemia trait.[23–25] In 2011, Yavarian M *et al*. demonstrated decrease in HbA2 was related to severity of anaemia. They highlighted the correlation between serum ferritin and HbA2 levels in beta thalassemia traits with contemporaneous moderate to severe iron deficiency while no correlation was found in mild deficiency.[26] To overcome this uncertainty, this present study focuses on to describe the relation between the amount of serum ferritin and the variation in HbA2 level.

In current research study we try to find out the correlation of HbA2 level in beta thalassemia traits with variable iron status, as no previous study from this region indicates correlation of these two parameters in beta thalassemia traits with normal or high body iron stores. We found participants with low serum ferritin showed notable decrease in haemoglobin A2 level and positive correlation between these two parameters but not to a level that can mask and hinder the diagnosis of beta thalassemia trait. It was also observed that none of the participants showed HbA2 level below 3.5% even in cases of iron deficiency anaemia. HbA2 level was found to be slightly high in participants with high serum ferritin levels as compared to beta thalassemia traits with normal iron stores but no statistically significant correlation was found between serum ferritin and HbA2 in these groups. Hence, further studies are needed to explore these findings on the basis of severity of decrease or increase in iron stores with large sample population.

CONCLUSION

Our study concluded, no reasonable correlation was found between serum ferritin and HbA2 levels in study participants that makes the detection of beta thalassemia trait difficult. Although, mean HbA2 level was found to be low in beta thalassemia traits with low serum ferritin as compared to beta thalassemia traits with normal or high ferritin levels, even the presence of iron deficiency did not thwart the diagnosis of classical beta thalassemia traits. However comprehensive study is needed at molecular level to determine correlation between HbA2 and serum ferritin level in beta thalassemia traits.

Conflict of interest: No potential conflict of interest to enunciate.

AUTHORS' CONTRIBUTION

MH: Study concept and design, data analysis and interpretation, drafting. FR, AM: Critical revision, proof reading. FR: Study supervision.

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