IMPACT OF IRON DEFICIENCY ON DIAGNOSIS OF BETA THALASSEMIA TRAIT.

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ABSTRACT... Objectives: We aimed to evaluate the effect of low serum ferritin levels on HbA2 values in Beta thalassemia trait (BTT) patients. Study Design: Cross-sectional study. Setting: Pathology department of University Medical & Dental College Faisalabad. Period: August, 2018 to July, 2019. Materials & Methods: One hundred and thirty seven subjects were included in the study after written informed consent. Those with serum ferritin < 10µg/L were taken as iron deficient. Based on serum ferritin levels, we divided our study participants into two groups (Group A Vs Group B). As ferritin is considered an acute-phase protein, 25/137 participants with leukocytosis were excluded from statistical analysis. We measured serum Ferritin on Cobas 6000 e611 and we assessed the red cell parameters on Sysmex (seven part differential XN 1000). Hb variants were analysed through High performance liquid chromatography (HPLC) based technique of BioRad D10. Results: After excluding 25 subjects with high Total leukocyte count (TLC), we were left with 112 subjects. We observed 26 participants in group A with Iron deficiency and 38 in group B with no Iron deficiency. Mean±SD serum ferritin in iron deficient group was 7.25±1.95 as compared to non-iron deficient group (87.63±7.35). Mean HbA2 value in group A was 4.56±0.04 and in group B it was 5.80±1.06 with significant statistical difference of P=0.0188. We also observed significant difference in the mean values of other Red cell indices (MCV, HCT MCHC, MCH) except for RBC count and RDW. Conclusion: This study shows that ID may reduce HbA2 levels. Overall, it does not essentially preclude the identification of BTT. It is recommended that Iron deficiency should be considered before measuring HbA2 levels in BTT.

Key Words: Beta Thalassemia Trait, HbA2, Iron Deficiency.

INTRODUCTION

Laboratory diagnosis of BTT can be made by estimating the concentration of HbA2 protein. Among genetic disorders, hemoglobinopathy is the only disorder that can be determined through its phenotype on Hb electrophoresis. Haemoglobin A2 (HbA2: a2δ2) estimation is the main parameter which is measured worldwide for identification of BTT. Comparable quantification of HbA2 has been achieved by High performance liquid chromatography (HPLC), Capillary zone electrophoresis, Cellulose Acetate electrophoresis-elution spectrophotometry or Microcolumn chromatography (MCC). So, any effect of assay interference is excluded.

Iron homeostasis has complex interplay with BTT. Researchers have observed that iron balance goes in favour of BTT patients as compared to controls. This is due to increased iron absorption in the background of small sized RBCs with less oxygen carrying capacity. However some of the studies observed the presence of Iron deficiency (ID) in about 30% cases. Iron is bound to heme portion of hemoglobin. Less iron availability definitely reduces the overall Hb concentration and might affect the HbA2 value either. This may mask the presence of BT trait and pose a major challenge for the screening laboratories in the diagnosis of BT trait. Situation is even gruesome in under-privileged settings like Pakistan where iron deficiency is highly prevalent. Since Concomitant iron deficiency has a potential impact on HbA2, therefore, the objective of this study was to evaluate the effect of low serum ferritin levels on HbA2 values in BTT patients.
MATERIALS & METHODS
This was a cross-sectional study and performed in Pathology department of University Medical & Dental College Faisalabad between August, 2018 to July, 2019.

One hundred and thirty seven subjects were included in the study after written informed consent. Based on serum ferritin levels we divided our study participants into two groups (Group A: Iron deficient with serum ferritin <10 µg/L Vs Group B: Non iron deficient with serum ferritin≥10 µg/L). As ferritin is considered an acute-phase protein, 25/137 participants with leukocytosis were excluded from statistical analysis. We measured serum ferritin on Cobas 6000 e611 and we assessed the red cell parameters on Sysmex (seven part differential XN 1000). Hb variants were analysed through High performance liquid chromatography (HPLC) based technique of BioRad D10. Statistal package of Social Sciences (SPPS) version 23.00 was used for statistical analysis. Red cell indices, serum ferritin and HbA2 were compared between two groups using independent sample t-test. The result were considered significant when P-value was <0.05.

RESULTS
After excluding 25 subjects with high total leukocyte count (TLC) count, we are left with 112 subjects for thalassemia screening. The subjects with high clinical suspicion of BTT were separated to assess for hemoglobinopathy. 64/112 subjects were observed with high HbA2 values (>3.5%). Majority of the participants (40/64) were female. Mean Hb levels of female were 9.0±0.5g/dL Vs male 10.5±0.10g/dL (P=0.231).

Twenty six subjects were found in group A with Iron deficiency and thirty six in group B without Iron deficiency. Mean serum ferritin in group A was 7.25±1.95 as compared to group B 87.63±7.35. Mean HbA2 value in group A Vs group B was: 4.56 ± 0.04(%) Vs 5.80±1.06 (%) with significant statistical difference of P=0.0188 (Figure 1). There was also significant difference in the mean values of other Red cell indices (MCV, HCT MCHC, MCH) except for RBC count and RDW. (Table-I).

| Red Cell indices | Group A (Mean±SD) | Group B(Mean±SD) | P-value |
|------------------|-------------------|------------------|---------|
| Hb(g/dL)         | 9.72±2.18         | 11.81±1.34       | 0.007   |
| RBC count (10¹²/L)| 5.93±0.57         | 6.50±0.38        | 0.497   |
| HCT (%)          | 32.32±5.36        | 37.95±4.82       | 0.010   |
| MCH (pg)         | 14.90±2.93        | 19.02±1.69       | < 0.001 |
| MCV (fL)         | 50.43±6.08        | 58.36±2.71       | 0.010   |
| MCHC (g/dL)      | 29.46±2.10        | 31.70±0.38       | 0.004   |
| RDW-CV (%)       | 21.18±4.13        | 18.62±2.39       | 0.09    |

Table-I. Red cell indices in Iron deficient Vs non-Iron deficient subjects with BTT.

| Group A: Iron deficient | Group B: Non-Iron deficient |
|------------------------|----------------------------|

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**DISCUSSION**

Beta Thalassemia Major (BTM) is an inherited hematological disorder with a global incidence of one in 100,000.\(^8\)\(^9\) Genetic derangement causes diminished synthesis of beta globin chain that leads to ineffective erythropoiesis requiring lifelong blood transfusion.\(^10\) However, BTT with single allele mutation usually remains asymptomatic. A probable diagnosis can be made on the basis of red cell indices but it needs to be confirmed on Hb electrophoresis.\(^11\)\(^12\) Detection of BT trait among the masses is critical to reduce the prevalence of BTM in the society by pre marriage counselling.

IDA and thalassemia are the main causes of Microcytic hypochromic blood picture in children and adults.\(^13\) Previously it was thought that ID is not present in thalassemic subjects including both thalassemia major and trait.\(^14\) But later studies showed concomitant existence of both iron deficiency and BTT. We found that 26(23%) BTT subjects were iron deficient in our study cohort which is relatively high percentage in any population. The high incidence of ID is substantiated by other studies. Sharma et al., and Dolai et al reported the frequencies of 20.7% and 19.3% respectively.\(^5\)\(^15\)

It has been observed that HbA2 levels fall in those BTT subjects who also have coexisting iron deficiency. However some of the studies did not show any significant effect of ID on HbA2 levels.\(^16\)\(^17\) Our study also showed relatively low concentration of HbA2 value in BTT subjects with coexisting ID but HbA2 value was still above its cutoff (>3.5%). It has been concluded by one of the studies that Hb levels in BTT cannot indicate the presence of co-existing ID. The process of erythropoieses becomes relatively active in BTT subjects with high RBC count. In contrast to this ID leads to low RBC count and slow rate of erythropoieses due to lack of Iron nutrient.\(^19\) Combined existence of these two conditions may lead to near normal RBC count. We observed this phenomenon very clearly in our study population where RBC count was relatively low in BTT subjects with ID. The low serum ferritin levels were observed in female subjects as compared to male. The iron supplementation is more critical for females than males.

We also observed two cases who had strong suspicion of BTT based on their red cell indices (RBC count, MCH, MCHC, RDW, RDW-I) and had low ferritin. Their HbA2 value was between 3.0-3.5. These borderline cases need to be re-evaluated after correcting IDA.

These borderline cases with coexisting ID should have been excluded from diagnosis of BTT based on genetic testing. Due to lack of genetic testing in our lab, we just excluded their diagnosis of BTT on the basis of HbA2 value. It is suggested that such cases should be tested again after correcting ID anemia through Hb electrophoresis where genetic testing is not available.

**CONCLUSION**

In summary, we conclude that that ID may decrease HbA2 levels. Generally, it does not preclude the diagnosis of BTT. This study also indicates the high incidence of coexisting ID and BTT. The diagnosis of BTT may be affected in these subjects. Therefore, it is very important to first correct the ID in the subjects with high suspicion of BTT before measuring HbA2.

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| 3     | Shireen Hamid                | Writing of review.                                                                       |                     |