Original Article

Association of Total Red Blood Cell Count with Hemoglobin A2 Level in Beta Thalassemia Trait

RBC with HbA2 in Thalassemia

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ABSTRACT

Objective: To evaluate the association of Red blood cells count with Hemoglobin A2 level in Thalassemia trait individuals.

Study Design: Descriptive / Observational / cross sectional study.

Place and Duration of Study: This study was conducted at the Diagnostic Laboratory, Rehman Medical Institute, Peshawar from April 2017 to October 2017.

Materials and Methods: A total of 200 beta Thalassemia trait and 100 normal healthy individuals as a control group were taken in the study. 2ml of blood was collected in EDTA tube and performed CBC and HbA2 from all subjects. All the data collected was recorded and analyzed in SPSS-22. Person correlation was used to find out association between the variables.

Results: We analyzed a total number of 200 thalassemia trait individuals and among them 116 (58%) were male and 84 (42%) were female participants. The study population age ranges from 1 year to 81 years with median age of 16 years. Highly significant correlation was found between RBC count and HbA2 level with P- value of 0.001. These finding reveals that increase RBC count is directly proportional to the Hb A2 level.

Conclusion: From the present study it is concluded that hemoglobin A2 level in Thalassemia trait individuals is highly associated with Red blood cell count. Moreover this study confirms that raised RBC count in contrast to Hb with low MCV, MCH and normal MCHC is indication for proceeding with Hb study for diagnosis and counseling them to prevent the birth of beta Thalassemia major children.

Key Words: Beta Thalassemia Trait, Hemoglobin A2 level, RBC count

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INTRODUCTION

Beta Thalassemia trait is a heterozygous form of genetic mutation of beta gene leading to compensatory hemolytic disease. Most of beta Thalassemia traits (BTT) are asymptomatic and some present with mild anemia[1].Approximately 5-7% of the globe populations carry a defected beta gene that responsible for propagation of Beta Thalassemia across the world as well as in Pakistan.[2] Although patient with BTT do not usually have increased morbidity and mortality but when both parents are beta Thalassemia trait (heterozygous) there is a 25% risk to give birth a child with beta Thalassemia major (homozygous) at each pregnancy.[3]

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Laboratory feature of BTT often present with normal to mild lower hemoglobin level, decrease MCV, MCH, normal MCHC and RDW.[4] Total RBC count is usually high (>5.0 million/µl)in contrast to hemoglobin concentration. Peripheral blood film reveals microcytic hypochromic red blood cells, occasional target cells and basophilic stippling. Raised Hemoglobin A2 level (>3.5%) on hemoglobin electrophoresis is diagnostic finding.[5]

In normal circumstances heme concentration regulates hemoglobin chain syntheses.[6] Decrease in heme concentration that activates the heme regulated inhibitor (HRI) that responsible for inhibition of globin chain synthesis and lead to depletion of hemoglobin A2 synthesis in iron deficiency anemia. When β-thalassemia trait individual present with iron deficiency anemia may shows normal or borderline A2 level and due to this impact of iron deficiency anemia on A2 level is cause of Beta gene propagation.[7, 8]

The association of total red blood cell count with hemoglobin A2 level is not fully understood. For stable hemoglobin molecules it is necessary that alpha chain, beta chain and heme must be in balanced with ratio of 2:2:4 respectively. Imbalance between these ratios lead to instability in hemoglobin quantity and quality.[9] Two beta genes (HBB) is responsible for beta chain synthesis, one gene is inherited from each

parents. In beta Thalassemia trait one gene is defected and one is normal.[10] In heterozygous state both beta genes are capable to synthesize up to 70-80% of beta globin chain. There is 20 to 30 % of beta chain deficiency in β-thalassemia trait.[11] Almost 70-80% of beta globine chain binds with alpha chain and heme molecules to formed a stable hemoglobin tetramer. The remaining 20-30 % free alpha chains have capability to form alpha and alpha chain tetramer.[12] Alpha chain tetramer is unstable and have tendency to precipitate with in a cell, which is cytotoxic for red blood cell and lead to ineffective erythropoiesis and peripheral erythrocytosis.[13] In Beta Thalassemia syndrome human body try to neutralize the free alpha chain by combing with alpha chain stabilizing protein, delta chain and gamma chain. Therefore it is observe that raised levels of hemoglobin F in beta Thalassemia major and Hemoglobin A2 in beta Thalassemia trait.[14]
Small amount of free alpha chain in beta Thalassemia trait individual become precipitate with in red blood cells that lead to premature destruction of erythrocytes. Premature red cell destruction causes the ineffective erythropoiesis with in compensation phenomena.[14] Compensatory ineffective erythropoiesis is leading cause of peripheral erythrocytosis in beta Thalassemia trait.[16] The present study is design to evaluate the association of total red blood cell count with hemoglobin A2.

MATERIALS AND METHODS

It was a descriptive comparative cross sectional study carried out at diagnostic laboratory Rehman Medical Institute, Peshawar Pakistan. Duration of this study was sixth months i.e. from April 2017 to October 2017.
A total of 200 beta Thalassemia trait and 100 normal healthy individual as a control for comparison were taken in the present study. Those beta thalassemia trait individuals were excluded who also iron deficiency anemia. A 2 ml of venous blood was collected from all diagnosed beta Thalassemia trait and normal healthy individuals in EDTA (Purple top, BD) vacutainer tube for CBC and Hemoglobin studies. Complete blood count (CBC) was performed by automated hematology analyzer (XN-1000, Sysmex, Japan) to determine red blood cell count and red cell indices. HbA2 level were evaluated by HPLC analyzer (D-10, Bio-Rad, USA),
All collected data was recorded and analyzed in SPSS-22. Chi-square and odd ratio were used for measurement of comparison between variables. Results were presented in tables and graphs. P value is less than 0.05 it was consider as statistical significant.

RESULTS

The total number of known thalassemia trait individual was 200. Out of total individuals116 (58%) were male and 84 (42%) were female individuals. The patients age ranges from 01 year to 81 years with median age were 16 years. All patient hemoglobin level (Hb), RBC count, MCV, MCH, MCHC, RDW and HbA^2 level were analyzed and show in table 1.
All beta thalassemia trait individual red cell indices and HbA2 mean level and standard deviation were compared .No statistical significant differences were found between HB, MCV, MCH, MCHC and RWD. Highly significant correlation was found between RBC count and HbA2 level with P- value is 0.001. Study result revealed that higher the RBC count is directly proportion to the Hb A2 level.

Table No.1: Red cells indices and Hb A2 level median, minimum and maximum levels.

S/No	Parameter	Median	Min.	Max.
1	Hemoglobin(Hb)	10.5000	4.36	18.70
2	RBC count	5.6350	2.26	8.31
3	MCV	59.850	45.8	86.2
4	MCH	18.300	12.6	29.8
5	MCHC	31.650	13.7	37.7
6	RDW	17.0000	00	36.80
7	HbA2 level	5.800	3.5	8.1

DISCUSSION

Present study result reveals that Thalassemia trait individual hemoglobin A2 level is highly interlinked with the total red cell count. In present study hemoglobin A2 is always higher than 3.5 percent in thalassemia trait. The mean value of MCV and MCH in the studied population is 59.850 fl and 18.300 pg that are lower than normal limit. The mean value of MCHC and RDW in included patients is 31.650 g/dl and 17 percent (CV) respectively that is within normal range. So it is also important that to measure the value of MCHC and RDW in diagnosing the thalassemia patient.
Elevated HbA2 level is due to βTT in almost all cases, and an elevated HbA2 level in the presence of microcytosis are indicative for ultimate diagnosis of βTT. Although elevated, the HbA2 level varies but rarely reaches 6%. It has been also suggested that the HbA2 levels in βTT may correlate with particular classes of β-globin chain gene mutations.[4,17] Zhanhui Ou et al study revealed that a large number of pregnant women with a mild increase in HbA2 levels without βTT. Elevated HbA2 level other then BTT also observed hyperthyroidism and HbS (sickle cell trait/disease).[18] In our studied population no symptoms of hyperthyroidism were documented and no TFTs (thyroid function tests) were advised and the possibility of hyperthyroidism cannot be definitely excluded.
Estrogen induces the expression of TBG (thyroxin-binding globulin). Total thyroid hormones are mostly increased during pregnancy. It is necessary to find that whether there is a relationship between the levels of TBG and HbA2.

Rarely βTT cases with certain β-globin gene mutations (CAP+1) may have normal HbA2 level with normal or abnormal RBC indices. In addition, HbA2 elevation is not a feature of the α-Thalassemia trait, and coexistence with an α-thalassemia mutation may give a normal level of HbA2. When HbA2 is used as the screening test, these cases will be missed. With doubtful result genetic studies must be done to actual result.[4]

Assessment of red blood cell parameters in the Complete blood count (CBC) is an important laboratory investigation and diagnostic workup for thalassaemias.[19,20] The red cell parameters good way for these mutations, lower hemoglobin concentration, low MCV and MCH, normal MCHC, RDW and increased TRBC.

CONCLUSION

From the present study it is concluded that hemoglobin A2 level in thalassemia trait patient is deeply associated with Red blood cell counts. This study further confirms that raised RBC count with low hemoglobin, MCV, MCH are indication for proceeding further with hemoglobin electrophoresis to screen for beta Thalassemia trait and further counseling them to prevent the birth of beta Thalassemia major children in Khyber Pakhtunkhwa.

Author's Contribution:

Concept & Design of Study:	Shahtaj Khan
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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