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EARLY DIAGNOSTIC ROLE OF HEMATOLOGICAL INDICES AND HBA2 LEVELS IN BETA THALASSEMIA TRAIT: A TERTIARY CARE PERSPECTIVE

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Abstract

The aim of study is screening of the beta thalassemia trait (BTT) with the help of CBC parameters. This ratio was used as a cost-effective screening tool for the identification of thalassemia carriers for the awareness and prevention of beta thalassemia major. A total of 150 individuals were selected who reported in AMTF for the screening of beta thalassemia trait (BTT). Among those 100 individuals were analyzed for complete blood count (CBC) and for that purpose, the blood samples were collected in Ethylenediaminetetraacetic acid (EDTA) tubes and analyzed on automated hematology analyzer K-4500. The ratio between Hb and RBCs was estimated for each sample for the screening of beta thalassemia trait. According to the results (n=50) had normal ratio while (n=50) showed thalassemia trait ratio, the subjects with normal ratio were used as control while the remaining as test subjects. The Hb-Electrophoresis done by Cellulose acetate method as confirmatory test of thalassemia trait. The data was analyzed on Statistical Package for the Social Sciences (SPSS) by T-Test. The cut off (3.5) or more for Hb-A2 was taken for the diagnosis of beta thalassemia trait (n =56) had normal amount of Hb-A2 and 50 % had a ratio of 2.2 or more, while (n= 44) had Hb-A2 level of 3.5 or more while 50 % had a ratio of less than 2.2. The p-value between the two arms was significant <0.05

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INTRODUCTION

Thalassemia is characterized as a genetic disorder in term of defaulted hemoglobin (Hb) chain alpha or beta synthesis. Numerous abnormalities in Hb collectively known as hemoglobinopathies. Beta thalassemia (BT) is one of the disorders which arises on the account of deficiency or inaccessibility of beta globulin production [1].This disease is ranked on top list of haemoglobinopathies followed by sickle cell anemia.Asian countries underscore the alarming higher prevalence of thalassemia. Approximately 80% of children who were suffering with this disorder. Although, developing countries enlisted Pakistan, but still burdened of healthcare issues included Beta thalassemia trait (BTT), where carrier of BTT had reported around 11% of the population. The Eastern Mediterranean Region become red zone producing half of the annual births with BTT [2]. The underlying reason behind the worst escalation of this genetic disease in Pakistan are lack of early screening, diagnosis, social alienation and cousin marriages [3].

BTT affected individuals required blood transfusion for the survival of life cause an increase rate of morbidity and mortality. Moreover, multiple transfusions would be attributed in developing alloimmunization. It could be dangerous in the future for matching blood group to the recipient [4]. Individuals with intermediate and minor category of beta thalassemia has shown less adverse outcome on the account of single copy of gene which act as a carrier. On the other hand, various types of mutations resulting in the significant beta globulin gene expression [5].

In Pakistan there are many public and private sectors involved in the management of thalassemia. Implementation of health care policies and proper health care measures adaptation by individuals would be supported in lowering the incidence of this disease [3]. Public awareness programs at lower class communities would be beneficial to decline the ration of this disease trait.

Early detection of thalassemia in pregnant women's ensure by screening in developed countries. Despite, in Pakistan situation is reverse due to unavailability of prenatal screening facilities, awareness and living in the ruler areas [6]. Therefore, present study is conducted to screen the Beta Thalassemia trait with the help of ratio outcome between hemoglobin and red blood cells, which is estimated from routine blood count.

Methodology

This study was conducted at Afzaal Memorial Thalassemia Foundation (AMTF) in the year. The initial sample size was one hundred fifty individuals, who were screened from outpatient department for Thalassemia. Individuals with no age limitation of age and gender were included. While individuals with transfusion of blood in the past three months were excluded. Hundred selected individuals followed by the detection of beta thalassemia trait who forwarded performed on routine blood count after their consent.

Blood samples of these individuals for the measurement of complete blood count were gathered in EDTA tubes. Complete blood count (CBC) was performed by automated hematology analyzer K-4500. Hb and RBCs were evaluated from each sample. The normal reference range of Red blood cells (RBCs) for Male was (4.32-5.72 million cells/μ), Female (3.90-5.03 million cells/μL) and Hemoglobin (Hb) for Male was (13.5-17.5 grams/dL), Female (12.0-15.5 grams/dL).

We analyzed fifty individuals as normal with >2.2 ratio, whereas remaining individuals were recognized as thalassemia trait with <2.2. Further, Hb-electrophoresis was performed for the ratio outcomes validation of each category. Hb-electrophoresis were performed by Cellulose acetate method as a corroborative test for the identification of beta Thalassemia trait. Individuals with normal or low Hb-A2 were taken as control arm while those with Hb-A2 of 3.5 or more were taken as test arm. The statistical analysis was performed on SPSS version 25. Mean difference was calculated with independent sample t-test. The results are reported as Mean ± deviation value and 95% Confidence interval

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(CI). P-value of <0.05 was considered as statistically significant.

Sensitivity and specificity formula:

Sensitivity=True positive / true positive + false negative x 100

Specificity= True negative / True negative + false positive x 100

Youden's index= (Sensitivity+ Specificity) -100

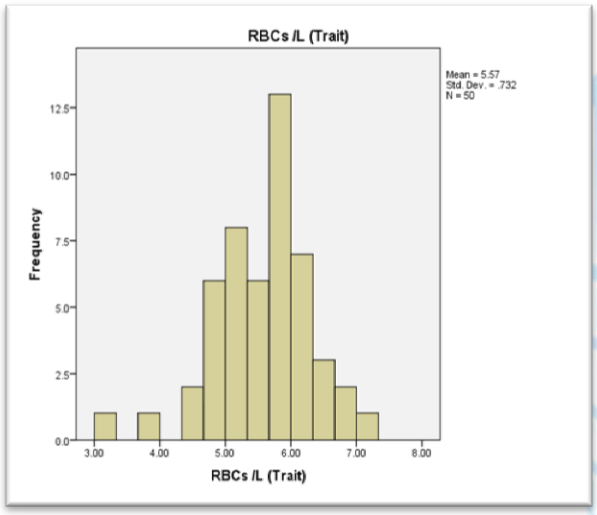
Present sample size sensitivity and specificity is 86% while Yourdon's index is 72%.

Results

The estimation of hemoglobin (Hb), red blood cell (RBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were analyzed between individuals with BTT trait and without BTT.

Estimation of Mean RBCs

The mean value of RBCs was shown significant difference. The mean value of RBCs was observed significantly higher (**5.57** ± 0.73/L),(**4.18** ± 1.43/L) in individuals with BTT as compared with normal individuals.



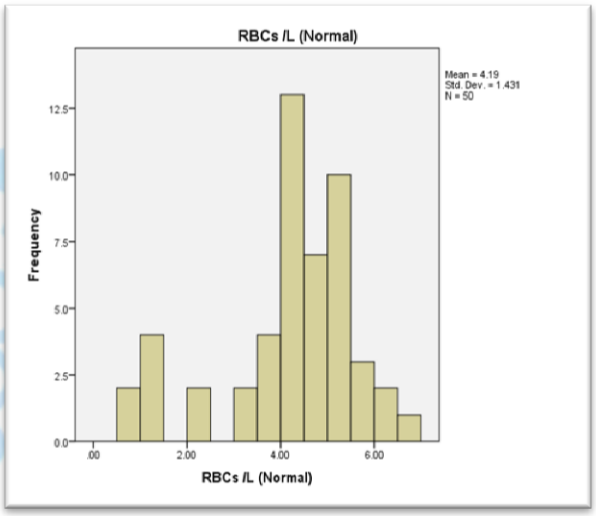


Table 1 Inequality of mean RBCs

Independent Sample t test										
		Levene's Test for Equality of varriances		t-test for Equality of Means						
									95% Confidence Interval of the	
		F	Sig.	t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
RBC	Equal variences assumed	10.798	0.001	6.092	98	0.000	1.3848	0.2273	0.93373	1.83587
	Equal variences not assumed			6.092	73.002	0.000	1.3848	0.2273	0.93179	1.83781

The statistical result was performed the inequality of mean between two groups by independent sample t-test. The t-test value was estimated as **6.092** (p <0.05) which shown significance and confidence level 95%.

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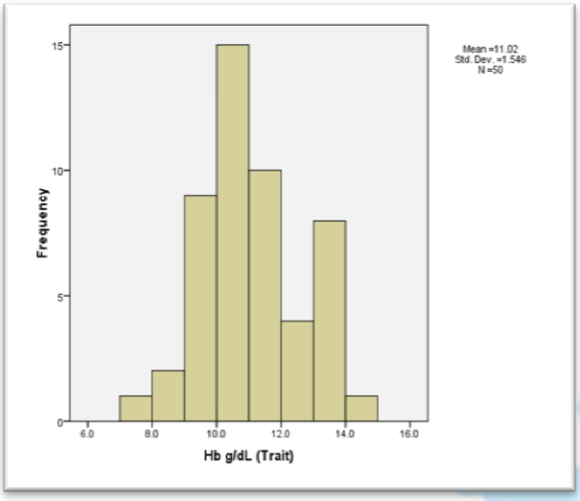
Estimation of Mean Hemoglobin

The mean value of Hemoglobin was analyzed as (11.02 ± 1.54g/dL),(11.15 ± 3.97g/dL) in individuals with BTT and without BTT.



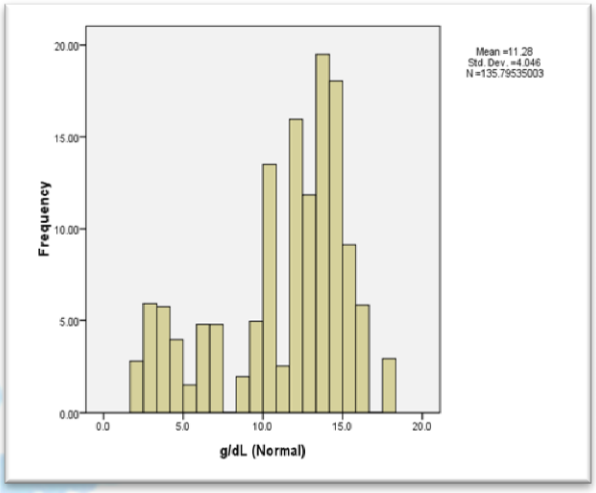


Table 2 Inequality of mean Hb g/dL

Independent Sample t test										
		Levene's Test for Equality of varriances		t-test for Equality of Means						
									95% Confidence Interval of the	
		F	Sig.	t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Hemoglobin	Equal varieces assumed	31.83	0.000	-0.2222	98	0.825	-0.134	0.60363	-1.33188	1.06388
	Equal varieces not assumed			-0.2222	63.468	0.825	-0.134	0.60363	-1.34008	1.07208

The statistical result shown no difference between individuals with BTT and without BTT.

Mean comparison of Ratio.

In comparison of ratio between Hemoglobin and Red blood cells, mean value of Ratio in Beta Thalassemia trait patients was estimated (**1.98**± 0.20) which is significantly different as compared to the mean value of Ratio of Normal individuals ($p < 0.05$) that is **2.71**±.0.481.

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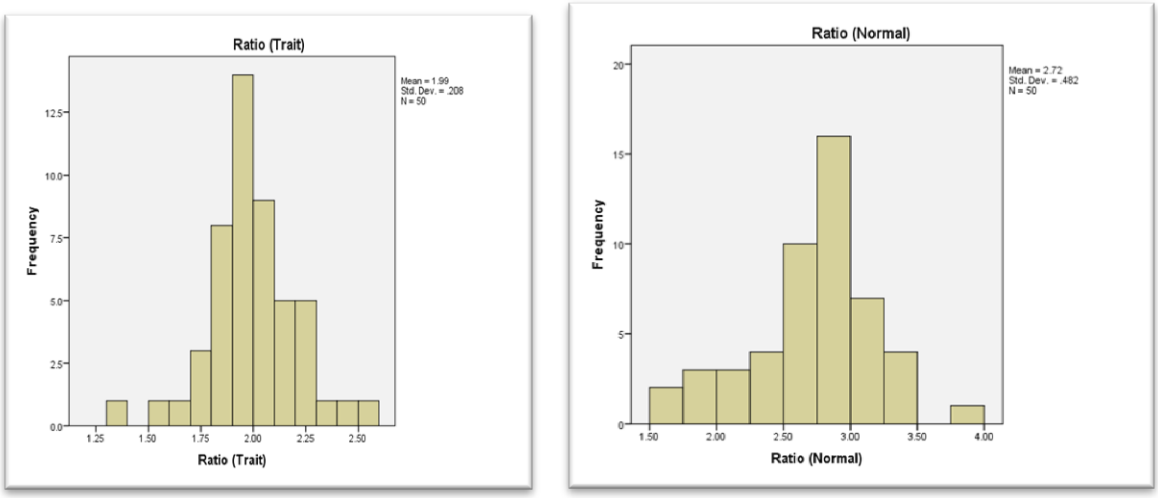

Estimation of ratio between Hemoglobin and Red blood cells by one sample t-test:

The results of one sample t-test were significantly proved the difference of ratio between Hemoglobin and Red blood cells in Beta thalassemia trait and Normal individuals.

1. Beta thalassemia trait subjects: Mean Ratio: **1.9** i.e. ≤ 2.2
2. Normal individuals: Mean Ratio: **2.7** i.e. >2.2

Table 3 One sample t test for beta thalassemia trait

One-Sample Test						
	Test Value= 2.2					
	t	df	Sig.(2-tailed)	Mean Difference	95% Confidance Interval of the Difference	
					Lower	Upper
Ratio	-7.174	49	0.000	-21105	-0.2702	-0.1519

The t-test values were -7.174 for beta thalassemia trait with $p<0.05$.

Table 4 One sample t-test for Control individuals

One-Sample Test						
	Test Value= 2.2					
	t	df	Sig.(2-tailed)	Mean Difference	95% Confidance Interval of the	
					Lower	Upper
Ratio N	7.573	49	0.000	0.51591	0.379	0.6528

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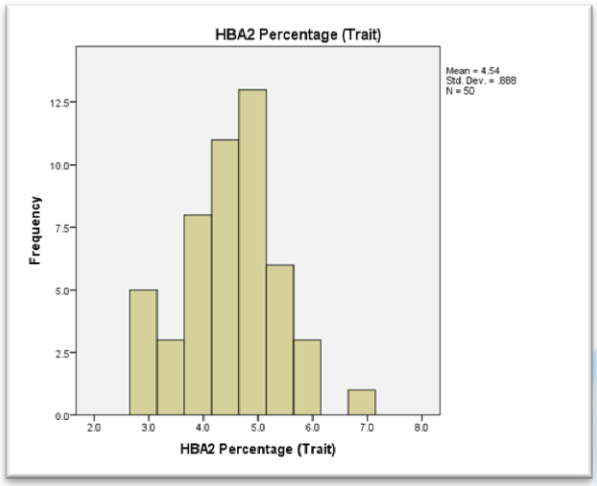
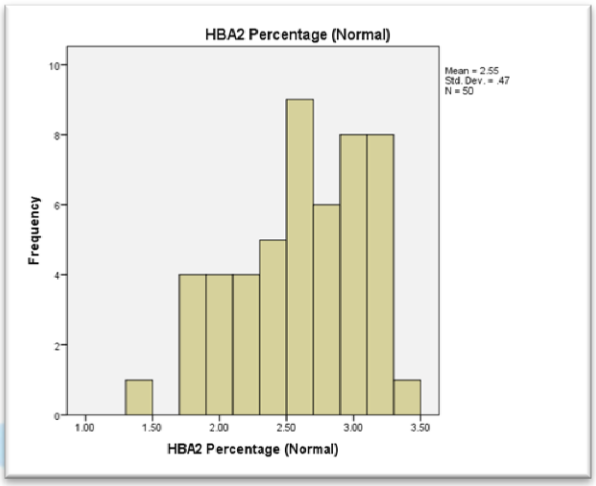
The t-test values were 7.573 respectively with p<0.05 and 95% confidence interval.

Mean comparison of HbA2 Level.

In comparison, patients who had thalassemia trait showed significantly higher HbA2 level (**4.54** ± 0.88%) as compared to Control individuals (**2.55** ± 0.47%).





The statistical result of t-test 14.00 (p<0.05) was measured to calculate the inequality of means between these two groups.

Table 5 Independent sample t-test for HbA2 level

Independent Sample t test										
		Levene's Test for Equality of		t-test for Equality of Means						
									95% Confidence Interval of the	
		F	Sig.	t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
HBA2	Equal variences assumed	13.368	0.000	14.005	98	0.000	1.99	0.14209	1.70802	227198
	Equal variences not assumed			14.005	74.453	0.000	1.99	0.14209	1.7069	227310

Estimation of mean MCV

The mean value of MCV was demonstrated a significant difference. The mean value of MCV in individuals with BTT was estimated as (**65.32** ± 5.65 fL) whereas, individuals without BTT was (**85.26** ± 10.78fL) (p<0.05). The statistical analysis was measured to check the inequality of means between these two groups.

Estimation of mean MCH

The comparison of MCV mean value in individuals with BTT was significantly lower as compared with normal individuals. The mean value of MCH was (**19.86** ± 2.070 Pg) and (**27.26** ± 4.47 Pg)

Discussion

This study investigated the CBC parameters Hb, RBCs, MCV and MCH as suspected trait of BTT. The significance differences in between groups. It would be contributed in the redundancy of BTT incidence by early cost-effective screening of individual as this tool has

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been validated since 90s [7]. Present study results determined a significance difference in CBC indices in individuals with BTT and without BTT. The decreased ratio of Hemoglobin and Red blood cells analyzed in individuals with BTT as compared to without BTT. The ratio between (Hb/RBCs) has shown their decreased levels, while RBCs and HbA2 assessed at elevated level in case of Beta thalassemia trait. Decreased production of beta chain result in increased HbA2 levels which ultimately cause thalassemia mentioned in literature.Previous study findings also supported these results [8, 9], Mean value of Hemoglobin (Hb) between individuals with beta thalassemia trait and normal individuals found equivalent. Hence suggested no significance difference which is accordance with previous study [7]. Hb electrophoresis has been used as a confirmatory test for beta thalassemia trait. This examination highlighted towards screening projects on large scale to evaluate thalassemia heterozygotes

Previous study conducted on pregnant anemic patients which reported thalassemia trait about 56.7%, is in line with our study findings 47(94%) beta thalassemia with 0.000 significance and 95% confidence interval ≤ 2.2[10]. According to Sharma et al., most of beta thalassemia carriers has shown lower mean MCV and MCH levels in the normal blood assessment which supported our findings [11].

On the account of examination and findings our study to pin back ones ear towards routine CBC in premarital counseling thereby hoping for a prevention of upcoming beta thalassemia majors. Ministry of health should be implemented health care policies at national level to ensure public health and prevention of future complications. Further interventional studies also recommended on large scale for identification of contributing factors in beta thalassemia, its treatment and early prevention.

Conclusion

Present study paves the way towards the detection of beta thalassemia traits and supports the opinion of population screening for thalassemia is recommended. Moreover, ensure the accessibility of resources such as for Hb-electrophoresis in health care institutions at public and private sectors.

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Authors' Contributions

RM; Designed, conceptualized and approved the final manuscript.

ZMA: Collected data, performed experiments, carried out the statistical analysis, wrote the manuscript.

SK&AK: Analyzed the data and manuscript writing.

NW, AB & SHQ: Reviewed and approved the final manuscript.

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