SCREENING OF BETA THALASSEMIA TRAIT BY COMPLETE BLOOD COUNT AND SINGLE TUBE OSMOTIC FRAGILITY TEST

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Abstract: Thalassemia is the most common inherited blood disorder with reduced rate of synthesis of either alpha or beta chain of hemoglobin. It most commonly occurs among Asian people, especially South East Asian and people of Mediterranean regions. In Pakistan, beta thalassemia is the commonest hemoglobinopathy disorder having carrier rate of 5% and 5-8% in KPK (Khyber Pakhtunkhwa). Therefore, prevention of thalassemia is needed to make it a thalassemia free region. Hemoglobin (Hb) electrophoresis is used to diagnose beta thalassemia but it is costly. Therefore, this study was conducted to find out the sensitivity and specificity of complete blood count and single tube osmotic fragility test in diagnosis of thalassemia trait.

Methods and material: In the present study we included 300 blood samples and 50 control samples from normal individual and 50 control samples from known thalassemia patients. Complete blood count was performed by using a hematological analyzer System KX 21. Single tube osmotic fragility test was performed by using a hypotonic solution of 0.36% concentration.

Result: SOFT (single osmotic fragility test) test was performed on 300 patients' samples and controls' samples. Normal controls showed negative result while in known thalassemia patient 48 samples showed positive result and 2 samples showed false negative result. In 300 samples 280 (93.33%) samples showed positive result with 93% sensitive while 20 samples showed false negative result having 87% specificity. In CBC (complete blood count) parameter, the sensitivity of RBC (red blood cell), red cell indices, Hb level and RDW (red blood cell distribution width) was 98%, 100%, 98% and 96% respectively.

Conclusion: As the sensitivity and specificity of single tube osmotic fragility test and CBC parameters is high therefore, it can be used for screening large populations in a cost effective way.

Key words: Beta Thalassemia, Complete Blood Count, Single Tube Osmotic Fragility Test

INTRODUCTION

Thalassemia is a group of inherited blood disorders characterized by decrease synthesis of one or more globin chains and reduce red blood cell indices. It is most prevalent in Asian people like Southeast Asian and Mediterranean. More than 100 million people are reported as thalassemia carrier throughout the world and about 100,000 children with thalassemia major are born each year.(1) In Pakistan Beta Thalassemia is the most common hemoglobinopathy disorder and major health problem with an estimated carrier rate of 5% while carrier rate in Khyber Pakhtunkhwa is 5-8%.(2) As in Pakistan, blood bank facility is very limited and blood supply to rural areas is a difficult task. Intense efforts are required for prevention of thalassemia and this is possible by mass education and various prevention strategies like, mass screening, family screening of the affected child, prenatal diagnosis and pre-marital screening.(3)

Premarital screening for thalassemia may be a good practice and require mass education in the preventive measurements. Prenatal diagnosis is required for those couples who are at risk and this is possible in first pregnancy by performing CVS (chorionic villus sampling). For a successful screening program proper awareness, education and support are require to eradicate thalassemia from this region.(4) The Beta thalassemia trait can be identified by mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH) determination combined with hemoglobin A_2 quantitation. Globin chain synthesis, analysis on fetal blood sample can also be used for the detection of thalassemia trait.(5) However, these techniques cannot be used for screening large populations as these techniques are very expensive and time consuming. Therefore, a simple, low cost, rapid and reliable technique for the screening of the mass population is required.(6)

To prevent thalassemia in KPK a good strategic approach for carrier screening is needed. For this purpose screening of school children, antenatal screening and screening of closed relatives of known thalassemia patients is required. This is the best way for screening, as large population can be screened with minimum efforts and in a cost effective way. In Pakistan, as a large population belongs to rural areas where diagnostic facilities are limited, there is unawareness due to lack of education, and due to cousin marriages cases of thalassemia are increasing day by day. For this reason screening in these areas are necessary to make it free from thalassemia. Microcytic red cells in thalassemia have a low surface to volume ratio and therefore when place in hypotonic solution show resistance to lysis. Several studies have proved the importance of this single tube osmotic fragility test in screening thalassemia trait.(7, 8)

The purpose of this study was to assess the usefulness of complete blood count (CBC) parameters and single tube osmotic fragility test as "rapid" screening method for screening of thalassemia's families.

METHODOLOGY

The present study was conducted in the Central Pathology Laboratory Peshawar from 2013 to 2015. We included 300 patients' samples, 50 samples from normal individuals and 50 samples from known thalassemia trait patients as a control. Complete blood count was performed on Sysmex KX 21. On the bases of CBC parameters, RBC count, Red cell indices, RDW and single tube osmotic fragility test were performed. For single tube osmotic fragility test, we used buffered saline solution of 0.36% concentration. A stock solution of 10% buffered saline was prepared in which 90 g NaCl, 13.655 g Na2PO4 and 2.4 g NaH2PO4.2H2O was dissolved in 1,000 ml of distilled water. To prepare 1% buffered saline form 10% stock solution, 1:10 dilution was used with distilled water. To prepare 0.36% solution from 1%, 36ml from 1% buffered saline was mixed with 64ml of distilled water. For confirmation of our results, these samples were tested for Hemoglobin electrophoresis on Bio rad D10, based on high pressure liquid chromatography technique.

In the present study, we included those patients whose RBC count was above 4.9 million/ul, red cell indices were below the lower limits of normal range and Hb levels was ranged from 9 to 11.5 g/dl. There was no limitation of age. Data was statistically analyzed by using SPSS version 16.0.

RESULTS

We included 300 samples in our study. We performed CBC, Hb electrophoresis and single tube osmotic fragility test on 300 samples and for references we processed 50 samples from normal individuals and 50 samples from known thalassemia patients. We determined that mean values for Hb, Hct (hematocrit), RBC, MCV, MCH, MCHC, RDW and Hb A2 level of patients were 10.25±1.23 g/dl, 35.81±3.67%, 5.06± 1.47 mil/ul, 70.85±3.43fl, 24.88±1.35pg, 30.11±0.70%, 19.60± 4.85% and 5.1±.90% respectively (table 1). We performed single tube osmotic fragility test on controls and patients samples and determined that 50 samples from normal individuals have negative results. In known patients of thalassemia trait 2 (4%) samples showed false negative results while 48 (96%) samples showed positive result. In 300 patients' samples 20 (6.66%) samples showed false negative results while 280 (93.33%) showed positive result which was confirmed after Hb electrophoresis (table 2).

TABLE: 1 MEAN VALUES OF CBC PARAMETER AND HB ELECTROPHORESIS REPORTS

Parameters	Normal control (50)	Known Thalassemia trait (50)	Patient samples (300)
Age (years)	20.00±8.00	12.00±7.00	10.00±5.00
Hb (g/dl)	14.05±1.45	9.67±0.79	10.25±1.23
Hct (%)	41.72±3.67	36.17±2.87	35.81±3.67
RBC(mil/μl)	4.26±1.57	5.43±2.21	5.06±1.47
MCV(fl)	88.67±4.21	62.43±3.13	70.85±3.43
MCH (pg)	30.43±2.11	22.31±2.16	24.88±1.35
MCHC (%)	34.32±1.10	24.65±1.40	30.11±0.70
RDW (%)	15.30±3.54	17.54±4.56	19.60±4.85
Hb A2 level (%)	2.10±0.81	4.80±1.10	5.10±.90

TABLE: 2 RESULT OF SINGLE TUBE OSMOTIC FRAGILITY AND HB ELECTROPHORESIS FROM CONTROL AND PATIENT SAMPLES

Test result	Control		Patient samples
	Normal individual (n=50)	Known thalassemia trait (n=50)	(n=300)
Negative SOFT test	50 (100%)	2 (4%)	20 (6.66%)
Positive SOFT test	0 (0%)	48 (96%)	280 (93.33%)
Hb A_2 level on Hb Electrophoresis	2.1±.81	4.8±1.1	5.1±.90

DISCUSSION

In our study, we determined the importance of complete blood count and single tube osmotic fragility test in diagnosis of beta thalassemia trait. We found in our study that in hematological parameters mean value of RBC count, MCV, MCH, MCHC and RDW was 5.06±1.47mill/ul, 70.85±3.43fl, 24.88±1.35 pg, 30.11±0.70% and 19.60±4.85% respectively. These hematology parameters can be used as initial screening for detection of beta thalassemia trait in areas where electrophoresis facilities are not available and for screening of large population.(9) We found that the sensitivity of RBC, red cell indices, Hb level and RDW was 98%, 100%, 98% and 96% respectively. Our results were similar to the study conducted by Yasar M and his colleague in 2010 at Pakistan who reported that the sensitivity of MCV was 100% while sensitivity of MCH was 99%.(10)

Single tube osmotic fragility test was performed on normal individual, known thalassemia patient and on 300 patients' samples. Samples from normal individuals showed negative results, while in 50 samples from known thalassemia trait patients showed positive results in 48 (96%) samples and false negative result in 2 (4%) samples. In 300 patients' samples, 280 (93.33%) samples showed positive result, while 20 (6.66%) samples showed false negative results. In our study the sensitivity of single tube osmotic fragility test was 93% and specificity was 87%. A study conducted by Singh S P and Gupta in 2008 reported that the sensitivity of the SOFT test was 97.7% while the specificity of the SOFT test was 83.3% which is very closed to our results.(6) Another study conducted by Mehta et, al. determined that the sensitivity of single tube osmotic fragility test was 95%, while specificity was 85%.(11) Similar study was conducted by Sirichotiyakul et al in 2004 who reported that the sensitivity of the SOFT test was 97.7% while the specificity of the SOFT test was72.9%.(12) Our result were different from the study conducted by Yazdani SM and Ahmed S in 2010 at Sargodha who reported that SOFT was positive in 79% and negative in 21% of cases.(13) This difference may be due to the low sample size they analyzed in their study. For confirmation, we performed Hb electrophoresis on 50 normal patients and 300 patients' samples. We found that in 50 normal patients' Hemoglobin A_2 level was normal while in 300 patients' hemoglobin A_2 was more than 3.5%. We conclude that Red cell count, MCV, MCH, MCHC, single tube osmotic fragility test can be useful tests for screening mass population in rapid and cost effective way.

CONCLUSION

Thalassemia remains a major health problem in Pakistan. A proper prevention program is required to make this region free from thalassemia. In order to reduce the rate of thalassemia proper screening is required. For this purpose Red cell count, red cell indices, red cell distribution width and Single Osmotic Fragility Test can be used as simple screening tests for the detection of thalassemia trait.

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