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SINGLE TUBE OSMOTIC FRAGILITY TEST: A SENSITIVE AND SPECIFIC TEST FOR SCREENING OF MICROCYTOSIS

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ABSTRACT

Aim and Objective: To evaluate usefulness of Single Tube Osmotic Fragility Test (SOFT) for detection of microcytosis and its further application for thalassemia trait screening. **Background:** Iron deficiency and thalassemia are common causes of microcytic anaemia in Pakistan. Easy microcytosis screening is necessary at a low resourced country like Pakistan, so that further investigations specially for thalassemia may be ordered. Thalassemia is one of the commonest genetic disorders of Pakistan that has both medical and social implication. **Materials and Methods:** A total of 387 students belonging to four different colleges of Gulshan-e-Hadid, were included in study. SOFT was performed on all 387 participants using 0.36% saline. Haemoglobin (Hb), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemglobin (MCH) was estimated in all individuals using Sysmexhaematology counter. **Results:** SOFT was positive in 26.87% of study population. Sensitivity and specificity of SOFT was found to be 93.94% and 96.18% for detection of microcytosis. Its positive and negative predictive values were 89.42% and 97.88% respectively. SOFT positive and negative groups showed statistically significant difference in Hb, MCV and MCH. **Conclusion:** SOFT can be used cost effectively for microcytosis screening. Its results can target individuals to be investigated for thalassemia.

Keywords: SOFT, Microcytosis, Thalassemia screening, Thalassemia trait

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INTRODUCTION

Iron deficiency & β –Thalassemia trait are the most frequent causes of microcytosis (anaemia of chronic disorders and sideroblastic anaemia being less common causes)[1]. Iron deficiency is more common in our country, but if it is ruled out, β –Thalassemia trait needs to be considered.β -Thalassemia is one of the commonest inherited disorders throughout the

world[2]. About 5% of Pakistani population are β-thalassemia carriers with 5000 to 9000 children being born with β –Thalassemia majorannually[3,4,5]. Trends of consanguineous marriages[6,7], high birth rate& early marriages with unawareness has led Pakistan towards a comparatively higher number of children with transfusion dependent Thalassemia in the world.[8]The birth of a thallasemic child, poses considerable physical & economic strain, not only on the affected child & its family, but also on the community & the nation at large[9]. Prenatal diagnosis followed by termination of pregnancy may not work in our society due to religious values[10].With these limitations, emphasis must shift from treatment to prevention of such births in future.[11,12]?- thalassemia trait is suspected when Mean Corpuscular Volume(MCV) and/or Mean Corpuscular Haemglobin(MCH) are low(Microcytosis and/or hypochromia).Microcytosis can reliably be detected by electronic counter with added peripheral film examination.These tests and HbA2 estimation (confirmatory test for beta thalassemia trait) are expensive and require sophisticated equipment and expertise[13]. The need, therefore, is for a simple, low cost, rapid and reliable test which can be applied for mass screening[14]. Microcytes, due to increased surface area to volume ratio resist osmotic lysis, and this property has helped development of Single Tube Osmotic Fragility Test (SOFT)[15]. We conducted this study to evaluate Single Tube Osmotic Fragility Test(SOFT) as screening test for microcytosis in Pakistan.

PATIENTS AND METHODS

TEST POPUATION AND SAMPLE COLLECTION:

The current study was undertaken in co-operation of four private colleges situated at Gulshan-e-Hadid, Karachi. Camps for screening were conducted at these four colleges during the month of March 2016.A total of 387 students were included in study. Consent was taken from parents before including a student in study (College administration helped in this regard). These 387 students were screened for Single Tube Osmotic Fragility Test(SOFT), Haemoglobin(Hb) level, MCV and MCH. Blood samples were collected by a team of two doctors, two laboratory technicians and two laboratory assistant. The laboratory personnel prepared the stock solution of Single Tube Osmotic Fragility Test(SOFT) in advance.

SINGLE TUBE OSMOTIC FRAGILITY TEST(SOFT):

SOFT was performed using 0.36% saline solution. 5ml of this solution was taken in one test tube and 5ml of distilled water was taken in another test tube. A drop of blood was added to each test tube. After initial mixing the tubes were allowed tostand for 5-10 minutes at room temperature. The results were read by placing the tube against written material. The written material was clearly visible through the contents of tube containing distilled water.If the written material was similarly visible through the contents of tube with buffered saline, the test was considered negative. If the written material was not clearly visible, the test was considered positive. A positive test indicates lowered red cell osmotic fragility, suggestive of thalassemia trait.

COMPLETE HAEMOGRAM:

Samples of blood taken in the EDTA tubes were numbered, sealed and transported in ice flasks back to laboratory. Complete haemogram including haemoglobin, 'PCV, RBC count, MCV and MCH were performed using Sysmexhaematology analyzerwithin 12 to 24 hours after collection.

DATA ANALYSIS:

SPSS version 16 was used to analyze data. Relative descriptive statistics, frequency, and/or percentages were computed for categorical variables like gender and SOFT. Mean and standard deviation were calculated for quantitative variables like Hb, MCV and MCH. Independent sample t-test was applied to compare the difference in Hb, MCV and MCH between SOFT negative and positive groups. Sensitivity analysis for SOFT was done to compute sensitivity, specificity, positive predictive value and negative predictive value by taking MCV as gold standard.

RESULTS

Of the total 387 individuals included in this study, 159 were male(41.1%) and 228 were female (58.9%). SOFT was positive in 104 (26.87%) individuals and negative in 283(73.13%) individuals. Mean MCV in SOFT positive individuals was 68.56fl which is significantly lower than Single Tube Osmotic Fragility Test(SOFT) negative group(Mean MCV in SOFT negative group was 86.56fl).Simillarly mean MCH in Single Tube Osmotic Fragility Test (SOFT) positive individuals was 21.51pg which is significantly lower than 27.85pg as seen in negative group.(Table 1)

Single Tube Osmotic Fragility Test(SOFT) was considered false positive when MCV was more than 75fl with positive Single Tube Osmotic Fragility

SINGLE TUBE OSMOTIC FRAGILITY TEST(SOFT)

TABLE 1:
Single Tube Osmotic Fragility Test: Results

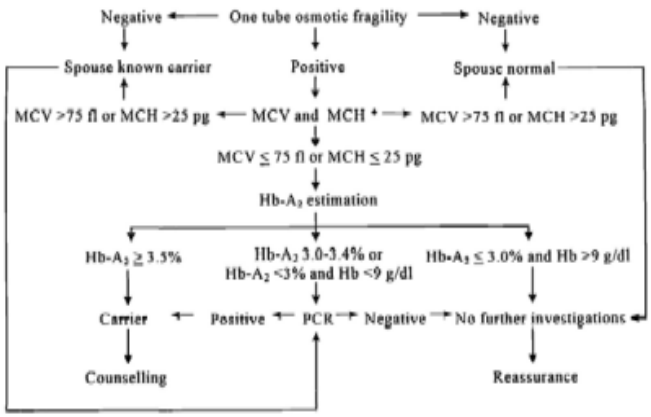
SOFT	Hb(gm/dl) Mean±SD	MCV(fl) Mean±SD	MCH Mean±SD
Positive	10.32±1.37	68.56±5.19	21.51±2.77
Negative	12.49±1.18	86.57±5.32	27.85±2.01

Test(SOFT) result. Alternatively, Single Tube Osmotic Fragility Test(SOFT) was considered false negative when test was negative with MCV was 75fl or lower. Out of total387 individuals 93(24.03%) were true positive and 277(71.58%)were true negative. 11(2.84%) were false positive and 6(1.55%) were false negative.Sensitivity analysis revealed that Single Tube Osmotic Fragility Test(SOFT) is 93.94% sensitive & 96.18% specific for detection of microcytosis.Positive predictive and negative predictive values were 89.42% and 97.88% respectively. So any individual with negative SOFT has 97.88% chances that he is free from conditions resulting in microcytosis. Diagnostic accuracy was 95.61%.

DISCUSSION

At Pakistan, strong cultural preference for consanguineous marriage has been responsible for relatively high prevalence of recessively inherited disorders like ?-thalassemia[2].Most children diagnosed with thalassemia belong to developed section of country whereas children born in less developed section of country die undiagnosed. Currently preventive strategy in most parts Pakistan runs in a retrospective manner, genetic counselling being considered after birth of thallasemic child in a family. We tested possibility of applying a simple and cost effective test for screening of ?-thalassemia trait at developing country, Pakistan where many areas lack basic necessities of life[17]. Haemoglobin electrophoresis is considered gold standard for diagnosis of thalassemia but it is costly and require expertise. Same is true for MCV and MCH estimation but they are comparatively cheaper. So we tried to test applicability of a simple Single Tube Osmotic Fragility Test(SOFT) for detection of microcytosis and thalassemia trait. Our study showed a strong association between microcytosis and SOFT positivity. Single Tube Osmotic Fragility Test(SOFT) with 0.36% saline concentration is found to be 93.94% sensitive and 96.18%specific for detection of microcytosis. Single Tube Osmotic Fragility Test(SOFT) positive and negative groups have statistically significant difference in MCH and MCV.So Single Tube Osmotic Fragility Test(SOFT) may be used to detect microcytosis, if found positive further investigation for thalassemia may be ordered. This has been proved in similar studies conducted at India[18], Jordon[19] and Iran[20]. A study conducted by Gomber S et al. in India showed comparative analysis of osmotic fragility test with red cell indices is most sensitive and specific test for diagnosis of thalassemia trait. They believed it is simple, inexpensive and easily reproducible test for population screening for ? thalassemia trait.[18]One similar study was conducted at Pakistan in2004[17] .One of the most significant finding is small number of false negative result i.e. 6(1.55%) with negative predictive value of 97.88%.It signifies that Single Tube Osmotic Fragility Test(SOFT) negative individuals can be declared thalassemia free with reasonable surety. This is quite strong implication that if both male and female members of married couple

FIG. 1
A proposed algorithm for screening in indexed families[22]



are SOFT negative, in a target family, then their descendants will not require further testing.Sumera A[21] et al observed that Single Tube Osmotic Fragility Test(SOFT) was positive only in 13% cases of microcytosis associated with iron deficiency. It shows this test is more specific for detecting microcytosis of thalassemia trait. Single Single Tube Osmotic Fragility Test(SOFT) costs only Rs.10 as compared to MCV estimation by automated analyser which costs about Rs.200 for one patient, making it ineffective for mass screening.an algorithm is proposed already for screening of indexed families of thalassemia[22].It includes Single Tube Osmotic Fragility Test(SOFT) as preliminary test. Recently government of Pakistan tried to make thalassemia screening mandatory before marriage, but practically it did not work because of cost of investigations. So, we believe that simple Single Tube Osmotic Fragility Test(SOFT) may be used for screening before marriage. This will change our current retrospective prevention strategy.

CONCLUSION

Single tube osmotic fragility test is a cost effective and simple test that can be done in the field without anyspecial equipment or expertise. It is very sensitive for the screening of ?-thalassemia carriers. The test has high negative predictive value and Single Tube Osmotic Fragility Test(SOFT) negative individuals can safely be declared free of thalassemia. Subject who are Single Tube Osmotic Fragility Test(SOFT) positive need further investigation to establish diagnosis.

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* Concept and design,Collection and assembly of data
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