ONE TUBE OSMOTIC FRAGILITY TEST: A SCREENING TEST FOR MICROCYTIC RED CELLS

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ABSTRACT

Objective: To evaluate the usefulness of One Tube Osmotic Fragility Test (OTOFT) for detection of microcytosis.

Design: Validation study.

Place and Duration of Study: The study was carried out from July 2004 to November 2004 at Pathology Department PNS Shifa, Karachi.

Patients and Methods: Five hundred and fifteen individuals were studied who reported to the reception of Pathology Dept. for blood complete picture. One drop of finger prick blood was added to a test tube containing 5 ml of 0.36% saline. The results were read at 10 minutes interval by visualizing a written material through the contents of the test tube. Three ml of blood was collected in the EDTA tube by venepuncture for determination of Haemoglobin (Hb), Mean Cell Volume(MCV) and Mean Cell Haemoglobin(MCH) by electronic haematology counter "Sysmex 4500".

Results: Out of total 515 subjects studied, OTOFT was positive in 130 (25.2%) and negative in 385 (74.8%) cases. OTOFT positive and negative groups revealed statistically significant difference (P-value of < 0.05) in MCV, MCH and Hb. OTOFT proved to be 92.5% sensitive and 95.2% specific for screening of microcytosis. It had positive predictive value of 85.38% and negative predictive value was 97.66%. The diagnostic accuracy was 94.6%.

Conclusion: One Tube Osmotic Fragility Test (OTOFT) is a simple and cost effective test, which is highly sensitive as well as specific for screening of microcytosis. It may prove a useful tool in targeted and/or population screening for thalassaemia.

Keywords: Microcytosis, thalassaemia screening, one tube osmotic fragility test, OTOFT, targeted screening

INTRODUCTION

The commonest causes of microcytosis (low MCV) are Iron deficiency and β-thalassaemia trait. Rarely it can be seen in chronic disorders and sideroblastic anaemia [1]. Iron deficiency is the commonest cause of anaemia worldwide [2] and has been labeled as the most common organic disorder seen in the clinical medicine [3]. The situation is even worse in developing countries like Pakistan [4]. Thalassaemia is the commonest genetic disorder in Pakistan. Taking an average figure of 5% for carrier prevalence of β-thalassaemia, there are 6 million carriers in the country [5,6]. However thalassaemia is a preventable disorder which implies identification of its carriers. β-thalassaemia trait can be suspected when MCV and/or MCH are low. The most accurate and reliable method to detect microcytosis is by electronic counter. RBC morphology in peripheral blood film is also an important adjunct. But these methods cannot be used in large scale population screening due to their inapplicability in the field. Microcytes due to increased surface area

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to volume ratio resist osmotic lysis [7,8]. This feature may be exploited in the development and evaluation of One Tube Osmotic Fragility Test (OTOFT) [9-11]. Many studies have shown OTOFT as an important tool for screening of microcytosis [12-15]. If this test is proved to be applicable in the field with considerable reliability for the detection of microcytosis then it can be used possibly as an initial tool in targeted screening or in large-scale population screening for β-thalassaemia trait. The objective of study was to evaluate OTOFT as a screening test for microcytosis in Pakistan.

PATIENTS AND METHODS

This study was conducted in Pathology department, PNS Shifa. This was a validation study and sampling was done by non-probability convenience sampling technique. Duration of study was from July, 2004 to November, 2004. All the individuals reporting for blood complete picture both males and females of all ages were included in the study. Patients reluctant to give blood samples were excluded from study. Total five hundred and fifteen individuals were studied. After taking consent their name, age and sex were recorded.

The reagent used was 0.36% NaCl solution, 5 ml of this reagent was taken in test tube and a drop of blood obtained by finger prick was added to it. After initial mixing the tubes were allowed to stand at room temperature. The results were read after 10 minutes by placing the tube against written material. In negative result due to lysis of red cells, solution became clear and written material was clearly visible. On the other hand in the tubes with positive result the cells were not lysed and it was not possible to see printed material through the tube (fig. 1). Three ml of blood is taken by venepuncture in a disposable syringe and the blood is shifted to EDTA anticoagulant containing CP bottle. The tube is inverted number of times for proper mixing of blood. Haemoglobin (Hb), Mean Cell Volume (MCV) and Mean Cell Haemoglobin (MCH) were determined by electronic counter "Sysmex 4500". The controls of the machine were run each day to monitor validity of results. All the observations were recorded. MCV was used as gold standard.

Statistical Package for Social Sciences (SPSS-10) was used to analyse the data. Relative descriptive statistics, frequency, and/or percentage were computed for qualitative variables like Sex and OTOFT. Mean and Standard deviation were computed for quantitative variables like age, Hb, MCV and MCH. Independent samples t-test was applied to to compare the difference in age, Hb, MCV and MCH between OTOFT negative and positive groups. Sensitivity analysis of OTOFT was done to calculate the Sensitivity, Specificity, Positive predictive value and Negative predictive value by taking MCV as gold standard.

RESULTS

Out of total 515 cases studied, 130 (25.2%) were positive while 385 (74.8%) were negative (table-1). These included 268 (52%) males and 247 (48%) females. The mean MCV in OTOFT positive individuals was 68.7 fl, which is significantly lower than 88.0 fl in the negative group (P-Value < 0.05). Very similar findings were noted for MCH. The mean MCH in OTOFT positive cases was 20.3 pg which is significantly lower (P-value < 0.05) than 28.5 pg as seen in negative group (table-1).

OTOFT is not a fool proof procedure. One can get false positive as well as negative result. False positive was considered when MCV was more than 75 fl, with positive result. Similarly result was labelled false negative when OTOFT was negative but the MCV was 75 fl or lower. Out of total 515 individuals, 111 (21.6%) were true positive and 376 (73.0%) were true negative. Nineteen out of 515 (3.7%) were false positive and only 9 (1.7%) were false negative (table-2).

Sensitivity analysis revealed that OTOFT is 92.5% sensitive and 95.2% specific for the detection of microcytosis. Positive Predictive Value and Negative Predictive Value were 85.38% and 97.66% respectively. So any individual with negative OTOFT have 97.66 % chances that he is free from the conditions resulting in microcytosis. Diagnostic accuracy was 94.6%.

Table-1: One tube osmotic fragility test: Results.

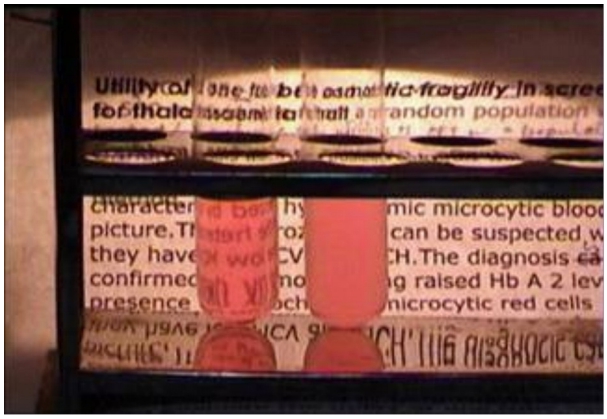
OTOFT	Hb (g/dl) Mean ± SD	MCV (fl) Mean ± SD	MCH (pg) Mean ± SD
Total (n=515)	12.1 ± 2.3	83.2 ± 10.8	26.4 ± 4.6
Positive 130 (25.2%)	10.4 ± 2.1	68.7 ± 7.2	20.3 ± 3.3
Negative 385 (74.8%)	12.7 ± 2.1	88.0 ± 6.7	28.5 ± 2.7
P-Value*	< 0.05*	< 0.05*	< 0.05*

Key: *: Significantly low if P-value is <0.05
Hb: Haemoglobin
MCV: Mean Cell Volume
MCH: Mean Cell Haemoglobin
OTOFT: One Tube Osmotic Fragility Test

Table-2: Sensitivity analysis of OTOFT.

MCV / OTOFT	Positive	Negative	Total
Positive	111	19	130
Negative	9	376	385
Total	120	395	515

Sensitivity = TP / TP + FN x 100 = 92.5%
Specificity = TN / TN + FP x 100 = 95.2%
Positive Predictive Value = TP / TP + FP x 100 = 85.38%
Negative Predictive Value = TN / FN + TN x 100 = 97.66%
Diagnostic accuracy = TP+TN/TP+TN+FP+FN x 100=94.6%
Key: TP: True positive TN: True negative
FP: False positive FN: False negative



DISCUSSION

Collectively iron deficiency and thalassaemia are the main causes of hypochromic and microcytic blood picture in Pakistan [4,6,16,17]. Thalassaemia is the commonest genetic disorder in Pakistan [6,17]. With a carrier rate of 5% it is estimated that about 5250 infants with thalassaemia major are born every year in the country [18]. In a married couple who are both carriers, there are 25% chances in each pregnancy that a child with thalassaemia major will be borne [18-20].

The majority of population in our country lives in rural areas where they lack even basic necessities of life. For the success of any health program and its delivery to the doorstep is of prime importance in this situation. In addition any prevention program for thalassaemia requires as a preliminary step, the reliable identification of carriers [17]. Screening for β-thalassaemia trait is extremely difficult because of unavailability of a single cost effective test that can be performed in the field (far flung rural areas lacking basic necessities) [21]. Many attempts have been made to establish a screening test capable of detecting β-thalassaemia carriers. These include MCV, MCH by electronic counter and estimation of Hb A2. But all of these require a well-equipped lab and technical expertise.

If a test is developed that is cost effective and simple to perform then it will be possible to screen for β-thalassaemia carriers at the doorstep of people. One tube osmotic fragility test was a proposed candidate and recommended in India [11-13,22], Jordan [23] and Iran [10] to be used as a screening test for β-thalassaemia. No such study has been published so far in Pakistan.

Our study has shown a very strong relationship between microcytosis and OTOFT positivity. OTOFT with 0.36% saline concentration is found to be 92.5% sensitive and 95.2% specific for detection of microcytosis. These results are comparable

Fig. 2: A proposed algorithm for screening in indexed families [17].

conducted [12,14,15,18]. OTOFT negative and positive groups have statistically significant differences in MCV, MCH and Hb.

One of the most significant finding is small number of false negative results (1.7%) with Negative Predictive Value of 97.66%. It signifies that OTOFT negative individuals can be declared there and then free from thalassaemia with reasonable surety. This has quite strong implications that if both male and female members of a married couple are OTOFT negative, in a target family, then their descendents will not require further testing. It further increases the cost effectiveness of the procedure. Since hypochromia is almost always associated with microcytosis the same is depicted in this study also.

The issue of cost is also very important in large scale screening. The cost of one test of OTOFT is less than one rupee as compared to the MCV determined by automated haematology analyser which costs at least more than thirty rupees on an average approximately (as per prevailing rates). An algorithm is proposed already for screening of the indexed families of thalassaemia (fig. 2) [17]. It includes OTOFT as a preliminary test.

CONCLUSION

One Tube Osmotic Fragility Test has emerged as a highly cost effective and simple test that neither requires any equipment nor any special expertise and on the other hand it is sensitive as well as specific enough that it can be used with reasonable reliability for the detection of microcytosis. We suggest that OTOFT is the most suitable preliminary test for targeted screening of extended families for thalassaemia in Pakistan. Subjects who are

OTOFT positive need further investigations to establish the diagnosis.

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