Evaluation of NESTROFT as a marker of differentiation between β- Thalassemia Trait & Iron Deficiency Anemia

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Abstract

Objective: To evaluate efficiency of Naked Eye Single Tube Red cell Osmotic Fragility Test (NESTROFT) as a marker of clinical differentiation between β -thalassemia trait (BTT) & Iron deficiency anemia; to rule out potential bias of NESTROFT with regards to its sensitivity and specificity in cases with coincident iron deficiency anemia.

Materials & Methods: This was a case control study on 503 subjects, carried out at Dow Diagnostics Research & Reference Lab (DDRRL) during the period from December 2009 to August 2010. Subjects were categorized into three groups based on red cell indices, preformed on automated hematology analyser (cell tac alpha, Nihon Kohden, Japan). Group I, comprised of control subjects with normal red cell indices, Group II comprised of case subjects with microcytosis MCV < 80 fL, normal ferritin levels and HbA₂ > 3.5% on Hb Electrophoresis and Group III included subjects with proven Iron Deficiency Anemia (IDA) i.e., low serum ferritin levels; hemogram & peripheral smears suggestive of iron deficiency anemia. NESTROFT was performed on all cases. Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value were calculated. Differential Diagnosis of IDA & BTT was justified by testing serum Ferritin and Hemoglobin Electrophoresis (showing increased HbA₂ (\geq 3.5%) on cellulose acetate at an alkaline pH in cases with BTT). Data analysis was done on SPSS version 16.0.

Results: Out of total 503 study subjects, microcytosis was found in 253 subjects. Majority of our microcytosis cases were with IDA n=174/253 (69%), while BTT cases were n=73/253 (29%). Sensitivity of NESTROFT was observed 93%, Specificity was 88% with Positive Predictive Value of 74% & Negative Predictive Value of 97%. NESTRODT was concomitantly positive in 13% IDA cases while it remained negative in 88% of subjects with IDA. For differentiation between IDA and BTT, red cell indices (MCV, MCH & MCHC), RBC count, & RDW% demonstrated statistically significant differences (P < 0.05).

Conclusion: We conclude that NESTROFT is sensitive and specific marker in differentiating beta-thalassemia trait from iron deficiency anemia.

Key Words: NESTROFT, β - Thalassemia trait, iron deficiency anemia, discriminant functions

Introduction

 β -Thalassemia is a progressive public health problem worldwide. In next 20 years, estimated 900,000 births with thalassemia disorders are expected.¹

 β -Thalassemia and Iron deficiency anemia are among the common causes of microcytic anemia. Rarely anemia of chronic disease also presents with microcytosis.² Differing management guidelines (e.g. iron supplementation) warrant correct differentiation between IDA and BTT.³

Several mathematical formulae have been used for identification of BTT, based on red blood cell indices.² The first step at discrimination between IDA and BTT relies upon identification of microcytosis and the whole blood red cell count. With the availability of electronic particle counters, red cell indices have become more clinically significant and are combined or used singly to identify possible heterozygotes. But due to technical requirements of method, red cell indices, done on automated analysers, cannot be used for mass screening, or field surveys.

Recently published data concludes that NESTROFT can be effectively used as screening marker for detection of β -Thalassemia trait.^{1, 4, 5, 6, 7} Different studies show that NESTROFT with 0.36% saline could detect 96-100% of heterozygotes with β -Thalassemia. A study published in Indian J Pathol Microbiol, 2002 concludes NESTROFT to be 92.5% sensitive and 95.2% specific for screening of red cell microcytosis.⁸ The test proves to be simple, cheap, easy to perform and adaptable for mass screening, coming close to an ideal screening test. According to a recent study conducted at PNS Shifa Hospital, Karachi, Pakistan, NESTROFT has a Positive Predictive Value of 85.38% and Negative Predictive Value of 97.66%, correlating to internationally published data.⁶ The diagnostic accuracy of NESTROFT was 94.6 %.¹ NESTROFT with 0.36 % saline is a sensitive solution and provides accurate results compared to other concentrations of saline.⁴ Routine use of hematological data from automated cell counters may complement the results of the NESTROFT.⁵

The objectives of this study were to evaluate efficiency of Naked Eye Single Tube Red cell Osmotic Fragility Test (NESTROFT) as a marker of clinical differentiation between β -thalassemia trait (BTT) & Iron deficiency anemia; to rule out potential bias of NESTROFT with regards to its sensitivity and specificity in cases with coincident iron deficiency anemia.

Materials & Methods

A case control study was conducted on total 503 subjects at Dow Diagnostics Research & Reference Lab (DDRRL), Karachi, Pakistan from December 2009 to August 2010. After informed consent, a questionnaire was filled by subjects. Participating subjects with MCV >80 and with Hb and ESR within normal ranges were taken for control subjects in our study. Subjects with gestation, having history of chronic inflammatory disorders or of blood transfusion within three months were excluded from study. Observing standard procedure,

venous blood (2.5 ml) was collected into a vacuum enabled tube containing K_2 EDTA anticoagulant (1.5 ± 0.25 mg/ml). Within two hours of sample collection, NESTROFT and Complete blood counts (CBC) were performed on all samples. CBC was done on Automated Hematology Analyser (Cell tac- α). NESTROFT was performed on all cases with fresh prepared 0.36% saline and reading was taken on specially designed SOFT test device (specially prepared for NESTROFT by Dr Moinuddin; Figure I). Whole blood (20 µl) of was pipetted out in a glass tube (12x75mm) and mixed by shaking with 4.0 ml of fresh prepared 0.36% buffered saline solution. The tube was left undisturbed at room temperature for about 20 minutes. After shaking again, result was read by viewing three sharp black lines visible (behind the tube) from a standardized distance. The results were recorded as 'NEGATIVE FOR BTT' if lines were clearly visible, 'POSITIVE FOR BTT' if lines were not visible and 'DOUBTFUL FOR BTT' when lines were partially visible.

There were 253 cases with microcytosis (MCV < 80 fL) and 250 controls with normal red cell indices. All subjects were further categorised into three groups. Group I comprised of normal control subjects with normal Hemogram values. Group II comprised of subjects with MCV < 80 fl, normal ferritin levels and HbA₂ > 3.5% on Hb Electrophoresis and Group III comprised of subjects with IDA proven by low serum ferritin levels while Hemogram & peripheral smears were also consistent with iron deficiency anemia. Data analysis was done on SPSS version 16.0; P value was taken statistically significant at < 0.05.

Results

Out of 253 subjects with microcytosis, IDA cases were n=174/253 (69%), subjects with BTT were n=73/253 (29%) & n=6/253 cases (2%) had normal serum ferritin & HbA₂ levels. NESTROFT was 93% sensitive, 88% specific with Positive Predictive Value of 74% & Negative predictive value of 97%. The NESTROFT positivity in IDA & BTT cases is shown in Figure II. In controls NESTROFT was reported negative for all subjects. The red cell indices, RBC count, MCV, MCH, MCHC & RDW% had statistically significant differences (P < 0.05) between IDA & BTT cases Table I.



Figure I: Single tube osmotic fragility test (SOFT) Device showing positive and negative samples in different tubes Tubes from L to R: NC=Negative control, PC=Positive control, Pos = Positive samples where black line is not visible through the solution, Neg = Negative samples where black line is clearly visible through the solution, DF =Doubtful sample where black line is partially visible.



Figure II: Sensitivity of NESTROFT in Subjects with Microcytosis.

Lab Parameters	IDA	BTT	P value
Hb, g/dl (±SD)	10.8 (±2.57)	10.9 (±2.31)	0.97
RBC, x10 ¹² /L (±SD)	4.6 (±0.88)	5.3 (±1.26)	0.02
HCT, % (±SD)	33.3 (±8.29)	33.1 (±6.88)	0.99
MCV, fL (±SD)	67.8 (±8.89)	62.1 (±6.38)	0.00
MCH, pg (±SD)	22.1 (±3.80)	20.2 (±2.79)	0.00
MCHC, g/L (±SD)	30.9 (±0.23)	32.0 (±0.26)	0.00
RDW, % (±SD)	15.6 (±2.52)	14.2 (±1.32)	0.00

 Table I: Hemogram Values in Iron deficiency anemia & B- thalassemia Trait.

Discussion

 β - Thalassemia is among the commonest inherited hemoglobinopathy worldwide with about 1.7% of world population carrying the mutated gene.⁹ Pakistan is resource limited country having prevalence of β - Thalassemia trait varying from 5-6% in various regions.¹⁰

The clinical presentation of IDA & BTT is usually same; to differentiate IDA from BTT is very important. Thalassemia trait detection should also be suspected in subjects who are resistant to iron therapy. It is difficult to suspect BTT on clinical examination as the classic heterozygote carrier of BTT is usually asymptomatic.² Usually the possibility of BTT is suspected on evaluation of positive family history, on hemogram variables or during population screening programs.⁽¹¹⁾ The correct identification of persons having BTT is an essential part of any screening program as significant number of people are unable to provide a positive family history of having affected individuals.²

The screening for BTT is very difficult mainly because of heterogeneity of β - Thalassemia and due to the fact that there is no single test to detect all variants. Despite these difficulties many researchers have tried to establish effective screening tests in order to address these problems viz. estimation of HbA, HbA₂, HbF & determination of red cell indices. However, screening by these techniques is time consuming and expansive for mass screening programmes.⁴

To qualify for a mass screening tool, an ideal screening test must be easy to perform, minimally invasive, less technically advanced, accurate in its objective, cheap to perform, and above all, must have on site result availability. Several studies recommend NESTROFT as screening test for detection of BTT in India^{12, 13, 14, & 15}, Iran¹⁶ and Jordan¹⁷.

With NESTROFT test sensitivity of 93% and specificity of 88% in detection of BTT and differentiating among cases of microcytosis, our results are comparable with studies carried out on same topic in other parts of the world.^{4, 6, 13, 18-22}

NESTROFT was positive only in 13% cases of IDA while it was negative in 88% of subjects with IDA. It was very sensitive to differentiate between IDA & BTT with Negative Predictive Value of 97%. Red blood cells in both IDA & BTT have an increased surface area to volume ratio, leading to a positive NESTROFT test. But we observed that NESTROFT was efficient enough to differentiate between both conditions. NESTROFT can be used as screening tool to rule the possibility of BTT by having a negative result. Positive NESTROFT results should be verified (for BTT) by standard Hb electrophoresis.

We understand that thalassemia can better be prevented, rather than treated. Considering the bleak outcome of commonly deployed methodologies for treating these patients, including latest therapies, promoting the best practice in thalassemia prevention and effective utilization of prenatal diagnosis is still the goal.

Conclusion

Based on observations in this study, we conclude that NESTROFT can be used effectively to differentiate BTT cases from iron deficiency if augmented with CBC variables by automated analyser. In remote local areas or where facilities of automated analysers are not available,

and also for the purpose of mass population screening, NESTROFT may serve its purpose as an efficient mass screening tool.

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References

- Annals of the New York Academy of Sciences Volume 1054 Issue Cooley's Anemia: Eighth Symposium, Pages 18 – 24 Published Online: 6 Jan 2006. © 2008 New York Academy of Sciences.
- Madan N, Meera S, Satendra S, Usha Rusia, Kusum Kela. Red cells Indices and Discriminant functions in the detection of B – Thalassemia Trait in a population with High prevalence of Iron Deficiency Anemia. Indian J Pathol Microbiol 1999; 42 (1): 55-61.
- 3. Sarnaik SA. Symposium-Thalassemia and related Hemoglobinopathies. Indian Pediatr 2005; 72 (4): 319-324.
- 4. Singh SP, Gupta S C, Effectiveness of red cell osmotic fragility test with Varying degrees of saline concentration in detecting β thalassaemia trait Singapore Med J. 2008 Oct; 49(10): 823-6.
- 5. Mamtani M, Das K, Jawahirani A. Is NESTROFT sufficient for mass screening for β-Thalassemia trait? J Med Screen 2007;14:169–73.
- 6. Yazdani SM, Ahmed S, Abbas F. One tube osmotic fragility test: a screening test for microcytic red cells Pak Armed Forces Med J Mar 2008; 58(1):45-50.
- 7. Viroj W. Osmotic fragility test for screening for thalassaemia in Thai pregnant subjects: a re-evaluation Haema 2004; 7(2): 205-7.
- 8. Bobhate. S, Gaikwad. S, Bhaledrao T, NESTROFT as a screening test for detection of B-Thalassemia trait. Indian J Pathol Microbiol. 2002 Jul; 45(3):265-7.
- 9. Shahid MB, Ayesha A, Hammad H, Jamshaid M , Muhammad A et al. Prenatal diagnosis of β-Thalassemia in Southern Punjab, Pakistan. Prenat Diagn 2006; 26: 903–5.
- 10. Angastiniotis MA, Hadjiminas MG. Prevention of thalassaemia in Cyprus. Lancet 1981;1:369-71. PMID:6109998 doi:10.1016/S0140-6736(81)91682-2.

- Caterina B, Galanello R. Thalassemia and Related Disorders: Quantitative. disorders of Hemoglobin Synthesis. John Gree (editor). Wintrobes Clinical Hematology, 11th edn, Lippincott Williams and Wilkins, 2004; 1319-66.
- 12. Gomber S, Sanjeev, Madan N. Validity of NESTROFT in screening and diagnosis of bthalassaemia trait. J Trop Pediatr 1997; 43(6): 363-6.
- 13. Manglani M, Lokeshwar MR, Vani VG, Bhatia N, Mhaskar V. NESTROFT- An effective screening test for b-thalassaemia trait. Indian Pediatr 1997; 34: 702-7.
- 14. Mahadik C, Kapadia C, Yagnik H, Sukumaran PK, Merchant S. One tube osmotic fragility as a useful screening test for thalassaemia carriers-A field experience. Indian J Hematol 1986; 4: 62-4.
- 15. Mehta BC. NESTROFT: a screening test for beta thalassemia trait. Indian J Med Sci 2002; 56(11): 537-45.
- Maram ES, Amiri ZM, Haghshenas M. Effectiveness of osmotic fragility screening with varying saline concentrations in detecting b-thalassaemia trait. Iran J Med Sci 2000; 25(1&2): 56-8.
- 17. Nayef S, Gharaibeh, Al-Sheyyab M, Batieha A. Detection of beta thalassaemia carriers in Jordan [[Abstract online]. Ann Saudi Med 1997 [cited 2002 Mar 15]; 184: 223-4.
- Thomas S, Srivastava A, Jeyaseelan L, Dennison D, Chandy M. NESTROFT as ascreening test for the detection of thalassaemia and common haemoglobinopathies - an evaluation against a high performance liquid chromatographic method. Indian J Med Res 1996; 104: 194-7.
- 19. Thool AA, Walde MS, Shrikhande AV, Talib VH. A simple screening test for the detection of heterozygous b-thalassaemia. Indian J Pathol Microbiol 1998; 41(4): 423-6.
- 20. Ahmed S, Saleem M, Modell B, Petrou M. Screening extended families for genetic hemoglobin disorders in Pakistan. N Eng J Med 2002; 347: 1162-8.
- 21. Mamtani M, Das K, Jawahirani. A. Is NESTROFT sufficient for mass screening for β-Thalassemia trait? J Med Screen 2007;14:169–73.
- 22. Aijaz J, Ahmed N, Sajid N, Natiq M, Nadeem R, Use of Naked Eye Single Tube Red Cell Osmotic Fragility Test as a Screening Tool for Beta Thalassemia Trait. Pak Paed J 2009; 33(3): 143-52.