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Possible application of Sickling test in haemoglobinopathy screening of Sri Lanka

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Abstract

The national screening programme for thalassaemia in Sri Lanka is currently using full blood count with red cell indices as the technique to identify haemoglobinopathies. This approach is likely to miss sickle haemoglobin (Hb-S) as it is well known that sickle haemoglobin is not associated with hypochromic microcytosis. Sickling test is a low cost microscopic screening test which detects sickle cell by its characteristic appearance. Therefore, the present study was undertaken to assess the performance of sickling test in identifying Hb-S among relatively high risk population in Hambantota district. A total of 581 school children (grade 11) were selected randomly from five (5) schools in Hambantota district. A blood sample (2ml) was collected from each participant after obtaining informed assent and consent. Screening panel comprises with sickling test by sodium metabisulphite method, one tube osmotic fragility test, dichlorophenol indophenol test, full blood count and Zinc protoporphyrin test. Haemoglobin phenotyping of each sample was confirmed by capillary electrophoresis technique. Four students out of 581 had sickle trait. Three other types of haemoglobinopathies were detected including β-thalassaemia trait (n=17), haemoglobin D trait (n=2) and haemoglobin E trait (n=1). Iron deficiency was observed in 8% (n=44) of students. All 4 cases with sickle trait were positive for sickling test and all other cases were negative. None of the Hb-S case demonstrated hypochromic microcytosis in full blood count. The average time taken for red cells with sickle haemoglobin to assume the characteristic sickle shape was 28 minutes and 45 seconds. Sickling test demonstrated 100 % sensitivity and 100 % specificity in identifying sickle trait. Inclusion of sickling test in screening individuals for haemoglobinopathies would have an additional advantage as highly specific, sensitive and low cost test for detecting sickle haemoglobin.

Keywords : *Sickling test; screening; haemoglobinopathies; Hambantota; Sri Lanka.*

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Introduction

Sickling test is a low cost microscopic test used to identify sickle cells in a wet preparation. The test encompasses the use of extraneous reducing agent to induce the formation of sickle shaped red cells. The test was first described by Daland and Castle with the use of Sodium metabisulphite as the reducing agent (Daland & Castle, 1948). Sickling test have demonstrated a high sensitivity and specificity with a greater diagnostic accuracy in detecting sickle haemoglobin (Hb-S) suggesting it's usage in preliminary screening of children for sickle cell disease. Besides, the procedure of the sickling test has been shown to be much easier than other screening tests such as sickle solubility test and peripheral blood film method (Okwi, Byarugaba, Parkes, & Ocaido, 2010).

National screening programme of thalassaemia in Sri Lanka currently rely on haemoglobin level and red cell indices (Mean corpuscular volume, mean corpuscular haemoglobin) when screening individuals for haemoglobinopathies. Nonetheless, relying merely on red cell indices and haemoglobin level is not appropriate as sickle haemoglobin carriers do not demonstrate reduction in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) (Castro & Scott, 1985; Chikhlikar & Wilkinson, 2014). Hence, sickle carriers could be missed and misidentified as normal individuals. Therefore, this study was conducted to determine the screening utility of sickling test in identifying sickle carriers among a relatively high risk population in Hambantota district of Sri Lanka.

Materials and methods

Grade 11 state school children were recruited for the study from randomly selected five schools in Hambantota district including; Weerakeatiya Rajapaksha central college, Angunakolapalassa Mahanaga national school, Suriyawewa central college, Ambalantota Theraputta national school and Debarawewa central college. A total of 581 students were selected. At least 100 students were randomly selected from each school with the help of teachers in charge of grade 11 in the respective school. Prior approval was obtained from the Education department of Southern province, Director of health service – Southern province and Director of health service – Hambantota district. Informed written consent from the participating student and informed assents were collected from a parent of students before data collection. A lecture was conducted on awareness and prevention of Sickle cell disease and thalassaemia for participating students and their parents in each school prior to data collection. 2ml of anti-coagulated blood sample was collected from each participating student.

Additionally, demographic data including; name, age, address, gender and ethnicity were also collected. Screening test panel in the present study incudes Full blood count (FBC), sickling test (Sodium metabisulphite method), Dichlorophenolindophenol test (DCIP), Zinc protoporphyrin test (ZPP) by Protofluro-Z Reagent method and single tube osmotic fragility test (SOFT). Haemoglobin phenotyping of each sample was confirmed by capillary electrophoresis technique (by Sebia Capillarys 2 flex piercing machine).

To estimate the co-existing Iron deficiency among recruited students ZPP was selected above serum ferritin estimation because along with heme; the ZPP/heme ratio has shown to foresee pre-anaemic iron deficiency better than serum ferritin levels which could affect by recent inflammations and infections (Crowell, Ferris, Wood, Joyce, & Slivka, 2006; Magge, Sprinz, Adams, Drainoni, & Meyers, 2013; Yu, 2011). Ratio of ZPP to Heme in the red blood cell was used to assess the iron status in the current study. Those who have values over 70 µmol ZPP/mole Heme were categorized as iron deficient individuals. Sickling test was conducted by 2% aqueous Sodium metabisulphite method as described by; (Old J & National Library of Medicine, 2012)

Protocol of sickling test

1. One drop of EDTA blood was mixed with 1 drop of 2% sodium metabisulphite solution on a microscope slide.
2. Smear was covered with a cover slip and edges were sealed with nail varnish.
3. Slide was immediately examined under the microscope with 40x magnification.
4. If negative for sickling, slide was examined intermittently up to 4 hours at room temperature before discarding.

Statistical Analysis

Statistical analyses were done using Statistical package for social sciences (SPSS) version 23 and Microsoft Excel programme (version 2013).

Ethical Approval

Ethical clearance for the study was obtained from Ethics Review Committee of Faculty of Medicine, University of Kelaniya. Ref No: p/01/01/2016.

Results

A total of 581 students were assessed in the present study including; 303 (52.2%) female students and 278 (47.8%) male students. Mean age was 15.83 (SD: 0.38) years. Four types of haemoglobinopathy carriers were identified by haemoglobin phenotyping. 24 out of 581 students (4%) were carriers of heamoglobinopathy. β-thalassaemia carriers (2.92%, n=17) reported at the highest frequency followed by Hb S trait (0.69%, n=4) in Hambantota district. Other haemoglobinopathy carriers identified include haemoglobin-D trait and haemoglobin-E trait. 44 (8%) students had ZPP values over 70 µmol /mole of Heme suggesting iron deficiency. Based on ZPP assessment 11 out of 17 β-thalassaemia trait subjects were identified to have iron deficiency.

Table 01: Different types of haemoglobinopathy carriers (n=581)

Haemoglobinopathy	**Count (n)**	**Prevalence**
β-thalassaemia trait	17	2.92%
Hb S trait	4	0.69%
Hb D trait	2	0.34%
Hb E trait	1	0.17%
Total	24	

Sickling test was positive in all 4 cases with Hb-S trait and was negative in others exhibiting 100% sensitivity and 100% specificity in identifying Hb-S trait. The average time taken for red cells with Hb-S to assume their characteristic sickle shape was 28 minutes and 45 seconds. DCIP test was positive in only one case with Hb-E trait. 85 (14.63%) out of 581 subjects were positive for single tube osmotic fragility test. Out of 17 β-thalassaemia trait subjects 16 had positive SOFT result. All the Hb-S and Hb-D trait subjects were negative for SOFT while the Hb-E trait individual was positive for SOFT. Sensitivity of SOFT to detect β-thalassaemia trait was 94.18%. 25 out of 44 individuals including 10 β-thalassaemia trait subjects with ZPP >70 iron deficiency had positive SOFT. Further, 46 out of 86 with positive SOFT did not have either β-thalassaemia trait or iron deficiency.

Table 02: Different interactions of screening tests in the panel

	β-trait	**S-trait**	**D-trait**	**E-trait**	**Iron deficiency**
ST (+)	00	4/4	00	00	00
SOFT (+)	16/17	00	00	1/1	25/44
DCIP (+)	00	00	00	1/1	00
Low MCV, MCH	17/17	00	00	1/1	44/44
ZPP (>70 u)	11/17	00	00	00	44/44
No of cases	17	4	2*	1	44

Discussion & conclusion

Present study identified the prevalence of Hb-S in Hambantota district as 0.69% among grade 11 students, while β-thalassaemia trait was detected at a prevalence of 2.92%. However, the prevalence of Hb-E trait and Hb-D trait were much lower. As table 02 demonstrates no screening test was able to pick up Hb-S except sickling test and it did not demonstrate any interaction with other haemoglobinopathies / iron deficiency except with Hb-S. Similarly, DCIP also did not demonstrate any interaction with other haemoglobinopathies/iron deficiency except with haemoglobin-E. Contrary to above mentioned tests; SOFT and low MCV/MCH values were not specific showing positive responses for both β-thalassaemia trait and iron deficiency. Nevertheless, the sensitivity of SOFT to detect β-thalassaemia trait was high (94.18%). But when compared with FBC and red cell indices it missed one individual with β-thalassaemia trait. Similar findings of low specificity of SOFT have been reported in earlier studies too (Ansari et al., 2014; Thomas, Srivastava, Jeyaseelan, Dennison, & Chandy, 1996). Additionally, no screening test in the panel were able to identify haemoglobin-D. The four students with Sickle-trait had normal haemoglobin levels (mean Hb: 13.6 g/dl) and red cell indices (mean MCV: 82.25 fL; mean MCH: 27.8 pg) suggesting if had they been screened for haemoglobinopathies solely with the indicators such as haemoglobin level and hypochromic microcytosis, all of them would have missed and incorrectly labeled as normal individuals. Previous study identified higher degree of false positivity of ZPP in individuals with β-thalassaemia trait (Rodrigo et al., 2018). In this study when cross checked with other indicators of iron deficiency like serum ferritin, transferrin receptors levels, ZPP seemed to be elevated in

most individuals with β-thalassaemia trait. Likewise, even in the present study most individuals with β-thalassaemia trait (11/17) had elevated ZPP. Thus this value needs to be interpreted with caution. The unexplained ZPP elevation may suggest individuals with a thalassaemia, who were not characterized in the present study.

Present study have demonstrated 100% sensitivity and specificity of sickling test by Sodium metabisulphite method. Essentially, our results have shown that sickling test is independent from various other interactions with different haemoglobinopathies. Besides, the procedure of the test is easier to follow and may perform by a qualified medical laboratory technologist without any hassle. Given the required laboratory items, sickling test can be performed in field setup too. Although haemoglobin phenotyping by high performance liquid chromatography or capillary electrophoresis are the confirmatory tests for haemoglobinopathies, the baring cost for these tests is paramount. Therefore, we recommend to include sickling test in the national thalassaemia screening programme of Sri Lanka as it has an additional value on detecting sickle haemoglobin as well.

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