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SPECTROPHOTOMETRIC MEASUREMENT OF SOLUBILITY TEST TURBIDITY AS AN IMPROVED DIAGNOSTIC TOOL FOR THE DIAGNOSIS OF SICKLE CELL TRAIT

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Abstract: The aim of the study is to establish new accurate Turbidometrical measurement of Sickle Hemoglobin using spectrophotometer instead of using naked eyes. Moreover, the study aimed to find out the most suitable filter and reducing reagent, which gives best result to improve the outcome of Solubility test. The study also intended to find out correlation between readings and previous transfusions as well as Jaundice. The study was carried out in Khartoum state among patients with sickle cell trait who were attending Khartoum educational hospital, Gafer Ibn Aouf Clinic and STAC International Centre Laboratory.

Forty, 26 female and 14 male patients were recruited for the study. Of them, 34 were children and 6 were adults over 20 years old. There were also 30 normal persons recruited as control group for comparison.

Results showed that 600 Nanometer is the best filter, which yielded highest light absorbance with significant statistical difference, and Na Meta-bisulphite is the best reducing agent because it produced turbidity more intense than Na Dithionate reagent. There is no significant correlation between reading and previous transfusion and jaundice. Therefore, the study recommend to use Na Meta-bisulphite for processing blood samples in Solubility test and to read the final reaction (Turbidity) by spectrophotometer using 600 Nanometer filter.

I. INTRODUCTION

The sickle cell mutation results in a single amino acid substitution in the globin chain; heterozygotes have one normal (A) and one affected chain (S) gene and produce about 60% Hb A and 40% Hb S [(1)]. Sickle Cell trait (AS) is an inherited condition in which both hemoglobin A and S are synthesized in red blood cells [(2)]. The amino acid substitution in the globin chain causes red cell sickling during de-oxygenation, leading to increased rigidity and aggregation in the microcirculation. These changes are reflected by a hemolytic anemia and episodes of tissue infarction [(1)].

Sickle cell trait occurs in approximately 300 million people worldwide, with the highest prevalence of 30% to 40% in sub-Saharan Africa [(3)] and is 8 to 10 percent in African Americans [(4,5)]. In Sudan the frequency of sickle cell trait and sickle cell disease were (52%) and (14%) respectively [(6)].

The sickling phenomenon may be demonstrated in a thin wet film of blood (sealed with a petroleum jelly/paraffin wax mixture or with nail varnish) which is known as Sickling Test. Tested red blood cells lose their smooth, round shape and become sickled if it contains Hb S. This process may take up to 12 hours in Hb S trait, whereas changes are apparent in homozygotes and compound heterozygotes after one hour at 37°C. These changes were hastened by the addition of a reducing agent such as sodium dithionite, which is known as Solubility Test that gives turbidity measured by the naked eye [(7)].

Measurement of the turbidity by the naked eye may lead to variable reading values as it may differ from person to another. The turbidity can be measured by the spectrophotometer in different wavelengths thus, accurate and invariable readings will be obtained. Therefore, the present study aimed to detect the optimal optical density that is crucial to determine the most suitable Wavelength as well as the best reducing reagent that surge reliability of the test.

II. ATERIAL AND METHODS:

The study was carried out in Khartoum state among Sickle Cell Trait patients who attending Khartoum Teaching Hospital, Gaafar Bin Auof Clinic and National Health Laboratory. A total of 40 blood samples were collected from (34) children and (6) adult patients with Sickle Cell Trait; of them, 26 were females and 14 were males.

Two milliliters of venous blood sample was collected from each participant using sterile disposable plastic syringe and applying aseptic standard vein puncture technique and processed with k_2 EDTA as anticoagulant before tested by automated analyzer (Sysmex-X_{21}) for Full Blood Count. The rest of the sample was then centrifuged and 10µl packed red cells was added to both Na-dithionate and Na-Meta-bisulphite in different test tubes, incubated for 5 minutes and read by spectrophotometer at wave length of 670, 635and 600.

Sickle cells percentage were calculated using Leishman stained thin blood film as the number of sickle cells was divided by the total number of red cells in 100 microscopic fields and multiplied by 100. Collected data were analyzed by the computer using SPSS software.

III. RESULTS

Table 1 Mean, Standard Deviation, Minimum, and Maximum values using Na Dithionate and Na Meta-bisulphite reagents and read by 670, 635 and 600 Spectrophotometer filters

Filters	Reagent	N	Mean	Std. D	Minim	Maxim
670	***Na Dithionate***	40	0.10	0.05	0.040	0.280
	Na Meta-bisulphite	40	0.13	0.06	0.049	0.303
635	***Na-Dithionate***	40	0.13	0.07	0.059	0.293
	Na Meta-bisulphite	40	0.17	0.07	0.062	0.392
600	***Na-Dithionate***	40	0.24	0.07	0.140	0.490
	Na Meta-bisulphite	40	0.29	0.09	0.164	0.594

As shown in table 1, readings obtained by 600 Nanometer filter is greater than that obtained by 635 and 679 Nanometer filter respectively. Similarly, reading results obtained by Na Meta-bisulphite is greater than that obtained by using Na Dithionate reagents.

Table 2 Statistical difference between the Na Dithionate and Na Meta-bisulphite reagents using 670, 635 and 600 Nanometer filters

Filters	**670**		**635**		**600**	
Reagent	Na Dithionate	Na Meta-bisulphite	Na Dithionate	Na Meta-bisulphite	Na Dithionate	Na Meta-bisulphite
Mean	0.11	0.13	0.13	0.17	0.24	0.29
SD	0.05	.06	0.06	0.07	0.07	0.09
PV	0.6		0.4		0.9	

Table 2 illustrates the insignificant statistical difference between Na Dithionate and Na Meta-bisulphite reagents when used as reducing agent and read by the three filters although, values obtained by using Na Meta-bisulphite obviously higher than that obtained by using Na Dithionate.

Table 3 Correlation between 670 and 635 Nanometer filters using Na Meta-bisulphite as reducing reagent

Filters	**670**	**635**
Mean	0.13	0.17
SD	0.06	0.07
p-value < 0.05		

The slight increase of the optical density readings obtained by 635 against 670 Nanometer filters, as indicated in table 3, is of no statistical significance.

Table 4 Correlation between 670 and 600 Nanometer filters using Na Meta-bisulphite reagent as a reducing agent

Filters	**670**	**600**
Mean	0.13	0.29
SD	0.06	0.09
p-value > 0.05		

Table 4 show the significant statistical increase of optical density readings obtained by 600 Nanometer filter compared to 670 Nanometer filter.

Table 5 Correlation between 600 and 635 Nanometer filters using Na Meta-bisulphite as a reducing agent reagent

Filters	**600**	**635**
Mean	0.29	0.17
SD	0.09	0.07
p-value < 0.05		

As shown in table 5, the optical density readings obtained by 600 Nanometer filter is significantly increased more than that obtained by 635 Nanometer filter.

Table 6 The effect of previous Transfusion and Jaundice on the reading of the three filters

Filters		670	635	600	p-value
Previous Transfusion	***Yes***	0.12	0.13	0.27	
	No	0.14	0.20	0.31	
Jaundice	***Yes***	0.14	0.16	0.30	
	No	0.11	0.13	0.24	

In table 6, optical density readings are slightly increased in patients who were not subjected for blood transfusion compared to transfused patients. In addition, patients with jaundice showing higher optical density compared to negative one but these differences are of no statistical significance.

IV. DISCUSSION

Premarital screening for sickle Cell disorders is now compulsory where consanguineous marriage is common as these, like other recessive diseases, have an increased prevalence in such populations [(8)]. Moreover, Surgeons and Anesthetists increase screening for Sickle cell hemoglobin before operative procedures [(9)].

Sickling test is cheap and simple to perform but false positive and false negative results are common [(10)].

In the present study, both reducing agents (Na Metabisulphite, Na Dithionate) were used for processing the same samples in order determine the best of them, thus, Na Metabisulphite harvest an intensive turbidity compared to Na Dithionate therefore, it can produce more reliable results.

The second aim of the study is to select the most suitable filter that can be used in Solubility test, which is depend on the extent of light absorbance. In this context, the 600 Nanometer filter showed absorbance efficacy that is significantly greater than 635 and 670 Nanometers filters. Hence, the 600 Nanometer filter considered as the most suitable for conducting spectrophotometric reading of Solubility test.

Adopting this new protocol will undoubtedly enhance the reliability of the solubility test and the obtained results will be stable regardless the person who perform these laboratory tests.

The study concluded that Sodium Meta-bisulphite is obviously better than Sodium Dithionate to be used as reducing agent and 600 Nanometer filter is significantly better than 635 and 670 Nanometer filters.

Therefore, the study recommends using Sodium Meta-bisulphite reagent as a reducing agent in the solubility test as well as Spectrophtometric reading with 600 Nanometer filter.

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