Prevalence of hemoglobin S in Wadi Eshati Region – South Libya

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ABSTRACT

This study is an attempt to evaluate the prevalence of hemoglobin S in Wadi Eshati Region.
The study group was composed of 2095 persons (984 males and 1111 females) between 2 and 85 years old were selected randomly from 16 towns and villages in Wadi Eshati Region, South of Libya who attended either Primary Health Care Unites, Brack Central Hospital, and from some primary and secondary schools in the region. All participants were subjected to complete blood count and screened for sickle cell anemia by sickling test, solubility test. Iso-Electric Focusing (IEF) tests were conducted on all positive sickling and solubility tests.
The prevalence of sickle cells in this region (4.15%) which is almost similar to those of other studies carried out in other regions in Libya, moreover, we observed that the prevalence of HbAS (2.53%) and Hb SS (0.14%) among females was higher in compare to males, (1.43% and 0.05% respectively).
A systematic neonatal screening programme for this disorders and all other abnormal hemoglobin, seems reasonable for early diagnosis and management.

Introduction

The sickle cell syndrome refers to a state in which red cells undergo sickling when deoxygenated. This occurs in homozygote state of hemoglobin S gene (sickle cell anaemia), hemoglobin SC, hemoglobin SD and hemoglobin S beta thalassaemia states. Sickle cell anaemia is the most severe sickle cell disorder, while hemoglobin SD is the mildest and hemoglobin SC and hemoglobin S-□-thalassaemia are in between with a variable overlap in severity (1).

Sickle cell disease is one of the commonest genetic disorders worldwide and is the most commonly inherited hematological disease affecting humans (2). It is one of the most global problems of hemoglobin disorder. It had spread through migration from its native areas in the Mediterranean Region, Africa and Asia and is now an endemic throughout Europe, America and Australia (3). Sickle cell anaemia results From substitution of a single amino acid, namely valine for glutamic acid in the sixth codon of beta globin gene in chromosome number 11. As a result of this substitution RBCs undergo sickling when deoxygenated (4). The susceptibility of red cells to sickle correlates well with the concentration of sickle hemoglobin within the red cells (5). Several other factors influence the course and severity of the disease. These include the presence or absence of alpha thalassaemia gene, oxygen content of the inspired air, cardiac and pulmonary status. Other factors such as dehydration, infection, acidosis and hypothermia may precipitate in some of the complications of this disease (6).

The clinical features of sickle cell disorders reflect the propensity of the red cells to assume a sickled configuration when blood is doxygenated, leading to a shortened red cell survival (chronic

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hemolysis) and a tendency to vaso-occlusion of many body organs (6). Although patients with sickle cell anaemia may adapt to their anaemia quite well, their illness is interspersed with acute episodes, or crises, which include episodes of sequestration of blood into the lung, liver or spleen, or the occlusion of cerebral vessels with resulting stroke. Furthermore, they are particularly prone to infection in early childhood and indeed at all ages (7). For reasons that are not yet fully understood, there is a remarkable variability in the severity of sickle cell disease (8).

But even in populations such as those of the Eastern Saudi Arabia and/or parts of India, which have a high frequency of thalassaemia and/or an unusual ability to produce Hb F in adult age (both of which when inherited with sickle cell disease tend to result in milder illness) (9). Although little is known about the mortality from ‘‘sickling’’ disorders in developing countries, it is clear that in sub-Saharan Africa, many children die of it in their early ages. Both in the USA and in Jamaica, the peak incidence of death among those affected with these disorders appears to be at 1–3 years of age, usually due to infection. Recent data from the USA suggest that among affected adults, the median age of death is 42 years for males and 48 years for females (10).

The sickle cell gene is distributed widely throughout sub-Saharan Africa, the Middle East and some parts of the Indian sub-continent, where carrier frequency ranges from 5% to 40% or more of the population. The most recent estimate suggests that annually there may be about 270 million carriers for important inherited disorders of hemoglobin and 300,000 –400,000 births of infants with sickle cell anaemia or serious forms of thalassaemia(11,12).

The number of people suffer from sickle cell anemia in Libya is unknown. The sickle cell gene with other abnormal hemoglobins in Libya was first described The first cases of abnormal hemoglobins reported in Libya in the medical litreture was in 1979 by Jain in a study carried on 545 subjects from the University of Garyounis, Benghazi. Abnormal haemoglobins were encountered in 23 subjects of unrelated families, giving an overall incidence of 4.2%. Sixteen of these subjects had Hb-AS trait (3.0%), Five subjects had Hb-AC trait (0.9%) and two subjects had Hb-AD trait (0.36%) (13), but now is recognized to be widespread, especially in the south of Libya, where the prevalence of sickle cell trait is around 4.4% and 1.2 for sickle cell diseas (14).

This study was designed to determine the prevalence of Hb S in Wadi Eshathi Regions in the South of Libya.

MATERIALS AND METHODS

Sample Collection:

Between July 2007and March 2008, a total of 2095 venous blood samples were randomly collected in EDTA tubes from persons of different age groups (ranging 2-85 years) belonging to different regions in Wadi Eshathi, using disposable syringes and needles for each individual. The samples were transported to Brack General Hospital Laboratory within two to three hours of collection for hematologic tests, solubility test and sickling test. These samples were subsequently analyzed at Human Histology Laboratory unit of the Biotechnology Research Center in Twesha-Tripoli, Libya using very sensitive test (Iso Electric focusing) to confirm the presence of hemoglobin S in these samples.

Hematological Analysis:

Hemoglobin concentration, RBC count, packed cell volume, mean corpuscular

volume, mean cell hemoglobin and mean cell hemoglobin concentration were conducted by automated cell counter (MINDRAY BC-3000 Plus Auto hematology Analyzer. Shenzhen Mindray–Biomedical). Hematological tests were conducted to show the statistical difference between normal and abnormal positive samples using t test.

Slide Sickling Test:

Whole blood was mixed, with strong reducing agent (Sodium metabisulfite) on a glass slide, which deoxygenated the Hemoglobin molecules. Under these conditions, hemoglobin S present in red blood cells causes the formation of sickle shaped red cells (15).

Solubility Test:

Whole blood was added to saponin-containing potassium buffer. Red blood cell is lysed due to saponin present in solution. Hemoglobin S and other sickling hemoglobins in reduced state, form liquid crystals and yield a turbid appearance in contrast to normal hemoglobin which gives transparent appearance (15).

Iso-Elctric Focusing (IEF)

Isoelectric focusing takes place in a pH gradient and is limited to molecules which can be either positively or negatively charged (amphoteric molecules), like proteins, enzymes and peptides. Separation takes place in a pH gradient which is formed by special amphoteric buffers (ampholytes) having high buffer capacities at their pI(isoelectric point). The pH gradient is produced by an electric field. Before an electric field is applied, the gel has a uniform pH-value and almost all the carrier ampholytes are charged. When an electric field is applied, the negatively charged ampholytes move towards the anode, the positively charged ones to the cathode and their velocity depends on the magnitude of their net charge. The carrier ampholytes align themselves in between the cathode and the anode according to their pI, and determine the pH of their environment. A stable and gradually increasing pH gradient depending on the initial mixture of ampholytes is formed. Isoelectric focusing (IEF) on agarose gels (Resolve System, Hemoglobin test kit; Perkin Elmer Life Sciences, Norton, OH, USA) was used to confirm Hb variants such as Hb S detected by positive sickling and solubility tests. About 5µl from each blood sample was eluted in 200µl of Hb elution solution supplied by Perkin Elmer for 30 minutes of continuous mixing in miro-wells plate. Then the samples were focused on agarose gels for 45 min. at 1.5 kV, 18 mA, after which the protein was fixed by immersion in 5% Trichloracetic acid in 35% methanol, and then the plate was stained and dried at 50o C to 60o C (16).

Age groups.

According to World Health Organization (WHO) standards, hematological parameters differ from one another depending on age and sex. The total samples were divided into 4 age groups. The first age group (<5 years) with 26 samples, (10 males and 16 females), the second age group (5– 11 years) with 210 samples (116 males and 94 females), the third age group (12- 14 years) contained 216 samples (120 males and 96 females), and the fourth age group (over 15 years) had 1643 samples (732 males and 911 females).

Results

Hemoglobin S is distributed worldwide, and this study was carried out to evaluate the prevalence of Hb S in the Wadi Eshati Region.

Venous blood samples were collected from 2095 persons belonging to Wadi Eshati Region, ranging from 2- 85 years (mean

age 29.9± 18.11). Out of the 2095 participants screened, 87 samples (4.15%) were found positive for HbS. On the other hand, 83 samples (3.96%) were found heterozygous for Hb S (Hb AS), and 4 samples (0.19%) were homozygous for HbS (Hb SS). Table 1 and figure 1 represent positive samples for Hb S in the total samples.

Table 1: Prevalence of HbS of total samples

Hemoglobin type	number	Percentage %
HbAS	83	3.96
HbSS	4	0.19
Total	87	4.15

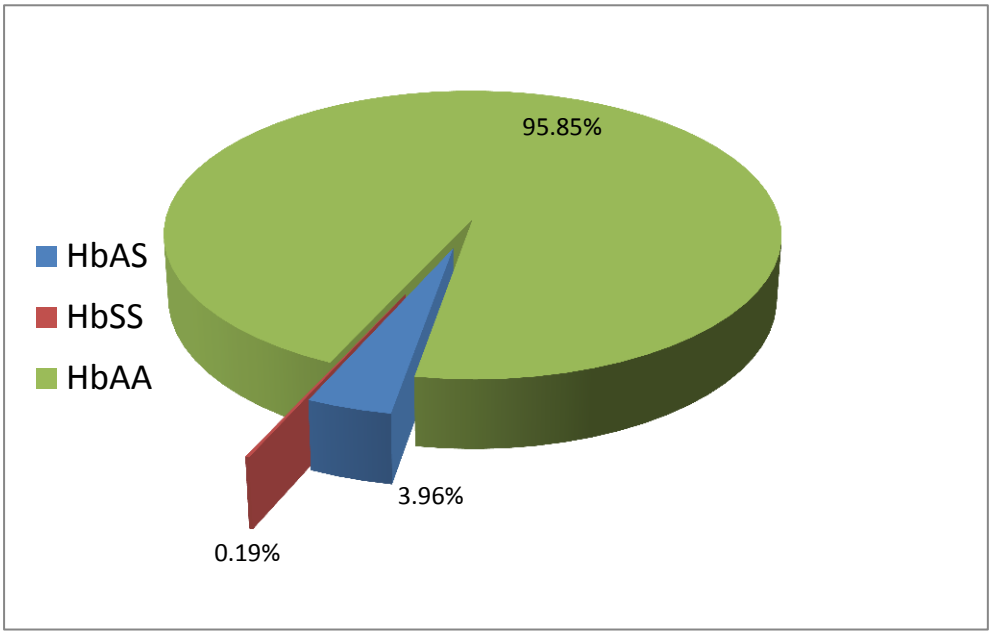

Figure 1: prevalence of HbS of total samples

According to the results of the tests carried out for this study 87 samples (4.15%) were found positive for HbS for both genders (Table 1, figure1).

Out of the total samples, 31 samples of males (1.48%) were positive for Hb S, out of which 30 samples (1.43%) were found heterozygous for HbS (HbAS), and only one sample (0.05%) was homozygous for HbS (HbSS). But, in females, 56 samples(2.67%) were found positive for Hb S, out of which 53 samples (2.53%), were found to be heterozygous for HbS (Hb AS), and 3 samples (0.14%) were homozygous for HbS (Hb SS). Table 2, figure 2 represent the prevalence of HbS in males and females in the total samples.

All the samples of the first age group were found negative for HbS (0%) (n= 26). In the second age group, 6 samples (2.86%) were positive for heterozygous HbS, (one sample 0.48% in males and 5 samples 2.38% in females). In the third age group, 11 samples (5.09%) were positive for heterozygous HbS (6 samples 2.78% in males and 5 samples 2.31% in females), while in the fourth age group, 70 samples (4.26%) were positive for HbS. 66 (4.02%)

positive samples were heterozygous for HbS of which, 23 samples (1.40%) were in males and 43 samples (2.62%) were in females, whereas, 4 samples (0.24%) were positive for homozygous HbS of which only one sample (0.06%) was in males and 3 samples (0.18%) were in females (n=1643)(Table 3).

Table 2:- Prevalence of HbS among sex (males & female) of total samples.

Sex	HbAS		HbSS		Total	
	N	%	N	%	N	%
Males (N = 984)	30	1.43	1	0.05	31	1.48
Females (N= 1111)	53	2.53	3	0.14	56	2.67
Total 2095	83	3.96	4	0.19	87	4.15

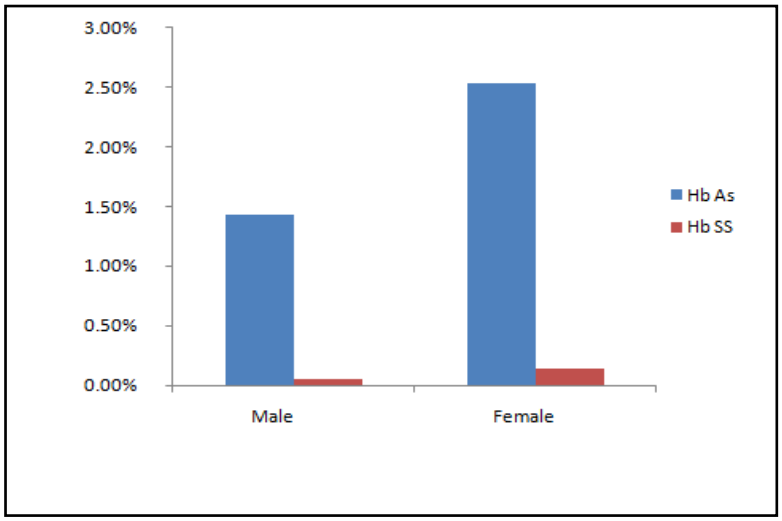

Figure 2: Prevalence of HbS in males & females.

Table 3: Prevalence of HbS in different age groups

M= males F= females

Hb	**2-4 years N=26**		**5-11 years N=210**		**12-14 years N=216**		**15 years & over N= 1643**	
	M N=16	**F N= 10**	**M N=116**	**F N = 94**	**M N= 120**	**F N = 96**	**M N= 732**	**F N = 911**
HbAS	N=0	N=0	N=1	N=5	N=6	N=5	N=23	N=43
%	0%	0%	0.48%	2.38%	2.78%	2.31%	1.40%	2.62%
HbSS	N=0	N=0	N=0	N=0	N=0	N=0	N=1	N=3
%	0%	0%	0%	0%	0%	0%	0.06%	0.18%
HbAA	N=16	N=10	N=115	N=89	N=114	N=91	N=708	N=865
Total	100%	100%	54.76%	42.38%	52.78%	42.13%	43.09%	52.65%

Statistical t test was done to compare the hematological parameters of heterozygous HbS and the negative samples in each age group. In the first age group, no positive sample for HbS was found, and the t test wasn't done for this age group, but in the second age group, statistically there was no significant difference in all the hematological parameters performed ($p>0.05$). In the third age group, there were significant differences in packed cell volume (PCV), mean corpuscular volume (MCV), and mean cell hemoglobin concentration (MCHC) ($p<0.05$), whereas no significant difference was found in the other hematological parameters performed ($p>0.05$).

In the fourth age group, no significant difference was detected between HbAS positive samples and negative samples in males ($p>0.05$), but a significant difference was detected in RBCs count and MCV ($p<0.05$). No significant difference was detected in the other hematological parameters performed ($p>0.05$).

Also t test was performed to compare hamatological parameters of homozygous HbS with negative samples, and statistical significant differences were detected in all the hematological parameters performed ($p>0.05$), but statistical significant differences were detected between homozygous HbS and Heterozygous HbS in all the hematological parameters performed ($p>0.05$), except in MCV($p>0.05$).

By comparing the tests carried out in this study (slide sickling test, solubility test and IEF test) to detect Hb S, it was found that the slide sickling tests and tube solubility tests were giving the same results in detecting HbS. These two tests gave similar results as IEF tests, but not like IEF tests . Sickling tests and solubility tests can't distinguish between homozygous and heterozygous HbS. Figures 3a & 3b show slides of sickling and and cloudy tubes of positive solubility tests due to the presence of HbS (Samples No:3, 4, & 5). and IEF tests (figure 4a, & 4b). which show transparent negative samples (samples No 1, & 2).

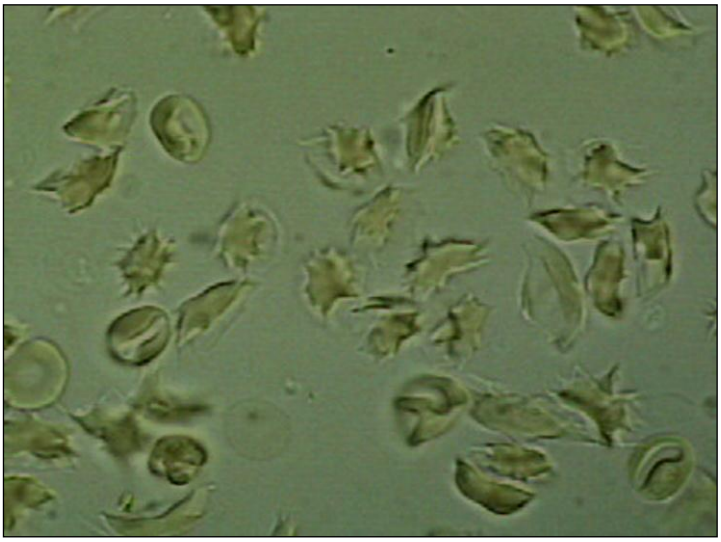

Figure 3: Sickling test using Sodium metabisulfate (rows represent sickled cells).

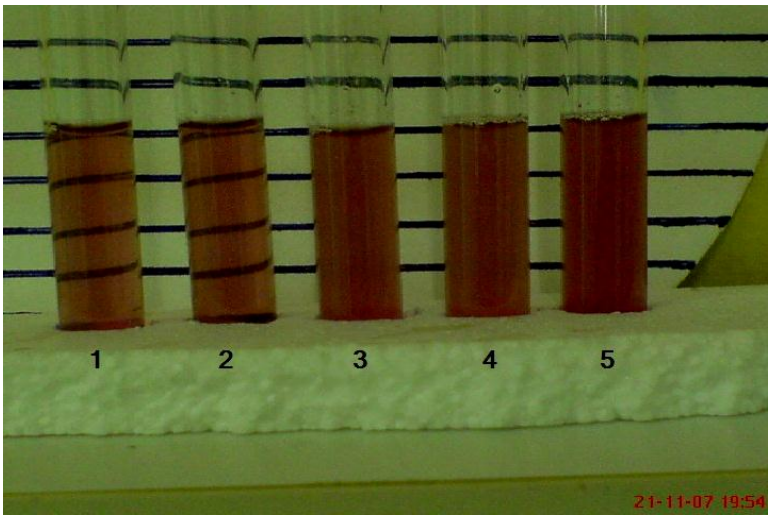

Figure 4: solubility test, 1= normal control, 2= normal sample, 3= positive control for HbAS, and 4 & 5 positive for HbAS..

Also IEF test considered to be more accurate for the detection of hemoglobin S, which precipitated on the gel as clear band in figure 5.

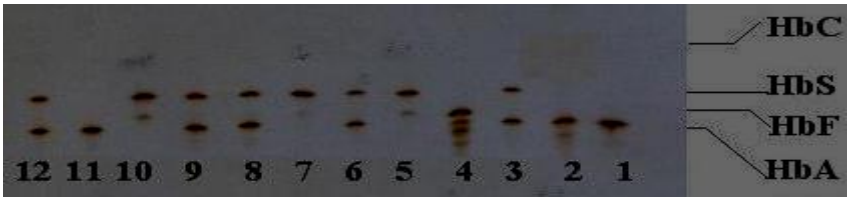

Figure (5) IEF gel (Sample No:1, 2 HbA control. Sample No:3 HbS control. Sample No:4 HbF control. Sample No: 6,8,9,12 positive HbAS, Whlie sample No:5,7,10 HbSS. and sample NO:11 HbA

Discussion:

Hb S is amongst the most prevalent hemoglobinopathies worldwide. The prevalence of hemoglobinobathies in different parts of Libya by other studies, was found to be 4.2% (17, 18). In this study, the prevalence of Hb S in the whole population screened was found to be 4.15%.

Out of all the samples, 83 samples (3.96%) were heterozygous for HbS (HbAS), and 0.19% were Homozygous HbS (HbSS). This prevalence is considered to be less when compared to the other previous studies carried out in Gariones University Bengazi- Libya on 545 students, which showed 3% positive for HbAS, and 0.33% HbAC (13).

On the other hand, most of the HbS positive samples were heterozygous (3.96%) whereas, the prevalence of HbSS was 0.19%, and this is confirmed by many other similar studies and this percentage is also less when compared to other studies in different parts of Libya which, demonstrate that the HbAS was 1.6%, and 0% for HbSS (18). Also the prevalence of Hb AS is slightly higher when compareed to other studies in Yemen by Al-Nood et al., 2004, which showed that the Hb AS was 2.2% (16).

According to age groups, the prevalence of HbS was found highest in the third age group (5.09%), while the first age group showed the lowest prevalence (0%), but in the second and the fourth age groups, it

was2.86% ,and 4.26% respectively. The prevalence of Hb S in the younger age group was more comon in children, which was in agreement with Deshmukh et al., 2006 (19), but different from Balgir, 2007, who didn't find any relation between age and prevalence of HbS in persons (20).

Depending on the t test, no significant difference was detected ($p>0.05$) in the second age group. This confirms that HbAS has no difference between hematological parameters with normal samples. In the third age group, there were significant differences between Hct, MCV and MCHC ($p<0.05$), where other parameters showed no significant difference ($p>0.05$). In the fourth age group, there was a significant difference in RBCs and MCV in females ($p<0.05$). That may be due to the effect of the other factors such as age, sex, physiological factors, sample collection time, site of blood collection and the nutritional status (21).

But HbSS samples showed a significant difference in HbAA, and this is considered to be the typical picture of a sickle cell disease (22). According to the tests carried out in this study, we found that sickle, and solubility tests were compatible with Isoelectric focusing tests in the detection of HbS. These tests may be used as primary screening tests, on account of their low cost, but they can't differentiate between HbAS and Hb SS in contrast with IEF test. On the other hand, sickle solubility tests are not sensitive for the detection of HbAC (19).

Conclusion

The prevalence of sickle cells in this region (4.15%) which is almost similar to those of other studies carried out in other regions in Libya, moreover, we observed that the prevalence of HbAS (2.53%) and Hb SS (0.14%) among females was higher in compare to males, (1.43% and 0.05% respectively).

Sickle cell disorders should be considered a public health problem in Wadi Eshati Region. A systematic neonatal screening programme for this disorders and all other abnormal hemoglobin, seems reasonable. Adapted management of hereditary sickle cell should be available as early identification, control and management of sickle cell disorders is necessary to prevent childhood deaths.

مدي انتشار الخضاب المنجلي في منطقة وادي الشاطئ – جنوب ليبيا

محمد مروان[1]، أبوبكر معروف ميلاد[2]، و عبدالسلام جمعة[3]

الملخص

تهدف هذه الدراسة إلي تقدير معدل انتشار الخضاب المنجلي (HbS) في منطقة وادي الشاطئ.

تم جمع عدد 2095 عينة عشوائية (984 ذكور، و1111 إناث) لأشخاص تراوحت أعمارهم بين 2-85 سنة من المترددين علي مراكز الرعاية الصحية الأولية ومستشفي براك العام ومن طلبة مدارس التعليم الأساسي والمتوسط بالمنطقة.

جميع العينات خضعت لاختبارات عد الدم الكامل (CBC) والمسح عن وجود الخلايا المتجلية بطريقة التمنجل والذوبانية، كما أن جميع العينات التي ظهرت موجبة بالاختبارين السابقين تم تأكيدها باختبار(Iso-Electric Focusing (IEF.

أظهرت النتائج أن نسب انتشار الخضاب المنجلي (HbS) في هذه المنطقة متقارب مع ما هو مسجل في بعض المناطق الأخرى من ليبيا. كما تم ملاحظة أن نسبة الانتشار بين النساء لسمة التمنجل والخضاب المتجانس (2.53% و 0.14% علي التوالي) كانت أعلى مما في الرجال (1.43%، و 0.05% علي التوالي).

وعليه نقترح ضرورة القيام بالمسح الدوري لجميع المواليد للكشف عن وجود الخضاب المنجلي من عدمه كأجراء احترازي مبكر لتشخيص واتخاذ التدابير اللازمة للعناية المبكرة بمن يكونوا موجبين.

1- مجموعة أبحاث أمراض الدم – مركز بحوث التقنيات الحيوية- الطويشة – ليبيا
2- قسم المختبرات الطبية – كلية العلوم الهندسية والتقنية – بيراك – جامعة سبها – ليبيا
3- قسم علم الحيوان – كلية العلوم جامعة الفاتح – طرابلس - ليبيا

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