A close-up photograph of a person's hands adjusting a microscope. The microscope is white and black, and the person is wearing a yellow lab coat. The background is a bright yellow wall.

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Research Article

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Mohammed Omer Gibreel, Mubarak El Saeed Elkarsani, Munsour Mohammed Munsoor and El Yasaa Ahmed Gubartella

Full Length Research Paper

Screening for hemoglobinopathy in Beja tribes and other minor groups living in Port Sudan City

Mohammed Omer Gibreel^{1*}, Mubarak El Saeed Elkarsani², Munsour Mohammed Munsoor³ and El Yasaa Ahmed Gubartella³

¹Haematology Department, Port Sudan Ahlia College, Port Sudan, Sudan.

²Medical Laboratory Sciences, University of Karary, Sudan.

³Hematology Department, Faculty of Medical laboratory Sciences, University of Sudan, Sudan.

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Hemoglobinopathies prevalence and distribution among ethnic groups inhabiting the Red Sea State in Sudan were not reported till date. Accordingly, this study has been conducted to address this problem in that area. The study was a cross-sectional descriptive one conducted in Port Sudan city to screen for hemoglobinopathy in anaemic patients referred to three major centers. The study population included 209 patients, 54% males and 46% females with age between 1 and 98 years. Five milliliters of blood were drawn from each subject for CBC (Sysmex KX21), peripheral blood films and hemoglobin electrophoresis using capillary electrophoresis. The results showed that hemoglobinopathy was detected in 59 (28.22%) subjects of whom 26 (44.06%) showed electrophoretic patterns of sickle cell anaemia, 29 (49.15%) Beta thalassaemia trait, 2 (3.38%) hemoglobin D trait, 1 (1.69%) hemoglobin E trait and 1 (1.69%) showed Beta thalassaemia major. The study concluded that occurrence of these frequencies in this population indicated that the target group have hemoglobinopathy and recommended that patients with hemoglobin below lower limits of normal, MCV and MCH lower than 78fl and 27 pg, respectively should be screened for hemoglobinopathy, besides the establishment of a center for diagnosis and control of hemoglobinopathy in that area.

Key words: Hemoglobinopathy, Beja tribe, red sea variants, thalassaemia.

INTRODUCTION

Sudan is a diverse country with a complex population originating from different ethnic groups. This reflects a matter of various intermarriage and social interaction status. Yet a local database table is not completed. Hence, the determination of the hemoglobin profile in Beja ethnic group of eastern Sudan will potentially fill the

gap in our local database table. Furthermore, thalassaemias and hemoglobinopathy are the most common inherited disorders among humans, and they represent a major public health problem in many areas of the world (Kaddah et al., 2009). It has been estimated that approximately 7% of the world population are carriers

*Corresponding author. E-mail: m_omer8164@yahoo.com. Tel: 00249912908164.

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of such disorders and that about 300,000 to 400,000 babies with severe forms of these disorders are born each year (Weatherall, 2004; Hoffbrand et al., 2005).

Most abnormal hemoglobin differ from the normal hemoglobin in the substitution of a single amino acid for another. In hemoglobin S, for example, valine replaces glutamic acid in the sixth position from the *N*-terminal of the β -globin chain. Many other examples of this kind have been discovered. 600 hemoglobin variants characterized by a single amino acid substitution have been recognized and characterized. Of these variants, more than 200 involve the α -chain, more than 400 involve the β -chain, more than 30 involve the δ -chain, and more than 70 involve other chains (Greer et al., 2003). Inherited hemoglobin disorders were originally characteristic of the tropics and subtropics, and are now common worldwide due to migration. Population migration and intermarriage between different ethnic groups has introduced thalassaemia in almost every country in the world. It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of beta thalassaemia, with about 60000 symptomatic individuals born annually, the greater majority in the developing world (Galanello and Offit, 2010).

The diversity and heterogeneous distribution of hemoglobin disorders make it necessary to develop strategies at country level (Modell et al., 2000). Since no population is completely free from hemoglobinopathies, and the condition is not limited to any particular racial group (Firkin et al., 1989), determination of the possible hemoglobinopathies among this ethnic group will add information to our local medical knowledge.

MATERIALS AND METHODS

Patients

Two hundred and nine anaemic patients referred to hematology laboratories in Port Sudan teaching hospital, Police Hospital and Sea Ports Corporation Hospital were recruited into the study after obtaining verbal consent. Included in this study are patients with haemoglobin concentrations below the lower limits of normal in respect to age and sex with mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values below 78 fl and 27 pg, respectively.

Specimens

Five milliliters blood specimens (anticoagulated with EDTA) were collected from each subject; 2.5 ml packed in transportation bags and sent to Khartoum by fast mail for capillary electrophoresis and 2.5 ml retained for hematological testing.

Methodology

All samples were subjected to measurement of complete blood count using the automatic hematology counter Sysmex KX 21 analyzer (Serial No. 87151, Japan) following manufacturer's instructions, thin blood smears were prepared and stained with Romanowsky

stain. Samples showed morphological features of polychromasia and nucleated red cells were subjected to reticulocytes count and sickling test by 2% sodium metabisulphite technique. Haemoglobin electrophoresis was done using the Capillary Electrophoresis (CE) technique with the Sebia CAPILLARY2 following manufacturer's instructions. CE technology utilizes liquid flow electrophoresis-buffer replaces agarose gel. Haemoglobin variants are separated with the principles of electrophoretic flow at an alkaline pH (9.4) with a negatively charged silica capillary support, and high voltage.

RESULTS

Two hundred and nine patients (112 males; 54% and 97 females; 46%) who met the inclusion criteria were included. Their mean age was 25 years with a range from 1 to 98 years. The results showed that 150 (71%) patients subjected to electrophoresis have normal hemoglobin patterns and 59 (29%) showed hemoglobin patterns indicative for hemoglobinopathy. Of the later, 14 (23.72%) showed electrophoretic patterns consistent with sickle cell trait (AS), 12 (20.34%) showed patterns consistent with sickle cell disease (SS), 29 (49.15%) showed patterns consistent with beta thalassaemia trait, 1 (1.7%) with beta thalassaemia major, 2 (3.39%) showed zones in the position of hemoglobin-D, and 1 (1.7%) showed a zone in the position of hemoglobin-E. The quantity of bands in the position of hemoglobin-D and E were not consistent to establish the diagnosis of an hemoglobin variant. The occurrence of sickle cell anemia was significantly affected by tribal origin (*P* value = 0.014) where the frequency is higher in the tribes originating from Western Sudan such as Taaisha and Hawsa than those of Eastern Sudan such as Bani Amer and Hadandawa. In contrast, beta thalassaemia trait was found to occur more frequently in Bani Amer and Hadandawa than in other tribes. The frequency of hemoglobinopathy was neither affected by age (*P* value = 0.66) nor by gender (*P* value = 0.23). Table 1 to 3.

DISCUSSION

Little is known till now about the occurrence and manifestations of hemoglobinopathies in the population of Eastern Sudan, namely Red Sea State. That is because these conditions are rare and their diagnosis is consistently more difficult especially in heterozygote subjects. So, paucity of their prevalence further complicated the matter. The present study is the first attempt to assess and determine the frequency of hemoglobinopathies in Eastern Sudan. The results of the present study showed that 71%, of patients have normal haemoglobin patterns and 29% of the patients have hemoglobinopathies. Beta thalassaemia trait was the most prevalent and constitutes an extremely high frequency of 49.15% among hemoglobinopathies (13.88% of study population) compared to the frequency reported for beta thalassaemia in the Sudan (1 to 10%), Jordan (3.5%), India (3.4%) and Turkey

Table 1. Quantity of different hemoglobin (Hb) among patients subjected to capillary electrophoresis.

Patient	Hb-A	Hb-A2	Hb-F	Hb-D	Hb-E	Hb-S
	(mean \pm 1SD)	(mean \pm 1SD)	(mean \pm 1SD)	(mean \pm 1SD)	(%)	(mean \pm 1SD)
Normal hemoglobin pattern	97.2 \pm 1	2.6 \pm 0.46	0.27 \pm 0.7	0	0	0
Sickle cell trait	65 \pm 13.7	3.1 \pm 0.52	2.9 \pm 9.4	0	0	30 \pm 17.1
Sickle cell disease	0	3.1 \pm 0.53	10 \pm 5.6	0	0	87.1 \pm 5.2
Hemoglobin-D trait	93.4 \pm 4	2.6 \pm 9.21	0.45 \pm 0.06	3.4 \pm 4.1	0	0
Hemoglobin-E trait (%)	95.8	2.7	0.9	0	0.6	0
Beta thalassemia trait	95.9 \pm 1.6	3.9 \pm 1	0.26 \pm 0.76	0	0	0

Table 2. Distribution of hemoglobinopathies among patients according to tribes.

Tribe	Number of patients with hemoglobin pattern					
	SS	AS	AD	AE	Beta thalassemia trait	Beta thalassemia major
Bani Amer	1	4	-	-	9	-
Hadandawa	2	1	2	1	11	-
Attman	-	1	-	-	-	-
Rashaida	-	1	-	-	-	-
Abderahmanab	-	-	-	-	3	-
Armada	-	-	-	-	1	-
Artega	-	-	-	-	1	-
Hawsa	4	4	-	-	1	-
Taaisa	3	-	-	-	-	-
Fallata	1	1	-	-	-	-
Foor	-	1	-	-	-	-
Rubatab	-	-	-	-	-	1
Jaafra	-	-	-	-	1	-
Danagla	-	-	-	-	1	-
Nuba	1	1	-	-	1	-

Table 3. Frequency of different types of hemoglobin among the study subjects as indicated by capillary electrophoresis and expressed as percent of the study population.

Type of hemoglobin	Frequency	Percentage of frequency
Normal hemoglobin	150	71.78
Sickle cell trait hemoglobin	14	06.70
Sickle cell disease	12	05.74
Hemoglobin D trait	02	01.35
Hehoglobin E trait	01	00.48
Beta thalassemia trait	29	13.88
Beta thalassemia major	01	00.48
Total	209	100

(1.2%) (Bain, 2006; Bashir et al., 1992; Nagar et al., 2014; Canatan, 2014), but comparable to that reported for Iran (Alizadeh et al., 2014) Figures (1 and 2).

The difference in frequency of beta thalassemia in this work and those reported in Sudan, Jordan, India and

Turkey may be attributed to study design which included known anemic patients in our study rather than a random sampling from subjects employed in those studies. The frequency of sickle cell trait in our work was 23.72%, a figure comparable to that reported in Nigeria, but lower

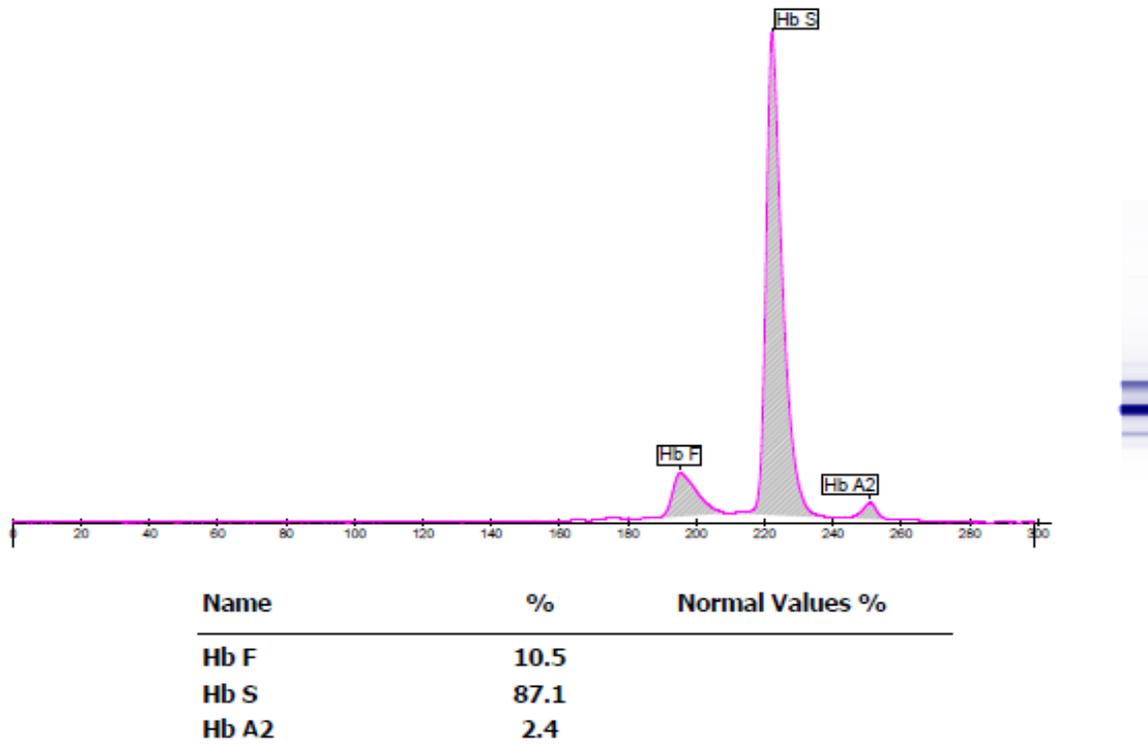


Figure 1. Capillary electrophoretic pattern of a 46 years old male belonging to Bani Amer tribe showing a typical pattern of hemoglobin in sickle cell disease (SS).

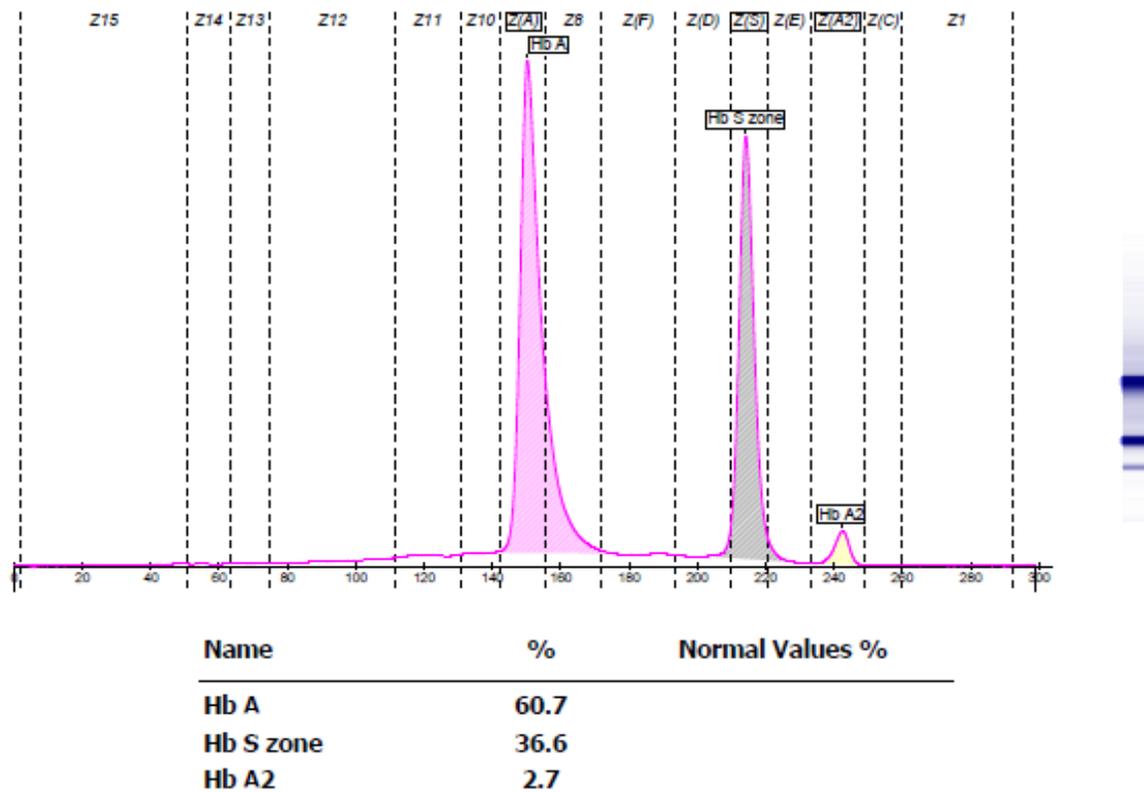


Figure 2. Capillary electrophoretic pattern of a 31 years old female belonging to Hawsa tribe showing a typical pattern of hemoglobin in sickle cell trait (AS).

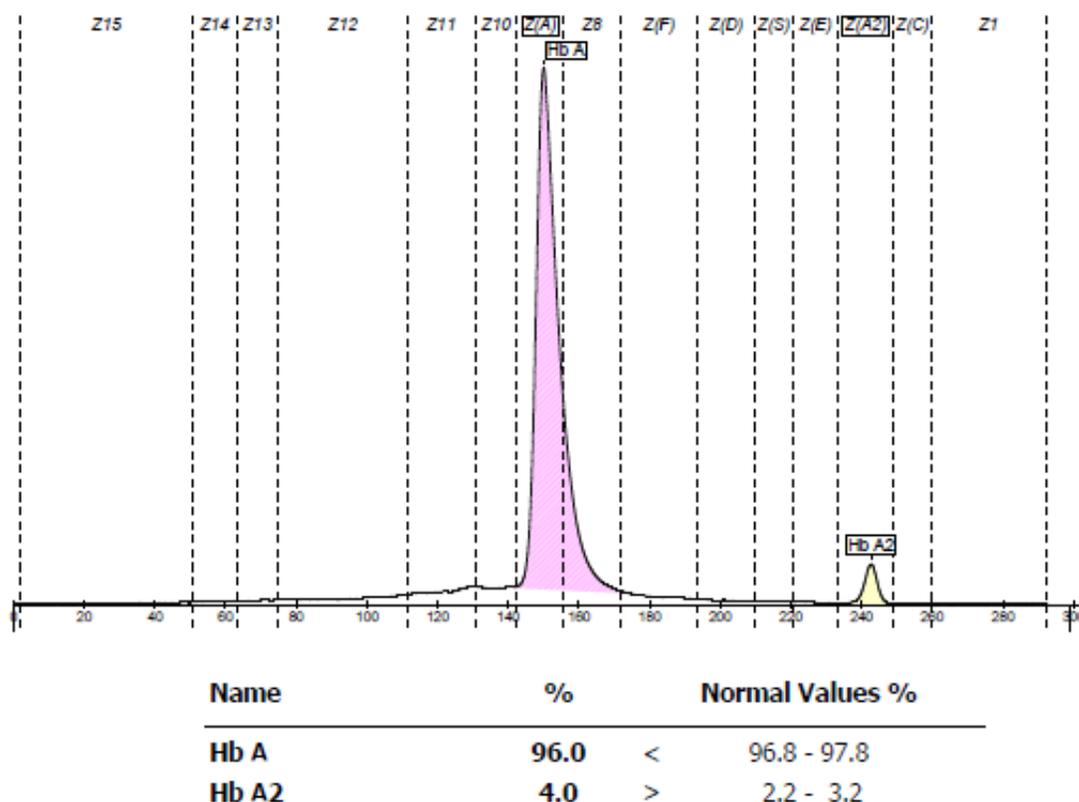


Figure 3. Capillary electrophoretic pattern of a 44 years old female belonging to Hadandawa tribe showing a typical pattern of hemoglobin in beta thalassaemia trait.

from that reported in Western Sudan (Kolita, 2010; Munsoor and Alabid, 2011). The frequency of the sickle cell disease was 20.34% which is also comparable to that reported in Nigeria and reflecting introduction of the West African hemoglobin SS gene in our study area. The frequency of beta thalassaemia major obtained in our study was 1.7% and it was comparable to that reported in Iran (Alizadeh et al., 2014). The results also showed, 3.39% of hemoglobinopathies with zones in the position of hemoglobin -D and 1.7% with a zone in the position of hemoglobin-E. The quantity of bands in the position of hemoglobin-D and hemoglobin-E were not consistent to establish a diagnosis of an hemoglobin variant. The occurrence of sickle cell anemia was significantly affected by tribal origin (P value = 0.014) where the frequency is higher in the tribes originating from Western Sudan such as Taasha and Hawsa than those of Eastern Sudan such as Bani Amer and Hadandawa (Elderderly et al., 2011; Elderderly et al., 2012; Mohammed et al., 2006). In contrast, beta thalassaemia trait was found to occur more frequently in Bani Amer and Hadandawa than in other tribes. Figure 3

The frequency of hemoglobinopathy was neither affected by age (P value = 0.66) nor by gender (P value = 0.23). The results reported from global previous studies have shown that, the incidence of beta thalassaemia gene

in Africa varied (Bain, 2006). Elderderly et al. (2011), studying the tribal distribution of hemoglobinopathies in a population of Sudanese patients, reported that the S gene was the most common variant found (6.1%) and was the most prevalent in the Western tribes of Sudan (12%). This observation was also reported by several works conducted in the Sudan (Mohammed et al., 2006; Munsoor and Alabid, 2011) and supported the findings observed in the present study. Kolita (2010), in a study conducted in Nigeria, mentioned that the prevalence of homozygous SCD (hemoglobin SS) and (hemoglobin S+C) disease is 3.1 and 1.1%, respectively, while the prevalence of sickle cell trait was 23.7%. The prevalence of sickle cell trait in Nigeria was significantly higher than our findings and that is because the S gene is much more prevalent in West African populations (Barbara, 2006).

The patterns of distribution of different types of hemoglobin in our study were consistent with that reported for the diagnosis of thalassaemia sickle cell disease and sickle cell trait (Alizadeh et al., 2014). The data obtained from this study indicates that haemoglobin variants and thalassaemia are common amongst the anaemic patients of the Eastern Sudan. This condition necessitates the establishment of a center for the diagnosis of the disease and implementation of control measures (Nagar et al., 2014) to aid reduction in the spreading of hemoglobinopathies

disorders in the Red Sea State in the Sudan.

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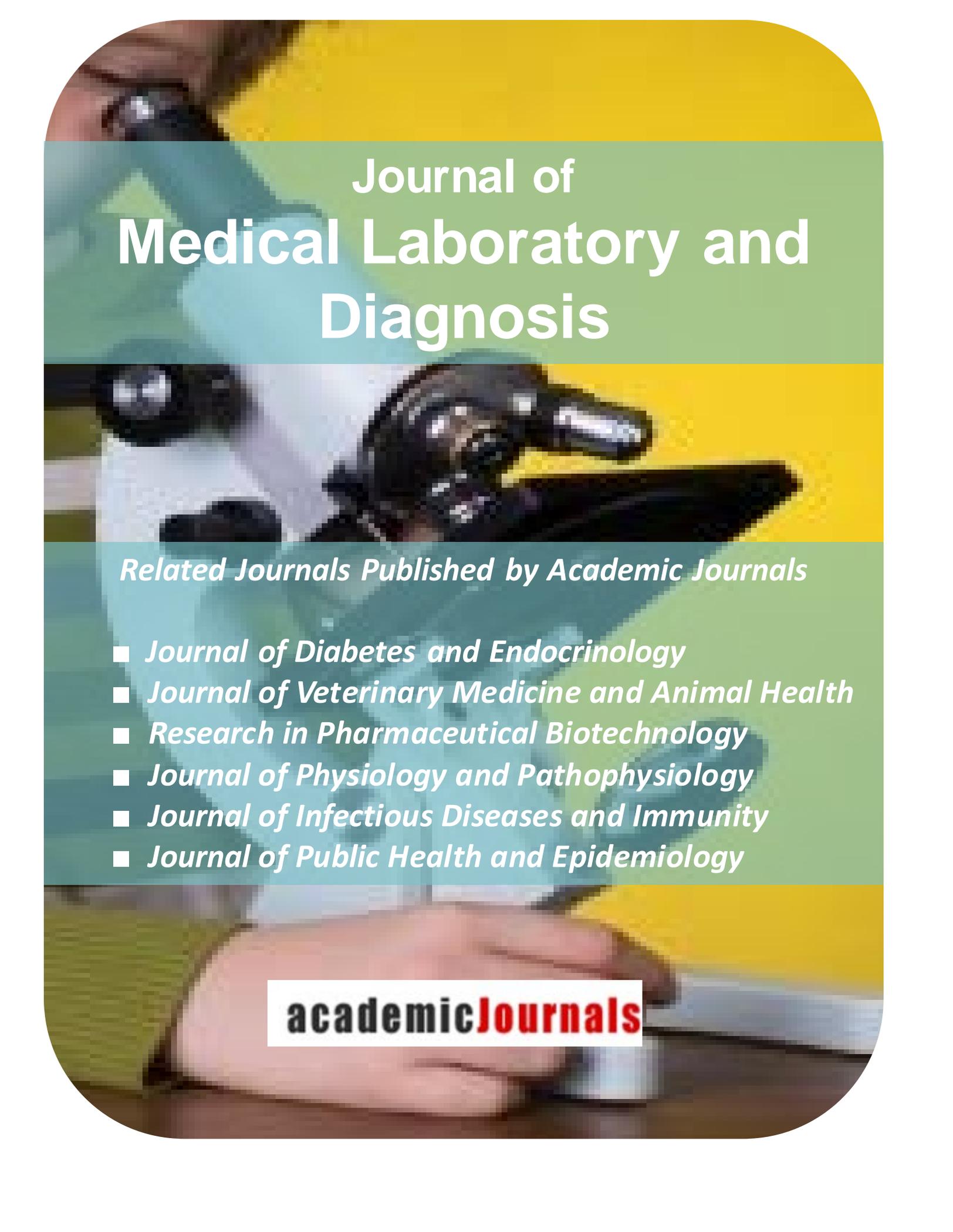
The authors would like to acknowledge Port Sudan Ahlia College for financial support and patients who have participated in this study and their families.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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