EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 6
118-123
Year: 2020

PREVALENCE OF SICKLE CELL TRAIT AMONG HEALTHY APPEARING MEDICAL UNIVERSITY STUDENTS IN HODIEDAH PROVINCE, YEMEN

Salem Bashanfer*, Abdullah Al-alimi, and Mohammed Morish

Department of Medical Laboratory, College of Medicine and Health Sciences, University of Hodiedah, Yemen.

***Corresponding Author: Dr. Salem Bashanfer**
Department of Medical Laboratory, College of Medicine and Health Sciences, University of Hodiedah, Yemen.

Article Received on 09/04/2020 **Article Revised on 29/04/2020** **Article Accepted on 20/05/2020**

ABSTRACT
Backgound: Sickle cell disease is one the most common types of hemoglobinopathies in the world, and is known to be prevalent inherited disorders in most Arab countries. It is a genetic, hereditary and chronic disease that affects the health of homozygotes sickle cell (SS) that manifest symptoms, while the asymptomatic heterozygotes (AS) are being referred to as sickle cell trait carriers. The aim of this study was to estimate the prevalence of sickle cell traits, gender differences, and red cells parameters among University students in Hodiedah Province, Yemen. **Methodology:** This cross sectional study was carried out in the department of medicine and health sciences of Univeristy of Hodiedah, Yemen. Five hundred blood samples were drawn from medical students and subjected to sickling and complete blood counts tests. **Results:** Of a total number of 500 students were screened for sickle cell trait (HbAS), there were 326 males (65.2%) and 174 females (34.8 %). The sickling test was positive in 40 samples and negative in 460 samples, and the overall prevalence of sickle cell trait among all students was 8.0% (4.8% males and 3.2 % females). CBC results of trait patient showed lower parameters compared to normal students. **Conclusion:** The sickle cell trait is highly frequent in Hodiedah city, and the spreading degree could be due to the high degree of consanguineous marriage in this city.

KEYWORDS: Sickle cell disease, sickle cell trait, Hodiedah, Yemen.

INTRODUCTION
Sickle cell disease (SCD) is one of the most common genetic hematological disorders worldwide, affecting approximately 4.4 million people, and 43 million have sickle cell trait as of 2015. (Vos, 2015; Vos, 2016).

Sickle cell anemia (SCA), the prototype disease of SCD is inherited as autosomal recessive genetic defects that result from mutations in both copies of the beta-globin gene of hemoglobin (HbSS) (Wilson, 2003). Individuals with only one mutated beta-globin gene and one normal working copy are known as sickle cell trait (SCT) or carriers (HbAS) (Wilson, 2003). Carriers are usually appearing healthy, asymptomatic and rarely develop sickling crises. However, there are many reports indicated sudden death or severe life-threatening complications such as hematuria, hyposthenuria, pulmonary embolism and splenic infarction, especially when carriers severely exposed to hypoxia in altitude and dehydrated conditions (Tsaras, 2009; Tripette, 2010; Eichner , 2007). Moreover, urinary tract infection (UTI) in women is also a well-defined complication in SCT patients compared to racially matched controls (Eichner , 2007).

Globally, It is estimated that 300 million people worldwide are heterozygous for SCT, reaching its highest prevalence in parts of Africa (Up to 40%) as well as among people with origins in equatorial Africa, the Mediterranean basin and Saudi Arabia (Kato, 2007). In 2004, Al-Nood et.al has showed the prevalence of SCT in Sana'a (Capital of Yemen) was 2.2% among 1700 subjects screened; while in 2011, he showed the prevalence of SCT in Taiz governorate (Southern) is about 8.2% (Al-Nood, 2004; Al-Nood, 2011).

Yemen is a developing country has next to no facilities, until today, there is no a neonatal screening program provided to detect hemoglobinopathy disorders. This study aimed to study the prevalence the SCT among medical educated students in the Western province (Hodiedah city), which has the highest prevalence of anemia, malaria and dengue fever.

METHODOLOGY
Study Design And Subjects
This a cross-sectional study was designed to determine the prevalence of sickle cell trait among medical university students, and to evaluate their hematological parameters, demographic information, family history of

anemia and blood transfusion through several experiments and questionnaire survey.

This study was conducted on 500 students, randomly selected between March and June 2019. Ethical approval was obtained by the department of laboratory medicine in the faculty of medicine and Health Sciences, Hodiedah University, Yemen.

Before starting blood sample collecting, participants were informed about the objectives of the study and types of experiments, their participation were totally voluntarily and the consent was obtained without coercion.

Three ml of venous blood were collected from each student, drawn into EDTA tube and used to analyze hematological parameters; complete blood count (CBC), blood film and screening sickling test.

Hematological Parameters Testing

Hemoglobin (Hb), red blood cell count (RBC), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), red cell distribution width (RDW) and platelets were determined and measured by using hematology analyzer Sysmex KX-21N.

Sickling test was performed to screen sickle cell anemia by using sodium metabisulfite as a reducing agent as previously described (Chikezie, 2011). One drop of 2% sodium- metabisulfite was added and mixed well with a drop of EDTA blood, placed on a slide, and covered with cover slip. Cover slip edge was sealed using candle wax or nail varnish and allowed to stand at room temperature in a moist petri dish for up to 24 hours. Each slide was examined under the microscope with dry (objective 40X, Olympus) with further observations were taken after 30 minutes, 2 hours and 24 hours. Positive samples indicate the presence of sickle-shape RBC, while no changes is observed in negative samples**.** For validation purposes ten positive samples and ten control samples were further examined by High performance liquid chromatography (HPLC) D-10 Hemoglobin Testing System (Bio-Rad).

Statistical Analysis

Statistical analysis of numerical data was achieved using Statistical Package for Social Sciences (SPSS) version 20. All quantitative variables were examined for normality by Shapiro-Wilks test before analysis. Continuous variables were presented as mean and standard deviations. The prevalence of the sickle cell trait was calculated along with the 95% confidence interval (95% CI). Student's "t" test was used to compare the proportions between sickle cells anemia and normal students in many parameters such as (RBCs, Hb, Hct, MCV, MCH, MCHC, and RDW). Statistical significance was identified at 0.05 levels. In addition, frequency tables, cross-tabulations, and chi-square tests were used for non-parametric variables and the statistically significant alpha p value ($p<0.05$) was used to determine the significant difference in clinical symptoms, past history of anemia, blood transfusion and socioeconomic status between anemic and normal group.

RESULTS

A total number of 500 university medical students were screened to detect sickle cell trait (HbAS) including 326 males (65.2%) and 174 females (34.8 %). The test was positive in 40 samples and negative in 460 samples, and the overall prevalence of sickle cell trait among all students was 8.0% (4.8% males and 3.2 % females).

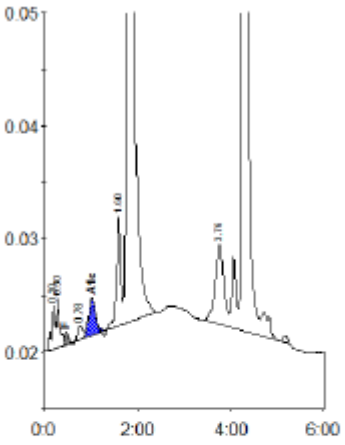
Table 1 shows out of 326 males students, 24 (7.90 %) and out of 174 females students, 16 (10.1%) had positive sickling test. The difference between affected males and females students was found to be statistically significant ($p<0.0001$). Also it was found higher prevalence of SCT (45.8%, 50.0%) among males and females students aged 17-19 years, respectively, than students aged 20-22 years (29.2%, 31.2%) and aged 23-25 year (25.0%, 18.8%). The difference between aged groups among SCT students was statistically significant too (P=0.013).

5 (20.8 %) out of 24 SCT males students and 3 (18.8%) out of 16 females students with SCT were found to have family history of SCT and past history of blood transfusion, with no statistical significant difference. Furthermore, 7 (29.2%) and 9 (37.5 %) males, and 4 (25.0%) and 5 (31.25%) females were found to be complain from fatigue and pallor, respectively, with statistically significant difference ($P <0.05$). Moreover, 5 (20.8%) males and 4 (25.0%) females' students were found suffering from chest and joints pain, with no statistical significant difference.

Table 1: The prevalence of sickle cell trait and variable parameters among 500 students (460 normal students versus 40 sickle students).

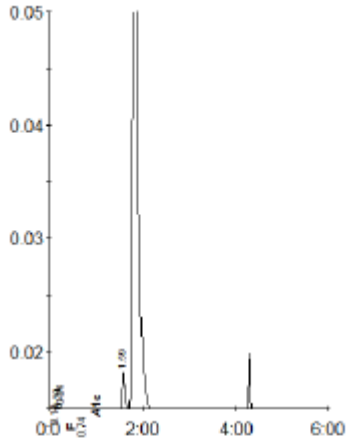
Parameters		Normal students		Sickle students		p-value
Gender		Males No. (%)	Females No. (%)	Males No. (%)	Females No. (%)	
		302 (92.6)	158 (90.8)	24 (7.90)	16 (10.1)	0.000
Age	17-19 years	81 (26.8)	60 (38.0)	11 (45.8)	8 (50.0)	0.013
	20-22 years	156 (51.7)	57 (36.1)	7 (29.2)	5 (31.2)	
	23-25 years	65 (21.5)	41 (25.9)	6 (25.0)	3 (18.8)	
Family History	Yes	0.0 (0.0)	0.0 (0.0)	5 (20.8)	3 (18.8)	0.643
	No	302 (100)	158 (100)	19 (79.2)	13 (81.2)	
Blood Transfusion	Yes	3 (1.0)	2 (1.3)	5 (20.8)	3 (18.8)	0.522
	No	299 (99.0)	155 (98.7)	19 (79.2)	13 (81.2)	
Fatigue	Yes	18 (6.0)	22 (13.9)	7 (29.2)	4 (25.0)	0.006
	No	284 (94.0)	136 (86.1)	17 (70.8)	12 (75.0)	
Pallor	Yes	20 (6.6)	25 (15.8)	9 (37.5)	5 (31.25)	0.000
	No	282 (93.4)	127 (84.2)	14 (62.5)	11 (68.75)	
Pain (Joints, chest)	Yes	4 (1.3)	2 (1.3)	5 (20.8)	4 (25.0)	0.521
	No	298 (98.7)	156 (98.7)	19 (79.2)	12 (75.0)	

For validation purposes 10 normal students samples and 10 sickle traits samples were selected for further evaluation. Figure 1 shows an example of High Performance Liquid Chromatography (HPLC) results of two samples (Trait and normal) which confirm the previous sickling test.



Peak table - ID: MOSTAFA

Peak	R.time	Height	Area	Area %
A1a	0.20	3876	19066	0.8
A1b	0.30	4361	16940	0.7
F	0.48	1122	7514	< 0.8 *
LA1c/CHb-1	0.78	1242	9449	0.4
A1c	1.03	3352	35332	4.0
P3	1.60	9658	61651	2.5
A0	1.80	176628	1166086	47.7
Unknown	3.76	6978	89357	3.7
S-Window	4.29	194161	1037882	42.5
Total Area:		2443278		



Peak table - ID: ASEIL

Peak	R.time	Height	Area	Area %
Unknown	0.14	2017	4443	0.7
A1a	0.20	3337	9664	1.5
A1b	0.26	3846	19820	3.1
F	0.49	1035	10438	1.4
LA1c/CHb-1	0.74	223	2381	0.4
A1c	1.04	1755	18263	4.2
P3	1.59	5268	37818	5.9
A0	1.81	80360	541513	84.0
Total Area:		644340 *		

Figure 1: High performance liquid chromatography (HPLC) samples of one sickle cell trait (Left) versus a control student (Right).

Table 2 shows the results of hematological parameters for SCT versus control group. On the one hand, the average means of hemoglobin concentration of SCT were found 11.89 g/dl (SD±0.62) and 10.90 g/dl (SD±0.37) among males and females respectively, were lower than normal males 14.10 g/dl (SD=1.17) and females 12.02 g/dl (SD=1.10), with statistically significant ($p<0.0001$).

On the other hand, SCT males and females students had lower mean of RBCs, Hct, MCV, MCH, and MCHC, comparing to their control groups. While RDW mean

were higher in SCT students comparing to control students with statistically significant (p<0.0001).

Table 2: Comparisons between mean, standard deviation (SD) and *p-value* of normal group (n=460), and sickle cell trait group (n =40) in the hematological parameters (RBCs, Hb, Hct, MCV, MCH, MCHC, and RDW).

Parameters	RBCs (10^{12}/L)		Hb (g/dL)		Hct %		MCV (fL)		MCH (pg)		MCHC %		RDW %		*P-value*
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Normal Males	5.23	0.51	14.10	1.17	46.02	5.21	87.62	7.10	27.23	2.64	31.03	1.40	13.44	1.33	0.000
SCT Males	4.68	0.40	11.89	0.62	42.20	2.15	83.42	5.20	25.61	2.40	30.20	1.30	14.20	1.58	
Normal Females	4.56	0.50	12.02	1.10	39.77	3.18	87.74	6.98	26.54	2.87	30.63	4.24	13.83	1.25	
SCT Females	3.85	0.30	10.90	0.37	36.95	1.70	82.06	2.87	25.48	1.26	29.80	1.23	14.33	1.51	0.000

SD= Standard Deviation; RBCs= Red Blood Cells; Hb= Hemoglobin; Hct= Hematocrit; MCV= Mean Corpuscular Volume; MCH= Mean Corpuscular Hemoglobin; MCHC= Mean Corpuscular Hemoglobin Concentration; RDW= Red Blood Cells Distribution Width

Table 3 presents the results of the differences in the means of hematological parameters between SCT male and female students. A statistically significant difference was found in the means of RBCs, Hb and Hct of males and females SCT. The males had a higher levels of means than females with SCT (P <0.0001). However, there was no statistical significance difference in the levels means of MCV, MCH, MCHC and RDW for males and females SCT.

Table 3. Mean, SD and P-value of hematological parameters among male and female SCT.

Parameters	Students with SCT				*P-value*
	Males		Females		
	Mean	SD	Mean	SD	
RBCs (1012/L)	4.68	0.40	3.85	0.30	0.000
Hb (g/dL)	11.89	0.62	10.90	0.37	0.000
Hct %	42.20	2.15	36.95	1.70	0.000
MCV (fL)	83.42	5.20	82.06	2.87	0.091
MCH (pg)	25.61	2.40	25.48	1.26	0.412
MCHC %	30.20	1.30	29.80	1.23	0.158
RDW %	14.20	1.58	14.33	1.51	0.406

DISCUSSION

Population-based screening programs for the detection of sickle cell anemia or other haemoglobinopathies has not yet implemented in Yemen to insuring health surveillance data that is essential for healthcare and public health planning. However, very few studies based on limited gene frequency were carried out on people with SCA and co-exiting with thalassemia in different parts of Yemen. (Nasher, 2013; Alnood, 2009).

To our knowledge this is the first study carried out in the West of Yemen (Hodaidah city) to survey the prevalence of SCT among health appearing University students. The frequency of sickle cell trait in the present study among all students aged 17-25 years was 8.0% with higher incidence among males (4.8%) as compared to females students (3.2%). The majority of SCT students (45.8% males and 50.0% females) have been found under age of twenty years.

The prevalence of SCT in this study was very similar to that study reported by Al-Nood in Taiz city in Yemen, and higher as compared with other results presented by the same author (2.2%) in Sana'a city (Capital of Yemen) (Al-Nood, 2004; Al-Nood, 2011).

Moreover, depending of city and geographical distribution there was some similarities and differences of this study's finding and other reported data in Arab countries such as 0.5% to 3.5% in Algeria, 13.0-16.4% Bahrain, 0.3% Egypt, 0.27-6.5% Iraq, 0.44-6.0% Jordan, 3.3-4.5% Libya, 1.2% Morocco, 4.8-10% Oman, 4.2-25.9% Saudi Arabia, 1.89-4.9% Tunisia, and 1.5% UAE (Hamamy, 2013).

The difference between the current findings and previous studies may be related to demographic changes, ethnic population, illiteracy, consanguineous marriage, and lack of medical counseling.

The present study showed higher prevalence of SCT among males (4.8% males and 3.2 % females) which similar to Charuhas et al finding (7.3% males and 5.9% females) and disagree with another study carried out by El Ariss et al where the prevalence was higher among females (3.2% males and 6.5% females). (Charuhas, 2013; El Ariss, 2016) (20.8 %) and (18.8%) of SCT males and females students respectively, were found to have family history of SCD and past history of blood transfusion, with no statistical significant difference. Furthermore, both males and females were found

suffering from fatigue and pallor, with statistically significant difference (P<0.05), which concurred with previous studies demonstrated that some SCT patients sickle cell trait complain of painful crisis, weakness, fatigue and pallor (Chikhlikar, 2014).

The average means values of RBCs, hemoglobin, Hct, MCV, MCH and MCHC, were low and high RDW was found among students of the SCT compared to normal students with statistically difference (p<0.0001) this results revealed that these students suffer from mild to moderately anemia. The current findings are in line with the previously reported findings by Datar S. et al. (Datar, 2015). Moreover, the hematological parameters of this study were also similar to those reported by El Ariss et al. (El Ariss, 2016).

The results of this study also demonstrate that there was significant difference in RBCs counts, Hb levels and Hct, and insignificant in regards of RBC indices (MCV, MCH, MCHC) among males and females SCT students, these finding were also similar to those reported by Datar et al. (Datar, 2015). Dissimilarity was reported in Iraq, Jaber et al reported a significantly lower MCV and MCH, while insignificantly were reported in regards of RBC, Hb, and MCHC among males and females SCT groups. (Jaber, 2019).

This difference among males and females could attributed to change in the demographics, environment, ethnic, education, type of gene variants, disagreement in the lifestyle, and other physiological factors such as menstrual cycles in females.

CONCLUSION

The incidence of sickle cell trait in the Western province of Yemen is 8.0% among University educated students. These results highlight the need population screening programs for different age levels (preschool and school children, adolescents and adults) to determine the carrier rates and gene frequencies in our province especially to whom premarital youths to reduce the disease incidence in our poor society. Also we need the policy efforts that target the information about the danger of disease and provide counseling to help, learn and educate as much about the disease as possible in schools, clinical centers and University people.

Competing interests: The authors declare no competing interest.

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