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The Prevalence of Sickle Cell Trait Among School Students Males and Females in Taiz City-Yemen

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Abstract:- Sickle cell disease (SCD) is the most common inherited disorders of hemoglobinopathies in the worldwide and represents a major public health problem in Arab countries especially Yemen. One of the most SCD is the asymptomatic heterozygotes (HbSA) sickle cell trait (SCT) carriers. The aim of the current study was to determine the prevalence of SCT, gender differences, and Full blood cells (FBC) parameters among school students in Taiz city, Yemen. A cross-sectional study was conducted on two hundred and seventy blood samples were subjected to sickling and FBC tests. One hundred thirty seven boys (50.7%) and one hundred thirty three girls (34.8%), nineteen blood samples were found sickling test positive, thirteen boys (68%) and six girls (32%) with overall prevalence of SCT among all students was 7.0% (4.8% boys and 2.2 % girls). 77% of boys and 33% of girls SCT carriers were found their parents having consanguineous marriage with statistically significant difference (p=0.007). FBC results of SCT students showed significantly lower compared to normal students(p=0.01). In conclusion: SCT carrier is highly prevalence among population in Taiz city as well as other parts of Yemen, these could be contributed to the highest degree of first-cousin consanguineous marriage and endemic malaria in this city.

Keywords;- *Sickle Cell Diseases (SCD), Sickle Cell Trait (SCT), Students, School, Taiz, Yemen.*

I. INTRODUCTION

Sickle cell disease (SCD) is on the top of a global health problems worldwide presents important challenges to our healthcare systems by causing a significant morbidity and mortality. It is group an autosomal recessive inherited genetic disorders of the erythrocytes, resulting in prevalence of a

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series of pathological phenotypes due to inherited of hemoglobin-S (HbS) such as sickle cell anemia (SCA)-HbS/S is a homozygous inheritance of two genes mutant and sickle cell trait (SCT)-HbS/A a heterozygous inheritance one mutant gene [1] and one normal gene of HbA (adult hemoglobin) are carriers for SCD, also known as sickle cell trait (SCT). Moreover, when a person inherited heterozygous gene of sickle hemoglobin (S) with other unusual hemoglobin gene variants such as D, E , C, Thalassemia [1] result in HbS/D, HbS/E, HbS/C and HbS/β thalassemia phenotypes. Individuals with HbS/β thalassemia, HbS/C, HbS/D and HbS/E may develop sickling complications due to the presence of one HbS gene, while HbS/S individuals having severe form of sickling complications due to the presence of two genes homozygous inheritance**[**4, 5, 25]. These SCD phenotypes having a variable clinical signs and symptoms of anemia with the earliest being appearing in infancy when sickled erythrocytes becomes deoxygenated resulting in polymerization of HbS to become more rigid, sticky and sickled shape and increasing vaso-oclusion as well as hemolytic crisis [1]. Therefore, the most reasons of hospitalization in patients (children, adolescent, adults) with SCD are suffering from severe pain crises due to acute and chronic vaso-oclusion which occurs when sickled erythrocytes block of blood vessels and capillaries, resulting in a reduction of oxygenated blood supplied to the tissues leads to ischemia, pain, and damage of most organs [26].

Individuals with SCD (HbS/β thalassemia, HbS/C, HbS/D and HbS/E) are not frequently have any clinical symptoms of anemia or severe pain crisis, however, mild to moderate anemia and some complications occur under severe dehydration and hypoxia/or severe infection due to HbS polymerization [25]. Similarity, individuals with SCA are usually having very low hemoglobin level and severe pain crisis, strokes, acute chest syndrome and sickling complications due to intensive HbSS polymerization **[**4].

Generally, individuals with SCT carriers are usually appearing healthy (asymptomatic) and almost having normal hemoglobin level with less or no clinical symptoms of anemia as well as rarely develop severe sickling complications after exposure to severe hypoxia and dehydration [4, 5]. However, mild to moderate pain crisis and anemia may occur under extensive dehydration, stressful conditions, hypoxia, and/or severe infection due to occur HbS sickling result in some complications of SCD [4, 5, 25]. Several studies were indicated to sudden death may be happened due to severe life-threatening sickling complications such as acute chest syndrome, venous thromboembolic events, renal papillary necrosis, splenic infarction and hyposthenuria, especially when carriers severely exposed to hypoxia during heavy exercise and severe dehydrated conditions [6, 7, 8**]**. Additionally, asymptomatic bacteriuria in pregnancy and non-pregnancy women as well as pyelonephritis and urinary tract infection were also a well-defined complications in carriers patients compared to controls [8].

SCD is mainly effect on the population whose mostly living and ancestors come from sub-Saharan Africa, South America, Asia and Mediterranean countries [24]. SCT carrier is the most common type of SCD with the highest prevalence rate in Africa, where up to 1 in 4 individuals having SCT [2, 3].

Globally, 300 million individuals were estimated heterozygous SCT carrier for SCD with the highest prevalence rate in parts of Africa and among population with origins in equatorial Africa as well as the Mediterranean basin, Saudi Arabia and Yemen (9, 10]. In Yemen at 2020, Bashanfer et al has demonstrated the prevalence of SCT in Hodiedah city was 8.0% among university educated students [10]; while other studies conducted in 2004 and 2011 by Al-Nood et al were carried in Sana'a on 1700 persons and in Taiz on thalassemia patients, he showed the prevalence of SCT was 2.2% and 8.2%, respectively [11, 12, 13].

However, until today there is no genetic program provided for neonatal and adult screening to detect and diagnosis hemoglobinopathies or other genetic disorders. On the other hand, it is necessary existence vital efforts to actually determine the prevalence rate of hemoglobinopathies disorders to promote awareness among the people about mode of inheritance and serious complications. Strong awareness is very necessary on SCT carrier status because carriers can pass their HbS gene to their children. This study aimed to estimate the prevalence the sickle cell trait among school students males and females from different parts in Taiz city, which is found in parallel with countries of African equatorial and has the highest prevalence of anemia, malaria and dengue fever.

II. METHODOLOGY

A. Study design and Subjects

A cross-sectional study was designed to determine the prevalence of sickle cell disease and trait it among adolescent school students, and to evaluate their blood parameters, demographic and socioeconomic information, history of family anemia, clinical symptoms and blood transfusion throughout several tests and questionnaire survey. 270 adolescent school students were conducted from different secondary schools, randomly selected between April and July 2022 after obtained ethical approval from the faculty of Medical health Sciences, Aljanad University for Science and Technology, education office at city and their parents.

Students participants were informed about the objectives of the study, types of tests and their participation were totally voluntarily before starting blood sample collection. The questionnaire were given to all volunteer students to fill up at home to obtained their parents' consent to participate in this study. 3 ml of venous blood were collected from each student, drawn into anticoagulant ethylene diamine tetra acetic acid (EDTA) tube and used to analyze blood parameters and carried out screening sickling test.

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B. Hematological Parameters Testing

FBC was performed to determined haemoglobin (Hb) concentration, red blood cells count (RBCs), 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 by using Auto Hematology Analyzer (Model: RT. 7600 , made in Germany).

All students blood samples were screened for sickle cell disease carriers by sickling test according to previously described protocol using reducing agents sodium-metabisulfite [13-6]. One drop was placed on a slide of sodium metabisulfite (2%) mixed well with a drop of EDTA venous blood, and covered with cover slip and edges of cover slip was carefully sealed using candle wax and allowed to stand at room temperature in a moist petri dish. After 30 minutes each slide was observed and examined under the microscope using (objective 40x), then after 2 and 24 hours, the presence of sickle-shape RBC indicate positive sample, while negative sample no changes was observed in shape of RBC. Actually, we didn't have any budget or support to further examination of all positive samples and some normal samples by electrophoresis so for validation purposes one sickle positive sample and one control sample were further examined by Hb electrophoresis.

C. Statistical Analysis

Statistical analysis 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 proportions, mean and standard deviations using independent "t" test for hematological parameters of sickle cell trait and compare with normal students. Statistical significance was identified at 0.05 levels. Nonparametric variables were analyzed using cross-tabulation test and chi-square used to determine the significant difference (p-value <0.05) in history of family anemia, clinical symptoms, blood transfusion and socioeconomic status for sickling and normal students.

III. RESULTS

Two hundred seventy school students were screened for sickle cell trait by sickling test, 137 of school students boys (50.8%) and 133 of school students girls (49.2 %). 19 blood samples were found positive for sickling test and 251 samples were negative with overall the prevalence of sickle cell trait among all students was 7.0% (4.8% boys and 2.2 % girls) as showed in "fig. I".

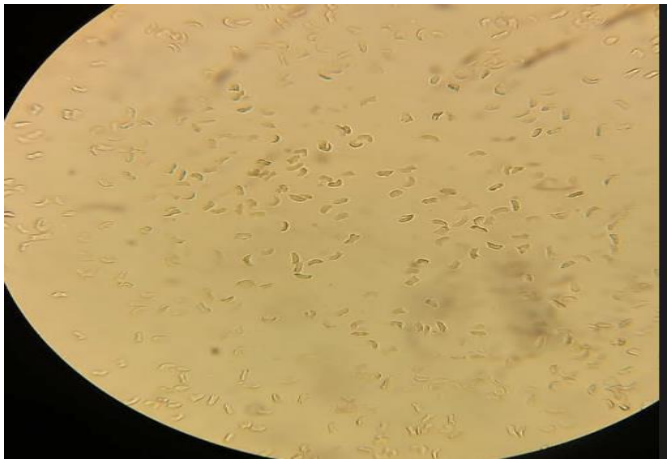

Fig 1 Shows an Example Positive Sickling Test Result.

Table 1 shows 13 (9.5 %) out of 137 of school students boys and 6 (4.5%) out of 133 school students girls were had positive sickling test, there was found statistically significant (p=0.002) difference in the prevalence rate of sickle cell SCT among affected boys and girls students. The highest prevalence rate was found 69% and 50.0% at age 14-16 year followed by 31% and 17% at age 16-18 year and at age 18-20 year 0.0% and 33% for boys and girls students, respectively. Statistically there was found a significant (P=0.038) difference in the prevalence rate among students aged groups. Moreover, 10 (77%) of boys and 2 (33%) of girls were found their parents having consanguineous marriage. 6 (46 %) out of 13 boys and 2 (33%) out of 6 girls students were found having awareness of anemia compared to 65 (52%) of normal boys and 81 (64%) of normal girls students with no significant difference (p=0.20). While 46 % and 50% of boys and girls students have family history of anemia compared to 18% and 21% of boys and girls normal students, respectively, with a higher significant difference (p=0.004). Similarity, 46 % of boys and 50% of girls students have history clinical symptoms of anemia compared to 19 % of normal boys and 28 % of normal girls students, with significant difference (p=0.024). Also 4 (31%) of boys and 1 (17%) of girls were found having history of SCD, however, there was no statistical significant difference (P=0.52) among boys and girls. Whereas, 3 (23%) of boys and 1 (17%) of girls were be found have history of blood transfusion for once time with no statistically significant difference (P=0.20).

Table II shows the comparison of the hematological parameters results for sickle cell trait students versus normal students. There was found a lower significant (p=0.01) in the means ±SD levels of Hb (14.0 ±1.1 g/dl and 12.5 ±0.5 g/dl) for boys and girls students with sickle cell disease than the means ±SD levels of Hb (15.3 ± 3.3 g/dl and 13.4 ±1.4 g/dl) of normal boys and girls, respectively. Moreover, sickle cell trait boys and girls had a lower significant (p=0.01) in the means levels of RBCs, Hct, MCV, MCH and RDW comparing to normal boys and girls students, whereas, the mean level of RDW was significantly (p=0.01) higher in sickle cell trait students comparing to normal students.

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For validation the results of the previous sickling test, "fig. II" shows an example of hemoglobin electrophoresis results of two samples (HbA/A normal on the left and HbS/Trait on the wright) .

IV. DSCUSSION

Yemen is the poorest country in the worldwide have next to not yet implemented programs based on population screening for the detection of haemoglobinopathies or any other genetic disorders. On the other hand, until today we are not known the exactly the prevalence of hematological disorders among our population, not only that, there is no policy to supporting of researches to insuring health surveillance data that is essential for healthcare providers and public health planning. However, there were very few studies carried out to determine the prevalence rat of SCT in the Northern part of Yemen in Sana'a (Capital city) on individuals having SCA co-exiting with thalassemia [13, 15] and other study carried out in the West of Yemen (Hodiedah city) on university educated students [10].

So our study was designed to survey the prevalence of sickle cell trait carrier among school students resident in the Taiz city aged between 14-20 years. The majority of sickle cell trait students (69% boys and 50% girls) were had been found at aged 14-16 years. The current results indicate to the overall prevalence of sickle cell trait was 7.0% among students, with a higher significant (p=0.002) distribution among boys (4.8%) compared to girls students (2.2%). These finding very similar

Table 1 The Distribution of Sickle Cell Trait and Normal Students According to Socio demographic Information and Clinical Symptoms

Characters		Sickle Cell Trait Students				Normal students				P-value
		Boys		Girls		Boys		Girls		
		No.	(%)	No.	(%)	No.	(%)	No.	(%)	
Gender		13	(9.5%)	6	(4.5%)	124	(90.5%)	127	(95.5)	0.002
Age (years)	14-16	9	(69%)	3	(50%)	44	(36%)	51	(40%)	0.038
	16-18	4	(31%)	1	(17%)	71	(57%)	71	(56%)	
	18-20	0	(0.0%)	2	(33%)	9	(7%)	5	(4%)	
Consanguineous Parents	Yes	10	(77%)	2	(33%)	53	(43%)	50	(39%)	0.007
	No	3	(23%)	4	(67%)	71	(57%)	77	(61%)	
Study Level	Primary School	6	(46%)	2	(33%)	53	(43%)	29	(23%)	0.70
	Secondary School	7	(54%)	1	(17%)	57	(46%)	47	(37%)	
	Tertiary School	0	(0.0%)	3	(50%)	14	(11%)	51	(40%)	
Awareness of Anemia	Yes	6	(46%)	2	(33%)	65	(52%)	81	(64%)	0.20
	No	7	(54%)	4	(67%)	59	(48%)	46	(36%)	
Family History Anemia	Yes	6	(46%)	3	(50%)	22	(18%)	26	(21%)	0.004
	No	7	(54%)	4	(50%)	102	(82%)	101	(79%)	
Symptoms of Anemia (Pallor, Fatigue)	Yes	6	(46%)	3	(50%)	24	(19%)	36	(28%)	0.024
	No	7	(54%)	4	(50%)	100	(81%)	91	(72%)	
History Blood Transfusion	Yes	3	(23%)	1	(17%)	20	(16%)	17	(14%)	0.20
	No	10	(77%)	5	(83%)	104	(84%)	110	(87%)	
History Affected by SCD	Yes	4	(31%)	1	(17%)					0.52
	No	9	(69%)	4	(83%)					

Table 2 Comparisons of the Hematological Parameters (Hb, Rbcs, Hct, MCV, MCH, and RDW) between Normal Group (N=251) and Sickle Cell Trait Group (N =19).

Parameters	Hb (g/dL)	RBCs (10^{12}/L)	Hct %	MCV (fL)	MCH (pg)	RDW %	*P-value*
	Mean ±SD	Mean ±SD	Mean ± SD	Mean ±SD	Mean ±SD	Mean ±SD	
Normal Males	15.3 ± 3.3	6.0 ± 1.0	48.0 ± 9.0	81.4 ± 6.3	26.3 ± 3.5	12.0 ± 0.5	**0.01**
SCT Males	14.0 ± 1.1	5.3 ± 0.6	42.0 ± 3.0	73.0 ± 9.6	23.4 ± 3.0	12.3 ± 0.3	
Normal Females	13.4 ± 1.4	5.4 ± 0.7	41.0 ± 4.0	80.0 ± 8.0	27.0 ± 2.7	12.1 ±0.47	
SCT Females	12.5 ± 0.5	4.6 ± 0.6	38.0 ± 1.8	72.0 ± 5.0	23.0 ± 2.4	12.6 ± 0.3	**0.01**

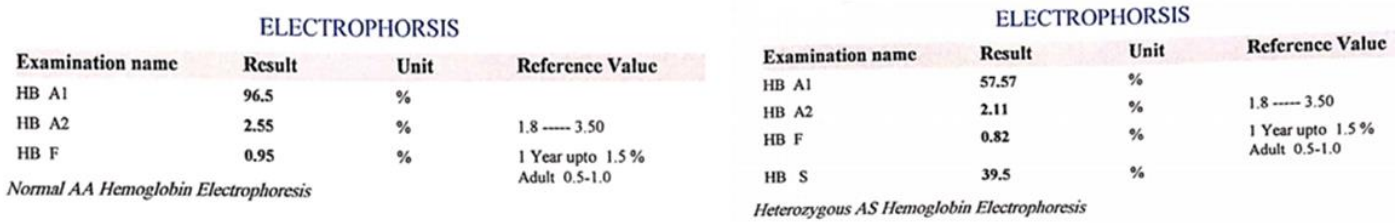

Fig 2 Shows an Example HB Electrophoresis for Two Samples One Normal on Left and Other Sample is SCT on the write Figure.

To result reported by Bashanfer et al in Hodiedah city (8%) as well as in similarity with other study reported by Al-Nood in Taiz city in which reported the prevalence of SCT (8.2%) among thalassemia patients, and also a higher than the result reported by the same author in Sana'a city (2.2%) [10, 11, 12, 13].

In addition, when we comparison our finding with the geographical and global prevalence of SCT, our prevalence rate of SCT (7.0%) was a agree to study reported by Charuhas et al he has been found 7.3% males and 5.9% females were had SCT [16]**,** also our result agree with study reported by Sanjay et al in which mentioned the prevalence of SCT among children was 7.7% **[**18]. However, our results disagree with study carried out by El Ariss et al where the prevalence of SCT carrier for SCD was higher among females (6.5%) than males (3.2%) [17].

Moreover, there was some constituents and differences in findings of the current study with several studies reported from neighboring Arab countries such as 6.5% Iraq, 6.0% Jordan, 4.8-10% Oman, 3.5% in Algeria,16.4% Bahrain, 4.5% Libya, 4.2-25.9% Saudi Arabia, 4.9% Tunisia [19]. Therefore, the difference between our findings and so far studies reported may be correlated with sociodemographic changes, ethnic of the Yemeni population, first-cousin consanguineous marriage, geographical location, population culture and habits, no neonatal program screening and lack of medical counseling.

The present results indicate to 46% of boys and 33 % of girls with sickle cell trait were found have awareness of anemia compared to 52% of boys and 64% of girls normal students with no significant difference (p=0.20). In similarity, there was found 46% of boys and 33% of girls with sickle cell trait have family history of anemia and clinical symptoms (pallor and fatigue), respectively, this is disagree with results reported by Bashenfer et al in which 20.8% of males and 18.8% of female students have their family history anemia and suffering from pallor and fatigue as well as disagree with result presented by Sanjay et al in which had been found 88% and 65% of children with SCT complain of pallor and fatigue, resecitvely [10, 18]. 4 (31%) of boy and 1 (17%) of girls with sickle cell trait were a knowing history of SCD and blood transfusion, these result was in line with result reported in Hodiedah by Bashanfer et al in which 20.8% of males and 18.8% of females university students with SCT having past history of blood transfusion [10].

The overall prevalence rate of consanguineous marriage among parents of study population was 43% (115/270) students (46% boys and 39% girls), these finding was in similarity to previous studies carried out on Yemeni population whose resident in the North and Western parts of our country in which there were found 37% [20] and 35% [21] of the prevalence of consanguineous marriage among first cousin. The highest prevalence rate of sickle cell trait was observed among boys (77%) compared to (33%) of girls students whose parents have consanguinity marriage, this is might be due to the first-cousin consanguineous marriage which considered as a deeply root in our social culture. The highest prevalence of consanguinity marriage reflected the higher frequency prevalence of x-linked genetic disorders that is markedly affected not just in our country population but in some regions of Middle East in which consanguineous marriage up to 70 % [19] when compared to other parts of the world.

The means levels of Hb, RBCs, Hct, MCV, and MCH were found lower among students with sickle cell trait compared to normal students with statistically difference (p=0.01) this is result shown that students with SCT suffer from mild anemia. These findings are in line with the previously reported findings by Bashanfer et al. and Datar et al, Chikhlikar, and El Ariss , respectively[10, 22, 23, 17]. This significant 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.

V. CONCLUSION

The prevalence of sickle cell trait among school students of males and females in Taiz city is 7.0%. This is findings may put some insight on the incidence of sickle cell diseases in Taiz city in the absence of health educational polices and screening programs (newborn preschool and school children, adolescents and adults or premarital couples) as well as lack definite data about prevalence of sickle cell diseases. Also these study highlight the need population screening programs for different age levels to determine the carrier rates in our province especially to whom premarital couples to those at risk of having children with sickle cell diseases and reduce the disease incidence in our poor society.

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