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Assessment of leukocytes, neutrophils, lymphocytes and monocytes level among sickle cell trait and sickle cell disease patients

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Sickle cell disease (SCD) is a blood disorder characterized by generation of abnormal haemoglobin (Hb) resulting in abnormally shaped (sickled) red blood cells (RBCs) leading to their life span reduction. It considers the most common single gene mutation affecting millions of people worldwide. Several number of studies found that the severity of sickle cell disease (SCD) has been found to be significantly associated with leukocytes count. In Saudi Arabia, there is a very limited research studies on leukocytes level between carrier and affected patients with sickle cell. Thus, the aim of this study was to assess the level of white blood cells, neutrophils, lymphocytes and monocytes among patients who diagnosed with either sickle cell trait or sickle cell anaemia between the aged 18 to 45 years. The result of this study showed that sickle cell carrier patients had normal level of total while blood cells, neutrophils and monocytes. However, slight increase in the lymphocytes count was detected among only 4% of carrier patients. The total level of white blood cells for patients with sickle cell anaemia showed higher results than both normal individuals (control samples) and sickle cell carrier patients. In addition, lymphocytes were reported with high results among 40% of sickle cell anaemia (SCA) patients while neutrophils and monocytes level was normal. Normal leukocyte, neutrophils and monocytes count was common among sickle cell carriers, which indicated that carrier patients have no disease complications or high risk of developing infection. On the other hand, abnormal level of leukocyte was common in majority of Saudi male and female SCA patients investigated in this study.

Keywords: Sickle cell trait, Sickle cell anaemia, White blood cells, Leukocytes, lymphocytes, neutrophils, monocytes

INTRODUCTION

Sickle cell disease (SCD) is a blood disorder characterized by generation of abnormal haemoglobin (Hb) resulting in abnormally shaped (sickled) red blood cells (RBCs) leading to their life span reduction (Al-Haggar et al., 2006; Roseff, 2009). It considers the most common single gene mutation affecting millions of people worldwide (Ballas and Lusardi, 2005). In normal healthy individual haemoglobin contains four protein molecules connected together and known as globulin chains. Adult haemoglobin consists of two alpha-globulin chains and two beta-globulin chains. However, when a point chromosomal abnormality occurs within the gene coding Hb beta-globulin chain this leads to a substitution of amino acid valine for glutamic acid, which, when existing on one or both chromosomes, resulted in sickle cell trait (Hb S) or sickle cell anaemia (Hb SS) respectively (Roseff, 2009).

In Saudi Arabia, the first sickle cell disease was detected in the 1960s at the Eastern province (Lehmann et al., 1963). In addition, medical features, epidemiology and frequency rate of this disease were noted to be vary among different regions of Saudi Arabia with the highest

prevalence reported in the Eastern province (Al-Qurashi et al, 2008). AlHamdan and colleagues have been reported that the carrier form of sickle cell disorder was ranged from 2% to 27%, and phenotype of sickle cell disease reached 1.4% among Saudi's population (AlHamdan et al., 2007).

Sickle cell trait patient with heterozygotes form presented with a single sickle cell haemoglobin gene (Hb S) and live a normal life without any health complications or disease symptoms. However, under dangerous conditions such as severe dehydration patients with sickle cell trait may safer from muscle breakdown, increased pressure in the eye and anaemia in pregnancy (Tsaras et al., 2009). Morbidity and mortality is occasionally associated with sickle cell trait (Platt et al., 1994).

On the other hand, patients with sickle cell anaemia have homozygote form of sickle cell haemoglobin genes (Hb SS) (Roseff, 2009). In homozygote patients, sickle RBCs become rigid with decreased deformability and has a short life span resulting in their prematurely removed from the circulation leading to chronic haemolytic anaemia. Such abnormalities will also cause a vaso-occlusive crisis (VOC), which is the most common complication of SCD in the bones and bone marrow. The severity of sickle cell disease (SCD) has been found to be significantly associated with leukocytes count (Olatunji and Davies, 2000). A study performed in 2007, found that the high level of white blood cells (WBCs) was significantly associated with SCD patients who had complications (Litos et al., 2007). Within the same study, they noted that the total WBCs level, lymphocyte count and monocytes count were higher in SCD patients than asymptomatic SCT patients (Litos et al., 2007). High WBCs values were also reported in SCD patients with acute chest syndrome with pain crises (Naprawa et al., 2005). More recent study in the Eastern region of Saudi Arabia showed abnormal elevated level of WBCs among Saudi male with SCD who had low haemoglobin level as well as cough and low heart rate (Ahmed et al., 2017). Accumulated evidences from several researches found that the base-line leukocyte count is a very important independent risk factor for sickle cell disease severity and incidence of chronic inflammation (Platt et al., 1994; Naprawa et al., 2005; Litos et al., 2007). Moreover, it has been found that leucocytosis, patient's age, over all heath and previous acute episodes of anaemia were highly linked with disease severity among SCA patients (Tassel et al., 2011).

The mortality rate was higher among SCD patients with high white blood cells count at children and younger age adult (Platt, 1994). Unfortunately, there is limited number of research studies on leukocytes level between carrier and affected patients with sickle cell in Saudi Arabia. Thus, the main aim of this study was to assess the white blood cells, neutrophils, lymphocytes and monocytes level and abnormality among Saudi male and female patients (aged 18-45 years) who diagnosed with either sickle cell trait or sickle cell anaemia.

MATERIALS AND METHODS

This is a cross sectional study performed between March to December 2018 in Makkah city at Western region of Saudi Arabia. It was conducted to assess the level of white blood cells, neutrophils, lymphocytes and monocytes among Saudi male and female patients aged between 18 to 45 years. A total of 52 blood samples were collected from patients who attending to before marriage clinic examination and had diagnosed with either sickle cell trait or sickle cell anaemia based on the results of the fully automated Variant V-II Haemoglobin Testing System which measures the concentration of HB A1, HB A2, HB F and HB S. Sample of five normal individuals (without haemoglobinopathy) were used as a control samples in this study.

Five ml of the venous blood specimens were collected from patients diagnosed with SCT or SCA, in ethylene demine tetra-acetic acid (EDTA) at Children and Maternity Hospital. All samples were examined for complete blood count (CBC) to investigate the level of RBCs, Hb, and total WBCs using CELL-DYN Ruby machine and calibrated by using standard quality assurance at the haematological laboratory. Additionally, microscopic examination of peripheral blood smear was performed to control and patients samples to assess the level (high or low) of neutrophils lymphocytes and monocytes.

Ethical Consideration

Ethical approval for this study was obtained from the ethics review committee of Applied Medical Sciences College at Al-Taif University. All information obtained at each course of the study was kept confidential.

Statistical analysis

Microsoft Office Excel was used for manually entered of data, which were analysed by

Statistical Package of Social Sciences (SPSS), version 16.0. (IBM Corporation, Armonk, NY, USA).

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RESULTS

The study was focused on assessment of white blood cells, neutrophils, lymphocytes and monocytes level and their abnormality among Saudi male and female patients (aged 18-45 years). Firstly, all samples for patients diagnosed with SCT or SCA during the period of the study were included. Exclusion criteria were including sickle cell anaemia patients who received blood unit since the result of complete blood count (CBC) will be affected. Five normal samples, which, were collected from healthy individuals (3 female and 2 male) were showed normal level of RBC, Hb and total WBC and normal distribution of haemoglobin. The majority of patient samples in this study were sickle cell carrier with 90% (47/52) (Table 1) while 10% (5/52) were having sickle cell anaemia (Table 2) according to the result of Variant V-II Haemoglobin Testing System.

The result of this study showed that all sickle cell carrier patients had normal level of leukocytes (3.70-10.1 $X10^9$/L), neutrophils (40-80%) and monocytes (2-10%). Only 4% (2/47) of sickle cell carrier patients represented a slight increase in the lymphocytes count on complete blood count and prephral blood smear stained with wrights stain. However, this was not significant increase. The level of total white blood cells was investigated for patients with sickle cell anaemia and showed higher results than both normal individuals (control samples) and sickle cell carrier patients.

Table 1: Shows an example for patient sample with sickle cell carrier investigated by using Variant V-II Haemoglobin Testing System.

Peak Name	Calibrated area %	Area%	Retention Time (min)	Peak Area
Unknown	---	0.0	1.00	1399
F	0.3	---	1.09	8538
Unknown	---	0.8	1.28	23678
P2	---	2.5	1.37	77545
P3	---	2.9	1.76	91463
Ao	---	54.4	2.39	1709049
A2	3.4*	---	3.67	108988
S-window	---	35.6	4.32	1118423

Table 2: Shows an example for patient sample with sickle cell anaemia investigated by using Variant V-II Haemoglobin Testing System.

Peak Name	Calibrated area %	Area%	Retention Time (min)	Peak Area
Unknown	---	0.0	0.99	871
F	0.3	---	1.09	6424
Unknown	---	0.5	1.21	10160
P2	---	2.0	1.34	39536
P3	---	2.1	1.71	42387
Ao	---	50.5	2.48	1019104
A2	3.5*	---	3.62	76189
S-window	---	40.8	4.37	822146

Moreover, lymphocytes were reported with high results among 40% of SCA patients while neutrophils and monocytes level was normal.

DISCUSSION

The severities of sickle cell disease (SCD) and mortality rate have been found to be significantly associated with WBCs count. Periodic assessment of leukocytes in primary health care clinics should be performed to recognize patients with increased or decreased level than the normal WBC values. This is essential to understand more about underlying etiology and allow physicians to impending SCD crisis and start the right treatment to avoid medical complications and sever symptoms related to high leukocytes.

Patients with sickle cell trait (SCT) (heterozygous form of the Hb S gene) are asymptomatic and they display a normal peripheral blood smear (PBS) and complete blood count (CBC) results. However, morphologic abnormality in the RBCs of patients affected by SCT was reported in a number of studies (Hoffman et al., 2004; Madjid et al., 2004; Jee et al., 2004). Sickle cell trait can be also associated with acute chest syndrome and sudden death under special circumstances such as extreme physical exertion, relative hypoxia and dehydration (Mitchell, 2007). In this study, it has been found that white blood cells, neutrophils, lymphocytes and monocytes count was within the normal ranges when examined for complete blood count (CBC) and differential analysis using CELL-DYN Ruby machine in patients with SCT. Our results are comparable to those previously published and reported normal leukocytes level among SCT patients (Schuetz et al,, 2004; Krailadsiri et al, 2001).

Multi-studies showed significant association between white blood cell count and severity, complications, poor prognosis and risk of mortality among sickle cell anaemia patients (homozygous form of the Hb SS gene) (Hoffman et al., 2004; Madjid et al., 2004; Jee et al., 2005). Anyaegbu and collages, reported a statistically significant association between high neutrophil level and severity of sickle cell anaemia (Anyaegbu et al, 1998). It has been suggested that the leucocytosis seen in sickle cell anaemia patients is responsible for the pain, anxiety, nausea and vomiting (Milhorat, 1942). Pediatric sickle cell anemia cohort study showed association between leukocytosis and decline in lung volumes and severity of the disease in children (Cle´ment Tassel et al., 2011).

CONCLUSION

Our results demonstrated that the total white blood cells count was higher than the normal values for adult patients with sickle cell anaemia when compared to SCT patients and normal healthy individuals in this study. Lymphocytes were reported with high results among 40% of SCA patients while neutrophils and monocytes level was normal. Similar to our result a study performed in USA on sickle cells anaemia patients had significantly higher WBC reported in their laboratory profile (Stewart et al., 1992). In 2007, high level of WBCs, lymphocyte and monocytes was significantly reported in SCD patients than asymptomatic SCT patients (Litos et al., 2007). Another more recent study found high concentration of white blood cells and lymphocyte in sickle cell anaemia patients (Solo et al., 2018).
This study has some limitations that it has been done in one area of the Saudi Arabia and small sample size, thus future studies should be performed on different regions of the Kingdom and to investigate a larger sample to overcome this limitation

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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