

## Reference Ranges of White Blood Cells Count among Sudanese Healthy Adults

Elmutaz H. Taha<sup>1\*</sup>, Mohammed Elshiekh<sup>1</sup>, Mohamed Ali Alzain<sup>2</sup>, Elnagi Y. Hajo<sup>3</sup>, Abdelmohisen Hussein<sup>4</sup>, Kamal M. Awad<sup>4</sup>, Ibrahim A. Ali<sup>5</sup>, Omer A. Musa<sup>5</sup>

<sup>1</sup>Department of Human Physiology, Director of Medical Research Center, Faculty of Medicine, Dongola University, Sudan

<sup>2</sup>Department of Community Medicine, Faculty of Medicine, Dongola University, Sudan

<sup>3</sup>Department of Physiology, Faculty of Medicine, The National University, Sudan

<sup>4</sup>Department of Physiology, Faculty of Medicine, Gadarif University, Sudan

<sup>5</sup>Department of Physiology, Faculty of Medicine, National Ribat University, Sudan

**\*Corresponding author**

*Elmutaz H. Taha*

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**Abstract:** The reference values of hematological markers currently used in Sudan have been obtained from researches on populations in developed countries and may not be applicable in Sudan. It has previously been shown that the normal hemoglobin level in Sudanese children is different from international values. The objectives of this study were to establish the reference ranges of white blood cells and differential count in healthy Sudanese adults. The present study included 1076 healthy Sudanese adults from both sexes and five states, with age range of 20 – 60 years. Clinical examination was performed, weight and height were measured, and BMI was calculated. Blood samples were obtained from brachial veins and drawn in EDTA tubes. WBCs and differential count were analyzed using Sysmex KX-21 automated hematology analyzer. SPSS version 25 statistical software was used for data analysis, P value < 0.05 was considered significant and 95% CI was accepted. The mean age of participants was 25.23±9.64 years. The median of WBCs count was  $5 \times 10^3/\mu\text{L}$  with reference range of ( $2.9-9.6 \times 10^3/\mu\text{L}$ ). Neutrophils count was  $2.3(1.7-3.1 \times 10^3/\mu\text{L})$ , while lymphocytes was  $2.1(1.8-2.5 \times 10^3/\mu\text{L})$ . The median of WBCs was significantly higher in female [ $5.1 \times 10^3/\mu\text{L}$  (range:  $4.2- 6.3 \times 10^3/\mu\text{L}$ )] than male [ $5.0 \times 10^3/\mu\text{L}$  (range:  $4.0- 6.0 \times 10^3/\mu\text{L}$ )] ( $p < 0.05$ ). Our results showed that WBCs count was positively correlated with BMI. The count was found to be significantly higher in Red sea and Darfur states compared with the other states. The reference ranges of WBCs count in Sudanese are lower than the international one and should be used in Sudan.

**Keywords:** WBCs, Differential count, BMI.

### INTRODUCTION

The local reference values for hematological parameters are essential for screening, diagnosis, treatment, and follow up [1]. The reference values of hematological markers currently used in Asia and Africa have been obtained from researches on populations in developed countries and may not be applicable in most local settings [1]. The Clinical and Laboratory Standards Institute (CLSI) recommended that reference range should be established for each region, because many different factors such as age, gender, ethnicity, geographical and environmental factors have an impact on the hematological parameters in different populations [2].

African origin population has lower white blood cells (WBCs) count compared with Caucasians origin population [3]. The WBCs count is directly associated with BMI and the count is significantly increased with BMI [4]. WBCs in obese subjects are found to be significantly higher than in underweight

subjects [4, 5]. A study done by A. Abbas and others in Gezira State, Sudan revealed that there is no significant difference in WBCs count between Sudanese people and international reference values [6]. In another study conducted in Khartoum state reported that WBCs count shows significantly higher mean TWBCs count in comparison with TWBCs counts of many African Countries [7]. In contrast to these results, WBCs count and differential count in Sudanese people showed significant lower value than the international references, but similar as those from African countries (4). It is necessary to conduct a study involving a large sample size from different states of the Sudan for more accurate determination of WBCs and differential count in Sudanese. Therefore, we have conducted this study to determine reference intervals of WBCs and differential counts in Sudanese people and to correlate them with age, sex and BMI.

**METHODS**

Sudan, one of the largest countries in Africa, has about 40 million individuals. Ethnic composition is the result of a complex mixing between Arabs and Africans. A cross-sectional study was conducted in five states of Sudan, representing the central, northern, western and eastern areas, from December 2016 to April 2018. Khartoum and Gezira states represent the central area with multi ethnicities... The Red sea state, near Eritria and Ethiopia, represent the eastern areas, North Darfur is near Chad and Central Africa and represent the western areas, while the Northern state, near Egypt and Libya represents the northern areas. Ethical approval was obtained from the research ethical committees of the Federal Ministry of Health and the National Ribat University, Sudan. Each participant provided a written informal consent after fully explaining to them the project. 1076 healthy adult Sudanese aged between 20-60 years old and resident for at least one year in their state were included. A questionnaire was filled by each participant. Any subject with chronic diseases (cardiac diseases, TB, asthma, thyroid disorders, diabetes mellitus, hypertension, renal failure, liver diseases .... etc.), recent acute diseases (malaria, typhoid fever, ...), recent surgery, drug abuse, pregnancy, lactation and heavy smokers were excluded.

Clinical examination was performed, weight and height were measured, and BMI was calculated as weight (kg)/height (m<sup>2</sup>).

Blood samples were drawn from the brachial veins by the standard procedure with a Vacutainer into

tubes containing K3E-EDTA and gently mixed by inverting the tube five to six times immediately after drawing. The samples were then placed in ice-box, transported to the laboratory and processed within 2 hours. A Sysmex KX-21 automated hematology analyzer was used for measuring WBCs and differential counts. Normality of continuous variable was determined by using the Shapiro-Wilk test. The non-parametric continuous (WBCs and differential counts) was presented as a median (interquartile range). 2.5th and 97.5th percentile was used to determine the reference value of WBCs and differential counts according to CLSI [2]. To determine differences between groups for continuous non-normally distributed variables, the medians was compared using the Mann-Whitney U test. All statistical tests were two sided, and  $p < 0.05$  was considered statistical significant. All statistical analyses were carried out using IBM SPSS Statistics software (version 25.0 from Armonk, NY: IBM Corp, USA).

**RESULTS**

The sample was selected from five states, namely Khartoum 437 subjects (40.6%), Northern 203 (18.9%), Red sea 174 (16.2%), North Darfur 171 (15.9%), and Gezira state 91 (8.5%). The age group 20-29 years represented 81.8%, 30-39 years was 7.3% While 40-49 years was 5.8% and 50-60 was 5.1%.

The reference range of WBCs count in adult Sudanese was  $2.9-9.6 \times 10^3/\mu\text{L}$  with a median of  $5.0 \times 10^3/\mu\text{L}$ . With 46.90% neutrophils and 42.85% lymphocytes (Table-1).

**Table-1: reference values of WBCs and differential count in adult Sudanese**

Parameter	Reference range (2.5 <sup>th</sup> - 97.5 <sup>th</sup> ) ( $\times 10^3/\mu\text{L}$ )	Median(IQR) ( $\times 10^3/\mu\text{L}$ )	Differential%
WBCs	(2.9-9.6)	5.0(4.2-6.1)	-
Neutrophils	(0.8-6.11)	2.34(1.7-3.1)	46.9%
Lymphocytes	(1.2-3.9)	2.14(1.8-2.5)	42.8%
Mixed cells	(0.1-1.7)	0.51(0.4-0.7)	10.2%

IQR: Interquartile range.

There was no significant difference in WBCs count and differential counts between age groups. The median of WBCs was significantly higher in females [ $5.1 \times 10^3/\mu\text{L}$  (range:  $4.2- 6.3 \times 10^3/\mu\text{L}$ )] than males [ $5.0 \times 10^3/\mu\text{L}$  (range:  $4.0- 6.0 \times 10^3/\mu\text{L}$ )] ( $p < 0.05$ ) (table 2) and (Figure-1). There was an increase in the WBCs count with an increase in educational level, but statistically not significant as shown in (table-2).

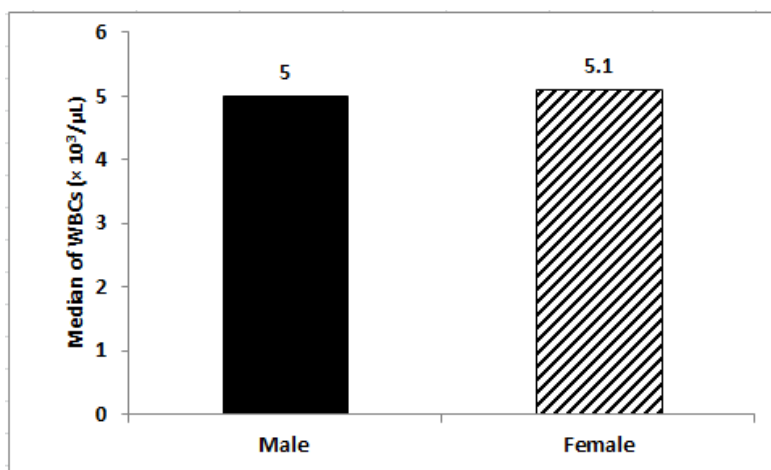
Our result showed that WBCs count was positively correlated with elevated BMI ( $p < 0.05$ ); the median of WBCs counts in underweight was (4.8),

normal weight (5.0), overweight was (5.4) while in obese was ( $5.7 \times 10^3/\mu\text{L}$ ); (figure-2).

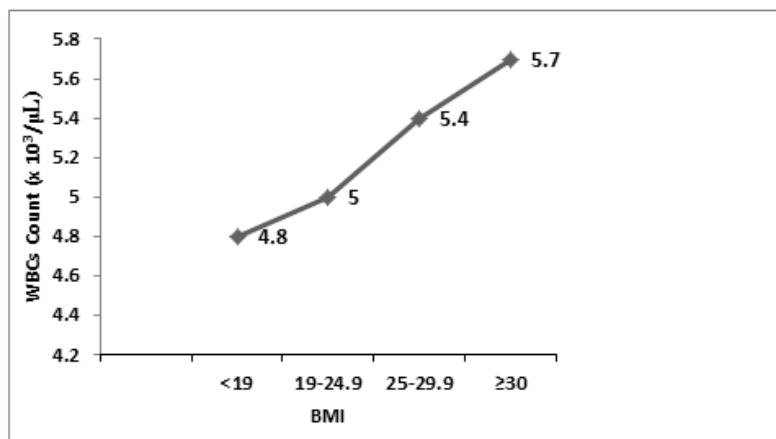
But when we consider gender, there was no statistical significant difference in WBCs count with increased BMI in males, while in females there was statistically significant ( $p$ . value  $< 0.05$ ) difference in WBCs count with increased BMI as shown in (figure-3). There was statistical significant ( $p$ . value  $< 0.05$ ) increase in WBCs count in Red sea state (East of Sudan) and Northern Darfur state (West of Sudan) compared to Northern state (northern of Sudan), Khartoum, and Gezira states (Central of Sudan) as shown in (figure-4).

**Table-2: WBCs count according to demographical characteristics of the study population**

Variables	n(%)	WBCs count Median (IQR) ( $\times 10^3/\mu\text{L}$ )	P. value
Age group			
20-29 years	848(81.8)	5.1(4.1-6.2)	0.767
30-39 years	76(7.3)	5.0(4.2-6.1)	
40-49 years	60(5.8)	5.1(4.2-5.9)	
50-60 years	53(5.1)	5.6(4.6-6.2)	
States			
Khartoum	437(40.6)	4.9(4.15-6.00)	<0.001
Red sea	174(16.2)	5.5(4.6-6.525)	
Northern	203(18.9)	4.7(3.8-5.9)	
Gezira	91(8.5)	4.8(4.0-5.85)	
N. Darfur	171(15.9)	5.6(4.7-6.5)	
Sex			
Male	261(24.6)	5.0(4.0-6.0)	0.034
Female	802(75.4)	5.1(4.2-6.3)	
Education			
Illiterate	19(1.8)	4.85(4.26-5.86)	0.229
Preprimary	19(1.8)	4.7(4.2-5.5)	
Primary	34(3.2)	4.75(3.57-6.6)	
Secondary	80(7.4)	4.86(4.0-5.7)	
University	844(78.4)	5.1(4.12-5.2)	
Post graduate	22(2.0)	5.65(4.57-6.7)	



**Fig-1: The median of WBCs counts between gender in adult Sudanese**



**Fig-2: Relation between WBCs count and BMI in adult Sudanese**

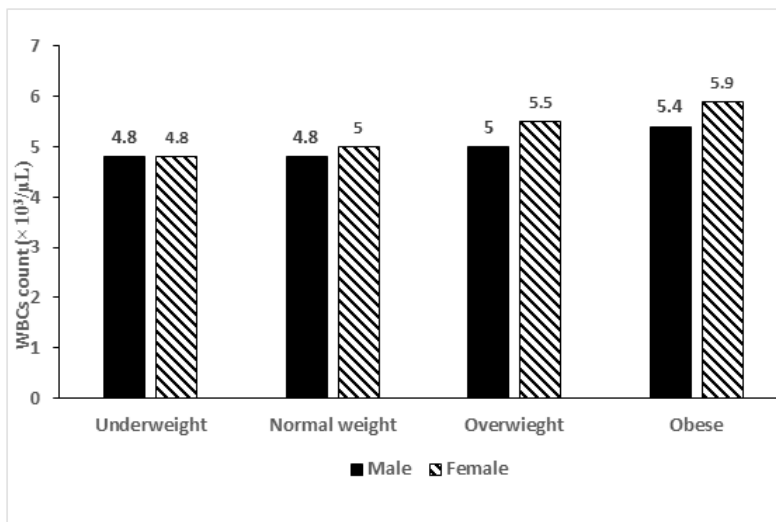


Fig-3: Comparison between WBCs count and BMI in gender

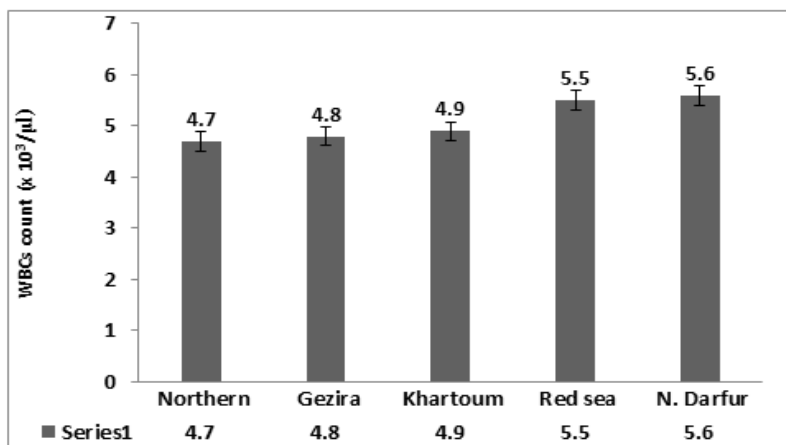


Fig-4: The median of WBCs counts in different states in Sudan

**DISCUSSION**

The aim of our study was to establish the reference range of WBCs count in adult Sudanese and identify factors that influence the WBCs count values. The reference range of our study was (2.9-9.4 × 10<sup>3</sup>/µL). This result was different from two studies done in Sudan; Khartoum state where the reference range is (3.2-9.4) [7]. and Gezira state where the reference range was (2.1-14.1 × 10<sup>3</sup>/µL) [6]. In these two studies, the sample size was small and not representative of the whole Sudanese, the sample sizes were 200 and 500 participants respectively, and each was done in one state without including the females in the study. The reference range of our study was lower than the international values but similar to several studies done in African countries [8-11]. which showed that persons of African descent have lower total WBC and absolute neutrophils counts compared with Caucasians. The WBC count in the South African’s study was higher [12]. The absolute neutrophils count was significantly lower in our study which was in accordance with the black population [13, 14]. Mechanisms by which black population have lower values of neutrophils is still not clear. The hypothesis of an excess of marginal

neutrophils pool is often proposed but a recent study did not confirm it [15]. Lymphocyte count in the current study was higher than Caucasian and African black populations [16-18], and this may be due complex mixing of many ethnicities and some environmental factors.(TABLES1&2) We have found that males had significantly lower WBCs counts values in comparison with females, and this agreed with a study done in Kenya [19], but in contrast to a study done in Ezurum, Turkey where females have significantly low WBCs count compared with males [20]. While in other studies done in Asmara, Ethiopia, and Northern Nigeria, which showed that the reference range of total WBC count for male and female did not show any significant variation [11, 21, 22] Our results showed that WBCs count was positively correlated with elevated BMI which is in contradiction to study conducted in Khartoum [7], and in line with several studies [23, 24]. This correlation was obviously in females, which might be due to an increase of adipose tissues. Adipose tissue is a major source of inflammatory factors, such as IL-6 and C-reactive protein (CRP), which are well established markers of systemic inflammation [25]. Pro-inflammatory cytokines, such as interleukin 6 (IL-6)

and interleukin 8 (IL-8), are important inducers of WBCs production [26]. There was statistical significant increase in WBCs count in Red sea and Northern Darfur states more than Northern, Khartoum, and Gezira states. This is might be due to geographical differences, environment, and ethnic background.

These results when compared with the reference values used in Sudanese laboratories which are the international ones we find major differences which can affect the diagnosis. The reference total WBCs count used is  $4-11 \times 10^3/\mu\text{L}$  and when compared to these results a patient with a count of three thousand will be considered leucopenic and another with 11000 will be considered as normal! Leading to misdiagnosis. The present study had some limitations. First, the mean age of participants was younger ( $25.23 \pm 9.64$  years) this is because most of elder participant were excluded due to the chronic diseases. Second, many states were not included in the study. Third the number of females was larger compared with males.

In conclusion the reference values of WBCs count in adult Sudanese is lower than the international one and these values should be referred to in Sudanese laboratories. A larger national scale should be performed to overcome the limitations.

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#### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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