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Correlations of Glycosylated Hemoglobin with White Blood Cells and Platelets Counts in Sudanese Non-Diabetics Population

[1]Ibrahim A. Ali and [2]Elmutaz H. Taha
[1]*Department of Physiology, Faculty of Medicine, The National Ribat University, Khartoum, Sudan*
[2]*Department of Physiology, Faculty of Medicine, University of Dongola, Dongola, Sudan*

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Corresponding Author:
Ibrahim Abdelrhim Ali
Department of Physiology, Faculty of Medicine, The National Ribat University, Khartoum, Sudan

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Abstract: Glycated Hemoglobin (HbA_{1c}) is now used largely for DM control. Previous studies showed different relationship of HbA_{1c} with White Blood Cells (WBCs) and platelets counts. The aim of this study was to investigate the correlation of glycated hemoglobin with WBCs and platelets counts in non-diabetic Sudanese population. A cross sectional study was conducted during 2016-2018 in Sudan covering Khartoum state, Northern state, Gezira state, Red Sea state and North Darfur state on adults of ages between 20-60 years and not known to be diabetic or suffering from any chronic illness. The 1096 participants were assessed by a questionnaire and physical examination. BMI and BP were calculated by standard scales. A sample of 5 mL venous blood was taken for FBG to exclude DM and measurement of HbA_{1c} using a modified ELISA reader known as Cobas Integra 800 (Roch) machine. A Sysmex KX-21 automated hematology analyzer was used for measuring WBCs and platelets counts. Correlations between the variables were estimated and $p<0.05$ was considered statistically significant. The mean age of the participants was found to be 25.1±9.2 years with a range of 20-60 years. The overall mean value of HbA_{1c} was 4.6% ±0.9 with a minimum value of 1.2% and maximums of 6.3. At 95% confidence interval the lower bound Mean was 4.5% and the upper bound mean was 4.7%. The reference range of WBCs count in adult Sudanese was 2.9-9.6×103 μL^{-1} with a median of 5.0×103 μL^{-1}. The reference range of Platelets count in adult Sudanese was (124-465)× 103 μL^{-1} with a median of 280×103 μL^{-1}. In overall Sudan samples there was no correlation between WBCs and HbA_{1c} ($r = -0.041$; $p = 0.200$) but in the samples of North Darfur and Northern states the WBCs count was positively correlated with HbA_{1c} with significant p value ($r = 0.185$; $p = 0.016$) and ($r = 0.241$; $p = 0.003$), respectively. In this study statistically significant

correlation was found between platelets count and HbA_{1c} level (r = 139; p = 0.000). The results suggest that WBCs levels need to be taken into account when using HbA_{1c} levels to screen pre-diabetes or diabetes. Thus, measuring Fasting Blood Glucose (FBG) or performing the Glucose Tolerance Test (GTT) recommended to be considered when diagnosing diabetes or pre-diabetes in patients in the upper normal range of WBCs and close to the diagnostic threshold of HbA_{1c} level. Further, study recommended to be done to investigate the level of WBCs and HbA_{1c} in Diabetic patients with measurement of inflammatory marker to ensure whether the association between WBCs level and HbA_{1c} is mediated through elevated glucose levels is caused by subclinical inflammation. The Mean Platelets Volume MPV values can be an effective marker for blood glucose. This exact scientific phenomenon needs further analysis and researches.

INTRODUCTION

Hemoglobin A_{1c} was first separated from other forms of hemoglobin by Huisman and Meyering in 1958 using a chromatographic column[1].

In 1969, Glycated Haemoglobin (HbA_{1c}) was initially identified as an "unusual" haemoglobin in diabetic patients by Samuel Rahbar, then he noticed a significant increase in the level of HbA_{1c} in diabetes[2]. Another cross sectional study conducted later by Rahbar *et al*.[3] at Tehran University found a similar abnormality in 57 diabetic patients.

After that discovery, numerous small studies were conducted correlating the HbA_{1c} level to the blood glucose level resulting in the idea that HbA_{1c} could be used as a positive objective factor to measure the glycaemic control. In a larger study of diabetic patients, Trivelli et al found a two-fold increase of HbA_{1c} over values observed in non-diabetic subjects[4].

Thus, by the mid 1970s, it was clear that HbA_{1c} is elevated in humans with diabetes mellitus, although the mechanism of this abnormality was not understood.

In 1975, Bunn *et al*.[5] described the reactions that lead to formation of HbA_{1c}, so, the natureof the chemical reaction had been explained. Glycation is as pontaneous non-enzymatic reaction in which glucose bindscovalently with haemoglobin at amino terminal of the globin chain.

In 1976, Anthony Cerami proposed the idea to use HbA_{1c} level for monitoring the degree of control of glucose metabolism in diabetic patients, then described HbA_{1c} as a useful mean for monitoring the glycaemic control in diabetic patients[6]. HbA_{1c} was introduced into clinical use in the 1980s and subsequently has become an important test in Clinical practice[7].

In 2017, Vinupritha *et al*.[8] performed a study aimed to identify the variations of the Complete Blood Count (CBC) parameters among the diabetic and normal individuals and to derive an empirical formula to estimate HbA_{1c} of an individual using CBC parameters. They found a negative correlation for Hb and Packed Cell Volume (PCV) against HbA_{1c}. The Hb, Erythrocyte Sedimentation Rate (ESR), PCV, red blood cells count, mean corpuscular volume and mean corpuscular Hb exhibited a statistically significant difference at the level between the normal and diabetic groups.

In 2017 Hussein *et al*.[9] performed a study aimed to compare the platelets indices in pregnant women with and without Gestational DM and to evaluate the relationship between Mean Platelets Volume MPV and HbA_{1c}. They found that MPV value was significantly higher in GDM group than normal pregnancies. Moreover, there was a positive correlation between MPV and HbA_{1c} values.

The aim of this study was to investigate the correlations of white blood cells and platelets counts levels in relation with glycaemic control of non-diabetic Sudanese adults.

MATERIALS AND METHODS

A cross sectional study was conducted during 2016-2018 in concomitant with another study on Healthy Sudanese subjects of both sexes with age group 20-60 years. The 1163 Healthy Sudanese adult volunteers were included and the study covered Khartoum state, Northern state, Gezira state, Red Nile state and North Darfur state.

The exclusion criteria of this study included: Pregnant ladies, abnormal Fasting Blood Glucose (FBG), Diabetes mellitus, Hypertension , Renal failure, Liver disease, Cancer, Chronic diseases (cardiac diseases, TB, asthma, thyroid disorders), Hematological disorders , Recent acute diseases (Malaria, typhoid fever..), Lactation., History of recent surgery or splencetomy, History of schistosomiasis, hemoglobinopathies, blood disorders and Subjects not consenting. Written consents were obtained from all participants after fully explaining to them the project.

A questionnaire was filled by all volunteers to obtain the data about name, age, address, medical history and drug use, dietary intake in the previous month, physical activityand lifestyle. Weight, height and blood pressure were measured with standard techniques. Complete clinical examination was performed.

After informed consent; 5 mL of venous blood was collected by a standard procedure from each participant under complete aseptic conditions in the morning and after an overnight fasting 0.2.5 mL was placed in fluoride oxalate containers and then used for FBG measurement with auto analyzer A 15. The remaining 2.5 mL was

placed in EDTA container and used for HbA_{1c} analysis. (Icteric, lipemic, hemolyzed or bacterially contaminated samples were not used).

HbA_{1c} was measured using modified ELISA reader known as COPAS Integra 800 using commercial reagent kits from Roche Company. A Sysmex KX-21 automated hematology analyzer was used for measuring WBCs and platelets counts. All techniques and equipment were standardized. All data collected in this study was analyzed using the SPSS computer programs. Correlation Coefficient (r) was used for continuous numerical variables and student t test and (ANOVA) statistics was used for categorical variables. The $p \leq 0.05$ was considered significant.

Ethical consideration: Ethical Approval of this study was obtained from the Federal Ministry of Health in Sudan (FMOH) and The National Ribat University (NRU). The objectives of the study were explained to all individuals participating in the study. An informed consent was obtained from each participant in the study

RESULTS

A total of 1096 volunteers were identified as eligible; according to the inclusion criteria and approved to be enrolled after filling the consent, questionnaire and were fit on the physical examination. About 67 subjects (5.7%) were excluded due to high Fasting Blood Glucose (FBG) and they were not known to be diabetics. About 808(73.7%) of the study sample were females and 288(24.8%) were males (Table 1).

The mean age of the participants was found to be 25.1±9.2 years with a range of 20-60 years. The mean of BMI was found to be 22.8±4.8 with a minimum of 18.5 and maximum of 51.4 (Table 2) .

The overall mean value of HbA_{1c} was 4.6% ±0.9 with a minimum value of 1.2% and maximums of 6.3. At 95% confidence interval the lower bound Mean was 4.5% and the upper bound mean was 4.7% as shown in Table 2.

The reference range of WBCs count in adult Sudanese was 2.9-9.6×103 μL^{-1} with a median of 5.0×103 μL^{-1}. The reference range of Platelets count in adult Sudanese was (124- 465)×103 μL^{-1} with a median of 280×103 μL^{-1} (Fig. 1). There was no correlation between HbA_{1c} with WBCs. There was correlation between HbA_{1c} and Platelets count with significant p = 0.00 (Table 4).

DISCUSSION

HbA_{1c} measurement is one of the diagnostic tests used in the diagnosis of diabetes and monitoring hyperglycemia in uncontrolled diabetic patients. HbA_{1c} is a relevant predictor of diabetes related complications and of mortality[10, 11].

Table 1: Distribution of studied population according to gender in the study

Gender	Frequencies	Percentage
Male	272	24.8
Female	808	73.7
Missing	16	1.5
Total	1096	100.0

Table 2: Descriptive statistics of age, BMI, pulse rate, SBP, DBP, FBG and HbA_{1c} of the studied population (n = 1096)

Test	Mean	SD	SE of mean	Median	Minimum	Maximum
Age	25.1	9.2	0.27	21.0	20	60
BMI	22.8	4.8	0.15	22.0	0.24	51.4
HbA_{1c}	4.6	0.98	0.03	4.75	1.2	6.3

Table 3: Mean of WBCs counts in Sudanese population in this study

Parameter	Mean for Sudanese (μL^{-1})
WBCs count	5.103
Male WBCs count	4.969
Female WBCs count	5.138

Table 4: HbA_{1c} correlations with white blood cells and platelets counts in Sudanese population

HbA_{1c}	WBCs	PLT
Pearson correlation	-0.041	0.139**
Sig. (2-tailed)	0.200	0.000

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed)

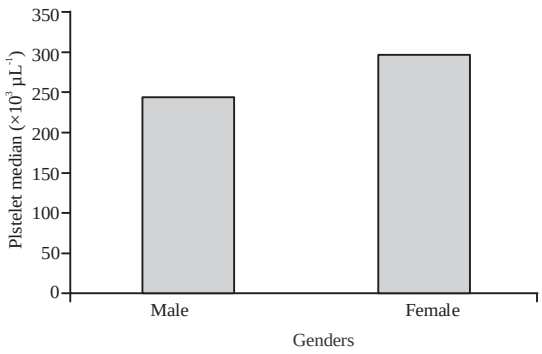

Fig. 1: Mean of platelets counts in Sudanese population in this study

As HbA_{1c}, white blood cells and Platelets counts is now used largely for infections and DM control, the correlations of HbA_{1c} with this blood values have been addressed by this study.

The reference values of HbA_{1c} currently used in Sudan have been adopted from textbooks or guidelines referring mainly to European or American populations; but the normal Sudanese reference intervals of hematological values[12-15], respiratory function tests parameters[16] renal functions test[17], serum electrolytes[18] and mean of glycated Hemoglobin (HbA_{1c})[19-23] have been found to be different from Caucasian reference values.

The purpose of this study was to establish the normal reference range for HbA_{1c} in Sudanese non-diabetic adults. Evermore, the study was designed to see the influence of white blood cells and platelets counts on the HbA_{1c} level.

In Sudan there was no correlation between WBCs and HbA_{1c} ($r = -0.041$; $p = 0.200$) but in the samples of North Darfur and Northern states the WBCs count was positively correlated with HbA_{1c} with significant p value ($r = 0.185$; $p = 0.016$) and ($r = 0.241$; $p = 0.003$), respectively.

This finding agreed with Hong *et al.*[24] study and Jiang *et al.*[25] who found HbA_{1c} levels were positively associated with WBCs levels within normal range in a general adult Korean and Chinese population.

In this study statistically significant correlation was found between platelets count and HbA_{1c} level ($r = 139$; $p = 0.000$). This finding is in agreement with Hussein *et al.*[9] study in which they reported positive correlation between platelets count and HbA_{1c} in normal pregnant ladies.

CONCLUSION

This results suggests that WBCs levels need to be taken into account when using HbA_{1c} levels to screen pre-diabetes or diabetes. Thus, measuring FBG or performing the GTT recommended to be considered when diagnosing diabetes or prediabetes in patients in the upper normal range of WBCs and close to the diagnostic threshold of HbA_{1c} level.

Further study recommended to be done to investigate the level of WBCs and HbA_{1c} in Diabetic patients with measurement of inflammatory marker to ensure whether the association between WBCs level and HbA_{1c} is mediated through elevated glucose levels is caused by subclinical inflammation. The Mean Platelets Volume MPV values can be an effective marker for blood glucose. This exact scientific phenomenon needs further analysis and researches.

This study has several strengths. First, this is the first large population-based study to investigate the reference value of HbA1c level in Sudan. The study was performed in a large sample size and in different states, selection of participants to fit the inclusion criteria was done through meticulous methods; history, physical clinical examinations and investigations and according to IFCC guidelines. Advanced laboratory standardized machines and techniques were used in this study. Our limitation was the children, age above 60 and age below 20 were not included.

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