Glycated hemoglobin and red blood cell indices in non-diabetic pregnant women

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Abstract

Hemoglobin A1c (HbA1c) is affected by the red blood cell (RBC) lifespan. The association of HbA1c with RBC indices in pregnant women is not widely investigated. In this study, we investigated the association between HbA1c and Hb/RBCs count and RBC indices; specifically, Hb/RBCs count and indices in non-diabetic pregnant women. Across-sectional study was carried-out at the antenatal care of Saad Abu Elela Hospital, Khartoum, Sudan. Obstetrics history was gathered using questionnaire and body mass index was calculated. Fasting blood sugar, at one hour, at two hours post prandial and HbA1c were investigated. Complete blood count parameters of RBCs count was performed, including, hematocrit, Hb, RBCs indices of MCV, MCH, and MCHC. One hundred twenty three women were enrolled. The mean age of the participants was 28±5.6 years and a mean body mass index was 27.65±6.8 kg/m². There was significant positive correlation between HbA1c and Hb (r=0.174, P=0.037), Hct (r=0.174, P=0.037), and MCHC (r=0.180, P=0.031). A negative correlation between HbA1c and the platelet index PDW (r=−0.198, P=0.017) was documented. The rest of the other factors showed no correlation with HbA1c. In linear regression analysis, HbA1c correlated positively with Hb (P=0.044) and Hct (P=0.047). The present study shows a significant positive correlation between HbA1c value with Hb, Hct, and MCHC. No significant correlation between HbA1c and other RBCs parameters was observed.

Introduction

HaemoglobinA1c (HbA1c) is a glycat- ed form of hemoglobin (Hb) that is produced as a result of non-enzymatic catalysis of the β chain of globin in mature Hb.1 The nature of the reaction between the Hb and glucose is a slow and irreversible.2,3 HbA1c is the predominant glycated Hb found in HbA1 fractions and maintained in the whole lifespan (120 day) of red blood cells (RBCs).2 The percentage value of HbA1c is positively related to the concentration of glucose in the blood. If there is an increase in the glucose level, a high value of HbA1c will be obtained. Normally, 4 to 6 RBCs in 100 have glucose attached to their globin chain; therefore the normal range of HbA1c in a healthy person is 4 to 6%. HbA1c can measure the average blood glucose level in the past 3 months. Hence, the percentage of HbA1c is mainly determined by the level of plasma glucose and the life span of the RBCs.2,3

The value of HbA1c is affected by three main factors: the Hb content of reticulo- cytes when they are released from the bone marrow, the mean age of RBCs in the circulation and the Hb glycation rate.5,6

However, there are many pathological conditions other than hyperglycemia that can interfere with HbA1c values. A low level of HbA1c may be seen in conditions that shorten the RBCs lifespan, irrespective of the method of the assay used.2 Anemia is a common problem worldwide and has higher prevalence among pregnant females as is estimated to be about 38% compared to 29% of non-pregnant women.7

Anemia can increase the RBCs turnover and lower HbA1c values as shown in blood loss, hemolysis, hemoglobinopathies, red cell disorders and myelodysplastic disease. On the other hand a reduction in RBCs turnover is accompanied with an increase in the glycation rate of Hb and ultimately will lead to high value of HbA1c.8

Essentially, the RBC survival term and its exposure to blood glucose are the determinants of glycated hemoglobin production. Based on this fact, HbA1c is not suitable for the diagnosis of diabetes in subjects with anemia, hemoglobinopathy and those, at risk of altered red cell survival.9 On the other hand, the age of the red blood cells is positively correlated with the mean corpuscular hemoglobin concentration (MCHC) and is negatively correlated with mean corpuscular volume (MCV) and hemoglobin content (mean corpuscular hemoglobin; MCH) due larger proportion of water loss compared to hemoglobin during survival age.16

RBCs indices; MCV is a measure of the volume of RBCs; MCH is a measure of Hb content in RBCs. It has a significant and an important role in the clinical practice. The red cell distribution width (RDW) measures the heterogeneity of the volume of RBCs (variation in cells size) that may help to differentiate between some types of anemia.11 Recent studies, documented a significant associations between an increased RDW and some clinical disorders, besides predicting mortality.2,12 Atrial fibrillation and heart failure are associated with high value of RDW,13,14 as well as adverse prognosis in patients with heart failure or coronary heart disease.15,16 The reasons to explain this association remain unclear.17 In one study, that recruiting healthy non-diabetic participants in the NHANES, a significant positive correlation between HbA1c and RDW was confirmed. Moreover, They proposed chronic hyperglycemia as the culprit for the association between high RDW and cardiovascular disease.18

Hyperglycemia has different effects on the RBC count. It is observed to be high in cases with pre-diabetes and low among those with established diabetes mellitus as compared to normal glucose homeostasis.19 The effects may be extended to include glycation of Hb, reduced the RBCs deformability,20,21 and lifespan.22-24 The exact degree of significant changes in the erythrocytes indices around the diagnostic value of HbA1c of 6.5% (48 mmol/mol) is not known yet nor the degree of severity.6

Thus, this study used the above-men- tioned principle to verify the influences of HbA1c values on blood count parameters; Hb/RBCs count and its indices, in non-diabetic pregnant women.

Materials and Methods

A cross-sectional study was conducted

Key words: Glycated hemoglobin; red blood cell; diabetes; pregnancy; Sudan.

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at Saad Abu Elela maternity hospital, Khartoum, Sudan during the period of August-November 2015. We investigated HbA1c level and parameters of complete blood count in a group of healthy pregnant women (No/123) who were seen at antenatal care unit. For exclusion of diabetes, the recruited subjects underwent a 75-g oral glucose tolerance test (OGTT), and impaired glucose tolerance test was diagnosed based on the World Health Organization (WHO) criteria The following criteria were used to diagnose patients with diabetes mellitus as per the American Diabetic Association (ADA): hemoglobin A1c (Hb A1c) ≥6.5% or fasting blood sugar (FBS) ≥126 mg/dL or 2-h plasma glucose ≥200 mg/dL during an oral glucose tolerance test (OGTT). The demographic and clinical data of each enrolled case were recorded in a pre-designed a questionnaire. The study was approved by the Ethical committee at the Department of Obstetrics and Gynecology, Medical College, University of Khartoum, Sudan. All cases gave written informed consent. We excluded women with lipid disorders, renal disease, hemolytic anemia/thalassemia/Hb variants, use of corticosteroids, recurrent miscarriage, preeclampsia, preterm birth (<34 weeks) and/or a stillbirth in a previous pregnancy. General physical and clinical examination was performed at Hospital.

Anticoagulated venous blood sample (5 mL) was collected, mixed gently, and was sent immediately after collection for laboratory investigations. Complete blood count by automated blood cell counter, Sysmex KX-21 was performed. HbA1c also was measured by enzymatic digestion (Abbott Chemistry) using colorimetric reaction.

Statistical analysis

Data were entered to an SPSS version 16 for Windows and was subsequently analyzed via it (Statistical Package for Scientific Studies). Data were statistically presented as means and standard deviations (SD) and in the case of non-normally distributed data, median and interquartile was used. After testing for normality of the data distribution with Shapiro-Wilk test, some of the variables were found to be not normally distributed. Correlation between the different variables was tested for, using Pearson moment correlation coefficient. Then the significant factors (if P value less than 0.05) affecting HbA1c (which was not normally distributed) were entered in linear regression analysis where HbA1c was the dependent variable and Hb, Hct, MCHC, PDW were the independent variables. A P value less than 0.05 was regarded statistically significant.

Results

The study recruited 145 pregnant Sudanese women but 22 were excluded because of incomplete data. Fifteen of the participants showed an impaired GTT. The mean age of the participants was 28±5.6 years, the mean gestational age was 26.6 (4.7) weeks, the mean body mass index of 27.65±6.8 k/m². The rest of the study group characteristics are shown in Table 1.

There was a significant positive correlation between HbA1c and Hb (r=0.174, P=0.037), Hct (r=0.174, P=0.037), and MCHC (r=0.180, P=0.031). There was a negative correlation between HbA1c and PDW (r= -0.198, P=0.017). The rest of the factors showed no correlation with HbA1c as seen Table 2. In linear regression analysis after log transformation, HbA1c the only 2 variables found to weakly predict HbA1c were Hb (P=0.044) and Hct (P=0.047) while the other two were found to be confounders. The regression model was HbA1c =23.45xHb-4.43xHct.

Discussion

HbA1c measurement is one of the diagnostic tests used in the diagnosis of diabetes and monitoring/managing hyperglycemia in uncontrolled diabetic patients. HbA1c is a relevant predictor of diabetes related complications and of mortality.25 The red blood cells indices are subjected to changes in conditions affecting the physiology of RBCs as seen in many types of anemias.

The current study showed a significant positive correlation between hematocrit (Hct), Hb, and MCHC values and HbA1c levels. (r=−0.174, P=0.037). Our findings are supported by one study that showed same observation between erythrocyte indices and HbA1C among both healthy pregnant and non-pregnant groups of participants.26 This also goes with the outcome in one study recruiting subjects with diabetes mellitus when were compared to control group.27 One Japanese study conducted on diabetic and non-diabetic pregnant females, confirmed a positive correlation between HbA1C and MCV and negative association with red blood cells count.28 On the same line, another study from Japan, evaluating a similar group of pregnant ladies with diabetes mellitus, documented a significant inverse correlation with MCH.29 Some studies enrolling individuals with diabetes mellitus, obtained negative relationship between HbA1C and red blood indices: Hb, MCV and MCH.32-30 Koga et al. showed a positive correlation between HbA1c and

| Table 1. Socio-demographic characteristics. |
|----------------------------------|
| **Mean (SD)**                  |
| Age, weeks                      | 26.6 (4.7) |
| Body mass index, k/m²           | 24.3 (2.2) |
| **Number (%)**                 |
| Primipara                       | 31 (35.6)  |
| Antenatal care within three time| 62 (71.3)  |
| Educational level ≤secondary    | 17 (19.5)  |
| Rural residency                 | 61 (70.1)  |
| Impaired GTT                    | 15 (0.12)  |
| Employment                      | 29 (0.24)  |
| **Median (interquartile)**      |
| Fasting blood glucose, mg/dL    | 70 (63-78.5) |
| Two hour blood glucose, mg/dL   | 11 (497-133) |
| Hemoglobin A1c%                 | 10.9 (10.4-11.5) |
| Hematocrit %                    | 35.5 (33.8-37.4) |
| MCV/µL                          | 82.8 (79.5-87.3) |
| MCH µg                          | 25.6 (24.7-27.4) |
| MCHC µg/µL                      | 30.6 (29.8-31.5) |
| RDW%                            | 13.6 (12.8-14.2) |
| MPV/µL                          | 8.1 (7.6-8.3) |

| Table 2. Showing the correlation between HbA1c and the different variables. |
|-----------------|-----------------|
| **HbA1c**      | P value         |
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| Age            | r=0.091         | 0.280 |
| BMI            | r=0.069         | 0.414 |
| Gravidity      | r=−0.061        | 0.471 |
| Parity         | r=−0.049        | 0.562 |
| Hb             | r=0.174         | 0.037 |
| FBS            | r=0.135         | 0.067 |
| 1 hPP          | r=0.155         | 0.067 |
| 2 hPP          | r=0.093         | 0.270 |

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RBCs count, besides a negative association with other erythrocytes indices (Hb, MCV, MCH) in pre-menopausal women. The same findings were reported in a study that was conducted by Hardikar et al. who assessed a non-diabetic population to show an inverse correlation between HbA1c and MCV, MCH and MCHC.

Anemia is an important factor that can affect HbA1c and erythrocytes indices in all subjects including pregnant women. This was strengthened by Sinha et al. who reported a significantly lower mean HbA1c value in a group of anemic patients compared that in control group. In addition to that, they observed a significant increase in the patients’ absolute HbA1c values after 2 months of treating iron deficiency anemia.32 Anemia during pregnancy in Sudan is known to be prevalent, where pregnant women are more prone to anemia (especially iron-deficiency anemia).33-35 Hence using HbA1c as a diagnostic tool for gestational diabetes may result in under diagnosing gestational diabetes. However, Koga et al. pointed to an increase in the glycation fraction in conditions with low MCH as a result of a decrease in the hemoglobin concentration.30 These facts may affect HbA1c as a diagnostic tool among such conditions, including pregnancy. Recently, some studies promoted HbA1c as screening tool for gestational diabetes at cut off value 5.1% with reasonable sensitivity (61% to 85.7%), specificity (61.1% to 68%) and higher negative predictive value (91% to 93%).36,37 The advantages of this test are more convenient to the patients, saving time and reducing financial load. The significant changes in the red blood cells indices may harbor an important predictive role. Jabeen and associates promoted an elevated level of erythrocyte indices as potential indicators to predict the risk of developing micro and macro vascular complications in diabetic patients. On the same issue, RDW has the same prognostic value despite the lack of significant association in the current study. RDW is considered to a new chronic inflammation mediator and a high-risk factor of pregnancy hypertension disease, besides being a predictor for its occurrence.38

Anemia may influence erythropoiesis; there may be less RBCs production, Hb synthesis and the RBCs volume or surface area also may be diminished. Anemia also can increase the rate of RBCs turnover and this itself can affect HbA1c value.

In general, features of iron deficiency anemia involve a reduction in RBCs indices e.g. MCV, MCH, and MCHC.39 Thus, as the Hb content inside a single RBC is reduced due insufficient amounts of iron, subse-

Conclusions

The present study shows a statistically significant positive correlation between Hb, Hct, and MCHC with HbA1c values. No significant correlation between HbA1c and other RBCs parameters was observed. More studies in this issue may help to explain the correlation between HbA1c and RBCs indices.

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