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 RBCs 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 k/m^2. 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 glycated 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).[4] 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 reticulocytes 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 HbAIc.[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, hemogloninpathy 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.[10]

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.[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-mentioned 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

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at Saad Abu Elela maternity hospital, Khartoum, Sudan during the period of August-November 2015. We investigated HbAIc 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/m^2. 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 see 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+4.5xHb-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.[27,29] 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/m^2	24.3 (2.2)
Number (%)	
Primipara	31 (35.6)
Antenatal care ≤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 A 1c%	4.6 (3.9-5.1)
Hemoglobin g/dL	10.9 (10.4-11.5)
Hematocrit %	35.5 (33.8-37.4)
MCV fL	82.8 (79.5-87.3)
MCH pg	25.6 (24-27.4)
MCHC g/dL	30.6 (29.8-31.5)
RDW%	13.6 (12.8-14.2)
MPV fL	8.1 (7.6-8.3)

Table 2. Showing the correlation between HbA1c and the different variables.

	HbA1c	P value		HbA1c	P value
Age	*r =0.091*	0.280	MCHC	*r =0.180*	0.031
BMI	*r =0.069*	0.414	MCH	*r =0.119*	0.154
Gravidity	*r = –0.061*	0.471	RDW	*r = –0.141*	0.091
Parity	*r = –0.049*	0.562	MPV	*r = –0.125*	0.134
Hb	*r =0.174*	0.037	PDW	*r = –0.198*	0.017
FBS	*r =0.135*	0.106	WBC	*r = –0.140*	0.094
1 hPP	*r =0.155*	0.067	Hct	*r =0.174*	0.037
2 hpp	*r =0.093*	0.270	MCV	*r =0.95*	0.256

RBCs count, besides a negative association with other erythrocytes indices (Hb, MCV, MCH) in pre-menopausal women.[30] 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.[31]

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.[27] 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, subsequently the concentration of the Hb in the total mass of the RBCs will be reduced too and this will affect HbA1c value.

This probably may explain the positive correlation between Hb, and MCHC with HbA1c in our findings.

The total mass of RBCs also is reduced in anemia and this leads to a reduction in the volume occupied by RBCs as they are measured in hematocrit; the later will be decreased in this situation, consequently the Hb content decreases as the mass of RBCs decreases and eventually HbA1c will be affected. This also may explain the positive correlation between Hct and HbA1c in the present study.

These discrepancies in such findings could be due to different analytical methods, sample criteria, and race/ethnic differences in populations studied as HbA1c can vary according to these factors.

Further studies containing large sample size are needed in to explain how RBCs parameters influence HbA1c levels. Once the correlation is established these parameters may be considered while interpreting HbA1c results.

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.

References

1 Konnert A, Arends S, Schubert S, et al. Uncertainty calculation for calibrators of the IFCC HbA1c standardization network. Accredit Qual Assur 2006;11:319-28.
2 Cohen Robert M, Franco Robert S, Khera Paramjit K, et al. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. Blood 2008;112:4284-91.
3 Higgins T. HbA1c - An analyte of increasing importance. Clin Biochem 2012;2012:1038-45.
4 Nitin S. HbA1c and factors other than diabetes mellitus affecting it. Singapore Med J 2010;51:616-22.
5 WHO. WHO - Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Abbrev Rep WHO Consult 2011;1-25.
6 English E, Idris I, Smith G, et al. The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review. Diabetologia 2015;1409-21.
7 Stevens Gretchen A, Finucane Mariel M, De-Regil Luz M, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. Lancet Glob Heal 2013;1:16-25.
8 Franco Robert S. The measurement and importance of red cell survival. Am J Hematol 2009;109-14.
9 Church D, Simmons D. More evidence of the problems of using HbA1c for diagnosing diabetes? The known knowns, the known unknowns and the unknown unknowns. J Intern Med 2014;276:171-3.
10 Bosch FH, Werre JM, Roerdinkholder-Stoelwinder B, et al. Characteristics of red blood cell populations fractionated with a combination of counterflow centrifugation and Percoll separation. Blood 1992;79:254-60.
11 Montagnana M, Cervellin G, Meschi T, Lippi G. The role of red blood cell distribution width in cardiovascular and thrombotic disorders. Clin Chem Lab Med 2012;50:635-41.
12 Perlstein TS, Weuve J, Pfeffer MA, Beckman JA. Red blood cell distribution width and mortality risk in a community-based prospective cohort. Arch Intern Med 2009;169:588-94.
13 Adamsson ES, Borné Y, Melander O, et al. Red blood cell distribution width is associated with incidence of atrial fibrillation. J Intern Med 2014;275:84-92.
14 Borne Y, Smith JG, Melander O, et al. Red cell distribution width and risk for first hospitalization due to heart failure: a population-based cohort study. Eur J Heart Fail 2011;13:1355-61.
15 Tonelli M, Sacks F, Arnold M, et al. Relation between red blood cell distribution width and cardiovascular event rate in people with coronary disease. Circulation 2008;117:163-8.
16 Felker GM, Allen LA, Pocock SJ, et al. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. College of Cardiology 2007;50:40-7.
17 Horne BD. A changing focus on the red cell distribution width: why does it predict mortality and other adverse medical outcomes?. Cardiology 2012;122:213-5.

18 Veeranna V, Zalawadiya SK, Panaich SS, et al The association of red cell distribution width with glycated hemoglobin among healthy adults without diabetes mellitus. Cardiology 2012;122:129-32.
19 Simmons D. Increased red cell count in diabetes and pre-diabetes. Diabetes Res Clin Pract 2010;90:3.
20 Singh DK, Winocour P, Farrington K. Erythropoietic stress and anemia in diabetes mellitus. Nat Rev Endocrinol 2009;5:204-10.
21 Singh M, Shin S. Changes in erythrocyte aggregation and deformability in diabetes mellitus: a brief review. Indian J Exp Biol 2009:7-15.
22 Virtue Mark A, Furne Julie K, Nuttall Frank Q, Levitt Michael D. Relationship between GHb concentration and erythrocyte survival determined from breath carbon monoxide concentration. Diabetes Care 2004;27:931-5.
23 Venerando B, Fiorilli A, Croci G, et al. Acidic and neutral sialidase in the erythrocyte membrane of type 2 diabetic patients. Blood 2002;99:1064-70.
24 Peterson CM, Jones RL, Koenig RJ, et al. Reversible hematologic sequelae of diabetes mellitus. Ann Intern Med 1977;86:425-9.
25 Tennyson C, Lee R, Attia R. Is there a role for HbA1c in predicting mortality and morbidity outcomes after coronary artery bypass graft surgery? Interact Cardiovasc Thorac Surg 2013;17:1-9.
26 Rafat D, Rabbani TK, Ahmad J, Ansari MA. Influence of iron metabolism indices on HbA1c in non-diabetic pregnant women with and without iron-deficiency anemia: effect of Iron supplementation. Diabetes Metab Syndr Clin Res Rev 2012 [Epub ahead of print].
27 Farah J, Husan AR, Farha AAZW. Hyperglycemic induced variations in Hematological indices in type 2 diabetics. Int J Adv Res 2013;1:322-34.
28 Hashimoto K, Osugi T, Noguchi S, et al. A1C but not serum glycated albumin is elevated because of iron deficiency in late pregnancy in diabetic women. Diabetes Care 2010;33:509-11.
29 Moriya T. Hemoglobin A1C but not glycated albumin overestimates glycemic control due to iron deficiency in pregnant women with diabetes. J Diabetes Metab 2014;5.
30 Koga M, Morita S, Saito H, et al. Association of erythrocyte indices with glycated haemoglobin in premenopausal women. Diabet Med 2007;24:843-7.
31 Hardikar PS, Joshi SM, Bhat DS, et al. Spuriously high prevalence of prediabetes diagnosed by HbA 1c in young Indians partly explained by hematological factors and iron deficiency anemia. Diabetes Care 2012;35:797-802.
32 Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. Ann Lab Med 2012;32:17-22.
33 Abdelgadir MA, Khalid AR, Ashmaig AL, et al. Epidemiology of anaemia among pregnant women in Geizera, central Sudan. J Obstet Gynaecol 2012;32:42-4.
34 Adam I, Elhassan EM, Haggaz AE, et al. A perspective of the epidemiology of malaria and anaemia and their impact on maternal and perinatal outcomes in Sudan. J Infect Dev Ctries 2011;5:83-7.
35 Adam I, Khamis AH, Elbashir MI. Prevalence and risk factors for anaemia in pregnant women of eastern Sudan. Trans R Soc Trop Med Hyg 2005;99:739-43.
36 Khalafallah A, Phuah E, Al-Barazan AM, et al. Glycosylated haemoglobin for screening and diagnosis of gestational diabetes mellitus. BMJ Open 2016;6:e011059.
37 Rajput R, Yogesh Y, Rajput M, Nanda S. Utility of HbA1c for diagnosis of gestational diabetes mellitus. Diabetes Res Clin Pract 2012;98:104-7.
38 Sen-Yu W, Chao X. Assessment of the relationship between red blood cell distribution width and preganecy hypertension disease. J Obs Gynaecol Res 2016;42:13067.
39 Gifford SC, Derganc J, Shevkoplyas SS, et al. A detailed study of time-dependent changes in human red blood cells: From reticulocyte maturation to erythrocyte senescence. Br J Haematol 2006;135:395-404.