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Effect of Microcytosis on HbA1c Values in Non-Diabetic Persons

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Abstract

Background: Glycated haemoglobin is a necessary test for assessment of control, compliance, modifying management, follows up and now-a-days for diagnosis of patients with prediabetes and diabetes mellitus. Microcytosis, which is a laboratory finding that found in a wide range of common diseases, appear to be found in a significant percent of diabetic and non-diabetic persons and may affect the measured glycated haemoglobin values.

Aim of study: This study tries to evaluate the effect of microcytosis in non-diabetic subjects on the level of haemoglobin A1c (HbA1c %).

Patients and Methods: HbA1c% measured in 80 non-diabetic patients with microcytosis after exclusion of factors that may affect the results compared to 80 healthy subjects with normocytic blood picture as a control group.

Results: The HbA1c level was significantly higher among patients with microcytosis as compared to normocytic control group. The mean HbA1c (5.8± 0.9%) in the patients was higher than that in the control group (5.3% ± 0.7) (p=0.001).

Conclusions: Microcytosis may affect significantly the HbA1c results and may wrongly affect management decisions in diabetic patients.

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Introduction

Hemoglobin (Hb) is a tetramer composed of two α (141 amino acid) and two β (146 amino acid) globin chains that bind four heme groups, each containing one iron atom. It founds in erythrocytes and it is critical for normal oxygen delivery to the tissues. Glycated hemoglobin or hemoglobin A1 represents a post-translational modification of hemoglobin A formed by the covalent attachment of glucose or other sugars to hemoglobin. Hemoglobin A1c (HbA1c) is formed by glucose attachment to the valine in the N-terminal of the β globin chain [1].

In general, all amino groups of the side chains of proteins can become glycated; however, many factors have an important impact on the concentrations of the final glycation end products: (a) the concentration of the protein, (b) the steric accessibility of the different side chain amino groups, (c) the glucose concentration in the compartment of the reaction, and (d) the lifespan of the glycated protein [2].

HbA1c% is currently commonly used marker for the determination of the glycemic status in people with diabetes and it is frequently used to guide therapy and especially medical treatment of people with diabetes. The measurement of HbA1c has reached a high level of analytical quality and, therefore, this biomarker is currently also suggested to be used for the diagnosis of diabetes [2]. In its 2010 Clinical Practice Recommendations, the American Diabetic Association (ADA) included an HbA1c of 6.5% (48 mmol/mol) or greater as one of the diagnostic criteria for diabetes, and 5.7-6.4% for prediabetics [3].

Regarding various disease entities that can affect the measurement, many studies have suggested that anemia, hemoglobinopathy, chronic liver disease, renal disease, and rheumatoid arthritis can infuence HbA1c and this influence is variable depending on the pathogenesis of the disease [4].

In Iraq, The prevalence of anemia among adolescents in High Socioeconomic Areas was 12.9% compared with 17.6% in Low Socioeconomic Areas. that also predict a high incidence of microcytosis [5].

Microcytosis defined as a mean corpuscular volume (MCV) of less than 80 μm^3 (80 fL) in adults. It is typically an incidental finding in asymptomatic patients who have a complete blood count for other reasons. The most common causes of microcytosis are iron deficiency anemia and thalassemia trait [6].

The concentration of HbA1c affected by the life span of erythrocytes,

also nutritional deficiencies are a major factor affecting erythrocyte survival. Among these, iron deficiency is the most common and affects >50% of the world's population during their life [7].

Evidence has accumulated, which supports the hypothesis that the glycation reaction, apart from the traditional chronic hyperglycemia, can be modulated by the iron status of the patient. If the degree of glycation of other proteins in anemic patients was similar to that of the glycated hemoglobin, it would have important clinical implications [8].

Other studies have shown that iron deficiency increases the erythrocyte survival and therefore disproportionately elevates HbA1c concentrations at a given glycemic level [9,10].

World Health Organization (WHO) and ADA have acknowledged that there is a limitation in using of HbA1c in the diagnosis of pre-diabetes and diabetes in nutritionally compromised populations, but they cannot assess the magnitude of this effect [11].

Aim of Study

The aim of this study is to evaluate the effect of microcytosis on the level of HbA1c % in non-diabetic patients.

Patients and Methods

This is a Case - control study conducted at AL-Sader Medical city, department of internal medicine, in An- Najaf Al-Ashraf city, during the period from the 15th May 2013 to the 28th February 2014.

Blood samples were obtained from 80 patients with microcytosis regardless for the age and sex and 80 healthy subjects as a control group. The patients with microcytosis were recruited from the Medicine Outpatients Department and visitors of emergency department at AL-Sader medical city. The patients with microcytosis were selected, based on peripheral blood picture (microcytic), and fasting blood glucose levels (FBG). The patients who had glucose tolerance abnormalities (impaired fasting glucose tolerance or diabetes mellitus), pregnancy, chronic renal failure, chronic liver disease and thalasaemia were excluded from the study.

Labratory Investigations

The blood specimens were drawn after an overnight fasting. A Sysmex KX-21automated hematology analyzer was used for the whole blood counts (hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH)); The HbA1c levels were determined by GHb ion-exchange resin using stan bio glycohaemoglobin PRE-FI kits.

For fasting blood glucose, blood urea and serum creatinine Abbott architect plus C4000 auto analyzer used.

Statistical Analysis

By using the statistical package for social sciences (SPSS) software for windows, version 20, data of all participants (cases and controls groups) were entered and analyzed with appropriate statistical tests. Descriptive statistics were presented as mean ± standard deviation (SD) for age, Hb, FBG, HbA1c and MCV, as frequencies (number) and percentages (%) for gender and HbA1c categories. Student's t test was used to compare means in between two groups; chi square test (X2) was used to compare frequencies of categories variables.

Pearson's correlation test and linear regression test was used to assess the correlation between MCV and HbA1c, the correlation coefficient (R) was calculated and the sign of R indicated the direction of correlation, negative sign indicated inverse correlation. Level of significance (P.value) of ≤ 0.05 indicated a significant difference or correlation.

Results

A total of 80 patients with microcytosis and another group of 80 healthy participants as control group were enrolled in this case - control study.

Male represented 45% of the cases and 48.8% of the controls while female represented 55% and 51.2%, respectively (Figure 1).

The mean age of the cases was 31.2 ± 9.3 years, compared to 32.4 ± 9.6 years of controls. The mean FBG of patients group was 87.5 ± 10.0 mg/dl and for controls it was 86.9 ±7.8, however, no statistically significant difference had been found neither in age nor Fasting blood sugar between the studied groups, in both comparison, P>0.05 (Table 1).

The HbA1c levels were significantly higher among the patients with microcytosis as compared to those in the control group. The mean HbA1c (5.8± 0.9%) in the patients was higher than that in the control group (5.3% ± 0.7) (p = 0.001) (Table 2) (Figure 2).

Hemoglobin level was significantly lower in cases group than controls (11.2 ± 1.8) *vs.* (13.2 ± 1.3) gm/dl, respectively. Similarly, the MCV was significantly lower among cases than controls, the mean MCV value was (71.4 ± 7.2) *vs.* (84.6 ± 9.2), respectively.

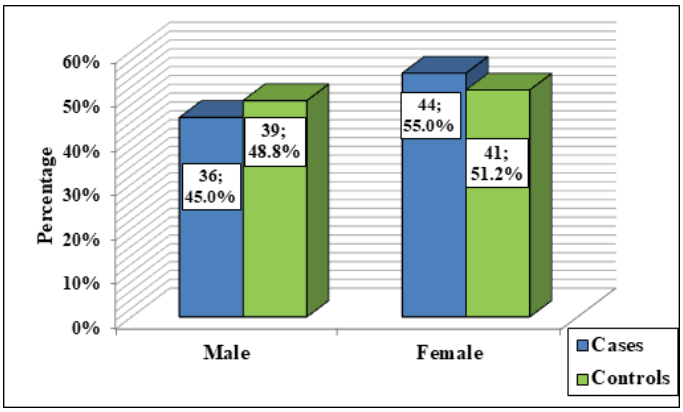

Figure 1: Sex distribution of study groups.

Table 1: Distribution of mean values of studied parameters of patients and control group.

Parameter (mean ± SD)	Cases	Controls	P value
Age (years)	31.2 ± 9.3	32.4 ± 9.6	0.21
FBG (mg/dl)	87.5 ± 10.0	86.9 ± 7.8	0.65
HbA1C (%)	5.8 ± 0.9	5.3 ± 0.7	0.001
Hb (gm/dl)	11.2 ± 1.8	13.2 ± 1.3	<0.001
MCV (fl)	71.4 ± 7.2	84.6 ± 9.2	<0.001

Table 2: Comparison of HbA1 categories between patients and control group.

HbA1c level (%)	Cases		Controls	
	No.	%	No.	%
≤ 5	13	16.3	34	42.5
5.1 - 5.5	15	18.8	21	26.3
5.6 - 6	19	23.8	13	16.3
6.1 - 6.5	15	18.8	12	15.0
> 6.5	18	22.5	0	0.0
Chi square (X2) = 22.9 , P.value < 0.001				

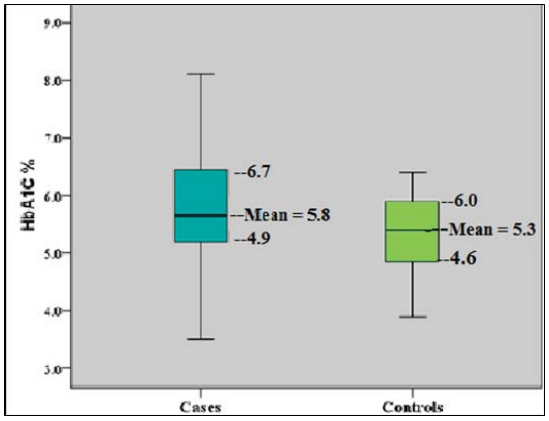

Figure 2: Comparison of mean HbA1c level of cases and control group.

Further analysis, by using the linear regression test, indicates an inverse significant correlation between MCV and HbA1c levels. (R = -0.16, P=0.041) (Figure 3).

On the other hand, the distribution of HbA1c levels into categories revealed the following: 13 patients (16.3%) had HbA1c ≤ 5% *vs.* 34 (42.5%) of the controls, 18 cases (22.5%) had HbA1c > 6.5% while all controls had HbA1c <6.5%, this finding indicates a significant correlation (P<0.001) between microcytosis and higher level of HbA1c, (Table 3).

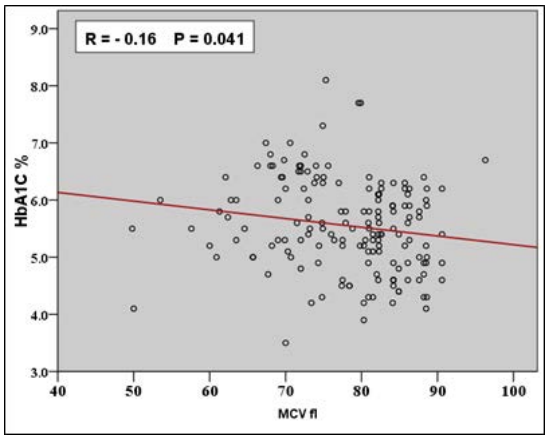

Figure 3: Correlation between HbA1c levels and MCV.

Table 3: Comparison of studied parameters in both genders of cases and control groups.

	Cases			Controls		
Parameter (Mean ± SD)	**Male**	**Female**	**P.value**	**Male**	**Female**	**P.value**
FBG (mg/dl)	90.2 ± 10.0	85.4 ± 9.5	0.031	86.4 ± 7.3	87.3 ± 8.4	0.59
HbA1C (%)	5.6 ± 0.9	5.9 ± 0.9	0.17	5.4 ± 0.7	5.3 ± 0.7	0.41
Hb (gm/dl)	11.8 ± 1.7	10.7 ± 1.6	0.003	13.6 ± 1.3	12.9 ± 1.2	0.017
MCV (fl)	72.1 ± 6.7	70.7 ± 7.5	0.38	84.2 ± 3.1	85.0 ± 2.7	0.20

MCV showed no significant difference between both sexes of both study group, (P>0.05).

Discussion

HbA1c% is currently the most commonly used marker for the determination of the glycemic status in people with diabetes and it is frequently used to guide therapy and especially medical treatment of people with diabetes. It is affected by erythrocyte size, life spine and Hb variants according to many studies [3,11,12]. In this study Microcytosis, defined as a mean corpuscular volume (MCV) of less than 80 μm3 (80 fL) in adults, evaluated as a parameter that may have an impact on HbA1c level [6].

Comparing Fasting blood glucose level (FBG), age and sex between patients with microcytosis and control group yielded no statistically significant difference. HbA1c results, when compared between patients with microcytosis and control group, yielded a high statistical significance (P. value < 0.001) and it was observed that HbA1c values was higher in patient with microcytosis than those of the control group. Same when mean HbA1c was compared between patients with microcytosis and control group where it was (5.8 ± 0.9%) and (5.3% ± 0.7) respectively with P.value < 0.001.

Furthermore, when comparing HbA1c between the two groups revealed that there was no one of the control group had HbA1c value above 6.5% compared to 22.5% of patient group also using the linear regression test indicates an inverse significant correlation between MCV and HbA1c levels. (R=-0.16, P=0.041).HbA1c levels were not significantly different between both genders in both study group, (P>0.05).

Hemoglobin level was significantly lower in patients group than controls, Similarly, the MCV was significantly lower among patients than controls, the mean MCV value was (71.4 ± 7.2) *vs.* (84.6 ± 9.2), respectively. This finding that may be due to selection criteria.

Ryo Maeda et al. had nearly similar findings for this study, regarding HbA1c as they found significant increase in HbA1c % (The HbA1c levels in patients with microcytic anemia were 2.0% higher than those in the control in men and 4.7% higher in women)in non-diabetic subjects with microcytic anemia [13].

The most common causes of microcytosis are iron deficiency anemia and thalassemia trait. Other diagnoses to be considered include anemia of chronic disease and lead toxicity [6].

In addition to average glycemia, factors that affect erythrocyte life span can affect HbA1c concentrations. For a fixed glycemic exposure, shortening of erythrocyte life span decrease HbA1c (hemolytic anemias, infections, acute blood loss, hypersplenism, malaria, and pregnancy), On the other hand, prolongation of erythrocyte survival (iron deficiency, splenectomy, aplastic anemia, and certain hemoglobinopathies) elevates HbA1c concentrations [11, 12].

Iron deficiency anemia and hemoglobinopathies will be discussed here, as they are the major causes for microcytosis, for their effect on HbA1c values. El-Agouza I, et al. (2002) and Coban E, et al. (2004) found that the HbA1c levels were higher in patients with iron deficiency anemia and decreased significantly upon treatment with iron. They assume that could be explained by the fact that if serum glucose remains constant, a decrease in the hemoglobin concentration might lead to an increase in the glycated fraction [9,14]

Sluiter WJ, et al. (1980) proposed that the formation of glycated hemoglobin is an irreversible process and hence, the concentration of HbA1 in one erythrocyte will increase linearly with the cell's age. However, if iron deficiency has persisted for a long time, the red cell production rate would fall, and leading not only to anemia but also to a higher-than-normal average age of circulating erythrocytes and, therefore, increased HbA1 levels and after treatment there will be new erythrocytes production which will decrease the HbA1c values [15].

On the contrary, Rai KB, Pattabiraman TN (1986) was unable

to find a difference in the mean concentrations of HbA1c between nondiabetic patients with IDA and controls [16]. Also the analysis of the National Health and Nutrition Examination Survey (NHANES) did not detect a significant difference in mean HbA1c level according to IDA status [17].

There is a complex interaction between insulin and iron in the body leading to several mechanisms, other than increase erythrocyte survival, may lead to increase HbA1c values in iron deficiency anemia [18-22].

Abbas SA, et al. (2011) study carried on non-diabetic thalasaemic patients, among other groups, stated that the HbA1c level was significantly higher in this group than for the control group. A finding that consistant with this study [23].

Given the global occurrence of both Hb variants and diabetes, it is important to understand the potential impact of Hb variants on RBC survival and ultimately how the most common Hb variants may affect the clinical interpretation of HbA1c test results used in the diagnosis and management of diabetes. Factors known to affect the interpretation of HbA1c results include analytical interferences resulting from the most common Hb variants and the effect of altered RBC turnover [24].

Most HbA1c measurements are performed using either ion exchange high pressure liquid chromatography (HPLC), by boronate affinity column methods or by immunoassays using antibodies against glycated hemoglobin and turbidimetry, for example, as the detection principle. It is important to note that certain routine laboratory methods may give inaccurate results in the presence of pathological hemoglobins variants such as HbC, HbS, HbE or HbD. There are at least two ways in which abnormal Hb may affect HbA1c values, first is the presence of an abnormal peak on ion exchange high pressure liquid chromatography (HPLC), making the estimation of the fraction of HbA1c unreliable (may cause false high results) by this method. Secondly, some abnormal forms of Hb (e.g. Thalassemia and sickle cell trait) make red blood cells more susceptible to hemolysis, Lead to decreased red cell lifespan consequently decreasing the time available for glycosylation of Hb chains. The two effects may coexist; a false increased or decreased HbA1c result may have a substantial influence on the instruction given to attain strict glycemic control [25].

The ADA stated that HbA1c can be used to assess glycemic control in patients with Hb S trait [26]; other groups have expanded this recommendation to include Hb C and Hb D trait [27-29].

Weykamp CW, et al. (1993) compared the HbA1c results of several hemoglobin variants in 102 laboratories using 16 methods of A1c assessment [30]. They found significant differences in results between laboratory and the methods used. For diabetics who are harboring Hb variant(s), proper care needs to be taken by the laboratory while choosing a method to test for A1c which has the least interference. Boronate affinity chromatography can be a method of choice.

Conclusion

Microcytosis significantly affect the accuracy of HbA1c% and may interfere with target level of HbA1c in monitoring and diagnosis in diabetic subjects.

Recommendations

Care must be taken when interpreting HbA1c% results in patients with microcytosis and anemic patients before making decisions concerning patient care. Instead of ion exchange high performance liquid chromatography, use analytic methods that less affected by microcytosis as Boronate affinity chromatography. Or offer alternative methods for assessing average glycemic control over prolonged periods as glycated albumin.

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