EFFECT OF MICROCYTIC ANAEMIA ON GLYCATED HAEMOGLOBIN (HbA1c) IN NON-DIABETIC ADULTS

Gokhularaj Baskar¹, Sundaramurthy Ganesan², Karthikeyan Thanjavur Sethuraman³, Ramesh Bhaskaran⁴

¹Postgraduate Student, Institute of Internal Medicine, Madras Medical College, Chennai.
²Professor, Institute of Internal Medicine, Madras Medical College, Chennai.
³Assistant Professor, Institute of Internal Medicine, Madras Medical College, Chennai.
⁴Assistant Professor, Institute of Internal Medicine, Madras Medical College, Chennai.

ABSTRACT

BACKGROUND
Glycated haemoglobin (HbA1c) is used as a gold standard for monitoring glycaemic control of patients and is used as a predictor of diabetes-related complications. The HbA1c fraction is abnormally elevated in chronic hyperglycaemic states, like diabetes mellitus, and it correlates positively with the glycaemic as well as metabolic control. Conditions that affect erythrocyte turnover affect HbA1c levels.

The aim of this study was to determine the effect of microcytic anaemia on the HbA1c levels in non-diabetic patients, so as to analyse whether microcytic anaemia influences the HbA1c levels.

MATERIALS AND METHODS
A descriptive comparative study with a convenient sample size of 100 non-diabetic, anaemic patients and 100 age-matched non-diabetic non-anaemic controls were chosen. The patients who had glucose tolerance abnormalities (impaired glucose tolerance or diabetes mellitus), blood loss, haemoglobinopathies, haemolytic anaemia, infestation, chronic alcohol ingestion and chronic liver or renal failure were excluded from the study. Relation of HbA1c with MCV was calculated using Chi square test. And also, HbA1c levels were compared between both the groups and analysed using independent t-test and its correlation with microcytic anaemia was calculated.

RESULTS
The mean HbA1c level of the non-diabetic patients with microcytic anaemia (6.89 ± 0.55 %) was higher than that in the non-anaemic controls (5.35 ± 0.28 %) (p<0.001).

CONCLUSION
Microcytic anaemia definitely has an impact on the HbA1c levels. In patients with microcytic anaemia, as the MCV decreases, the values of HbA1c tends to rise spuriously, probably because, the glycation of the globin chain, in the relative absence of iron would occur more readily.

KEYWORDS
Glycated Haemoglobin (HbA1c), Microcytic Anaemia, Diabetes Mellitus.


BACKGROUND
Glycated haemoglobin (HbA1c) is produced by a ketamine reaction occurring between glucose molecule and the valine in the N-terminal end of β-chains of the haemoglobin molecule. HbA1c levels help clinicians to get an overall picture of the average blood sugar levels over a period of three months.

The earliest study that investigated the effects of iron deficiency anaemia on HbA1c levels was conducted by Brooks et al. Assessment of HbA1c values was done before and after treatment with iron.

The observation was that HbA1c levels in iron deficiency anaemia patients were higher and the HbA1c values decreased after treatment with iron.[1] The glycation of the globin chain, in the relative absence of iron would occur more readily.

Sluiter et al proposed that the formation of glycated haemoglobin (HbA1c) within an erythrocyte was an irreversible process. Hence, the concentration of HbA1c within a single erythrocyte would linearly increase with the age of the red cell.[2] Later, Hansen et al also demonstrated that HbA1c levels tend to decrease upon treatment of the anaemia,[3] adding evidence to the study done by Sluiter et al.

El-Agoza et al argued that elevated HbA1c levels in iron deficiency anaemia was due to the fact that, if the serum glucose remains constant, a decrease in the haemoglobin concentration increases the glycation of haemoglobin.[4]

India being the diabetic capital of the world, along with microcytic anaemia prevailing so common in the community, needs certain measures to prevent false reporting of diabetic cases. Thus, the main aim of our study was to determine...
whether microcytic anaemia affects HbA1c levels in patients without diabetes.

MATERIALS AND METHODS
A descriptive comparative study was conducted in the Department of Internal Medicine, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai after the approval of ethical committee and after obtaining consent from the participants.

Cases
Cases were selected based on the inclusion and exclusion criteria as mentioned below.

Inclusion Criteria
Confirmed cases of microcytic anaemia evidenced by-
1. Hb < 12 g/dL (women); < 13 g/dL (men).
2. MCV < 80 fl.
3. Peripheral smear showing microcytosis.

Exclusion Criteria
The following patients were excluded-
1. Acute/Chronic blood loss.
2. Haemolytic anaemia.
3. Haemoglobinopathies.
5. Chronic liver disease.
8. Impaired fasting glucose.
9. Impaired glucose tolerance.
11. Obesity.
12. MCV > 100 fl.

Controls
Age and sex matched subjects who did not have microcytic anaemia, and MCV ranging between 81 – 100 fl, but still meeting the exclusion criteria were chosen as control subjects.

Data Collection & Analysis
A convenient sample size of 100 cases and 100 controls from general medical ward/OPD are selected according to inclusion and exclusion criteria and patients were subjected to following investigations-
1. Complete haemogram.
2. Peripheral smear study.
3. Fasting blood glucose (FBG).
5. Oral Glucose Tolerance Test (OGTT).
6. Glycated haemoglobin (HbA1c).

HbA1c levels were then compared between both the groups and its correlation with microcytic anaemia was calculated.

Statistical Analysis
A descriptive comparative study was conducted and statistical analysis was done using independent t test. Range, Median, Mean, Standard Deviation were calculated. The effect of MCV on HbA1c was calculated using Chi Square test, and ‘p’ values were calculated. Statistical significance was considered if the ‘p’ value was below 0.05. HbA1c values were compared between cases and controls using independent t test. Pearson’s coefficient of correlation was also calculated between HbA1c and MCV.

RESULTS
Cases and controls were selected between age of 20 – 80 years. 31% of the cases and 24% of the controls fell within the age group of 20 – 40 years. 47% of the cases and 42% of the controls fell within the age group of 41 – 60 years. 22% of the cases and 34% of the controls fell within the age group of 61 – 80 years. 49.5% of the cases were males and 50.5% were females. Of the controls, 50.5% were males and 49.5% were females.

<table>
<thead>
<tr>
<th>MCV (fl)</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;6.5</td>
</tr>
<tr>
<td>Count</td>
<td>% of Total</td>
</tr>
<tr>
<td>50 – 60</td>
<td>0</td>
</tr>
<tr>
<td>61 – 70</td>
<td>9</td>
</tr>
<tr>
<td>71 – 80</td>
<td>6</td>
</tr>
<tr>
<td>81 – 90</td>
<td>70</td>
</tr>
<tr>
<td>91 – 100</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
</tr>
</tbody>
</table>

Table 1. MCV and HbA1c Levels

Pearson Chi-Square=141.058*p<0.001
From the above data, 44.2% of the patients with MCV values ranging between 61 – 70 fl had an HbA1c values > 6.5% and 46.5% of the patients with MCV values ranging between 71 – 80 fl had HbA1c values > 6.5%. And also, 61.4% of the patients with MCV values ranging between 81 – 90 fl had HbA1c values < 6.5% and 25.4% of the patients with MCV values 90-100 fl had an HbA1c values < 6.5%.

Thus, putting it together, 90.7% of the patients with HbA1c levels >6.5% had an MCV 61 – 80 fl. 86.8% of the patients with HbA1c levels <6.5% had an MCV 81 – 100 fl. The effect of MCV values on HbA1c was then analysed using the Chi Square test. The Chi Square statistic obtained was 141.058. The ‘p’ value of the Chi Square statistic was <0.001, which is statistically significant.

Figure 1. Bar Diagram Depicting the HbA1c Levels of Patients across the Various Ranges of MCV
The mean MCV levels of the cases observed was 70.6 fl with a standard deviation of 5.85 and a standard error of mean of 0.58. The mean MCV levels of the controls was 88.4 fl with a standard deviation of 4.58 and a standard error of mean of 0.45.

The mean HbA1c levels of the cases was 6.89% with a standard deviation of 0.55 and a standard error of mean of 0.055. The mean HbA1c levels of the controls was 5.35 % with a standard deviation of 0.45. The mean HbA1c levels between both the groups were compared using independent t test.

The Pearson correlation between MCV and HbA1c is **-0.770** with a 'p' value of <0.01, which is statistically significant, thus signifying an inverse correlation.

All the mechanisms result in the production of reactive oxygen species and thus mitochondrial injury at the cellular level, thus resulting in tissue changes. High glucose produces super oxide anion from the endothelial cells, which may quench nitric oxide, a potent endothelial derived vasodilator. Oxidative stress also interferes with endothelial dependent relaxation and cell replication, all of which culminating in the vascular complications of diabetes mellitus.

**DISCUSSION**

**Diabetes and its Complications**

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia, which, overtime leads to multiple complications. Raised glucose levels in the blood speeds up the process of atherosclerosis through various mechanisms such as oxidative stress, protein glycation and many others.

Long term complications of diabetes can be grouped into two types, as microvascular and macrovascular. Microvascular complications includes, neuropathy (autonomic, sensorimotor, plexopathy and neuronopathy), nephropathy, ocular (macular oedema, proliferative retinopathy and neovascular glaucoma). Macrovascular complications includes, coronary artery, cerebrovascular and peripheral arterial disease.

The pathogenesis of diabetes mellitus fall under two categories: vascular and metabolic. Vascular changes occurring in diabetic patients include, reduced blood vessel contractility, increased vascular permeability, thickened basement membrane, endothelial dysfunction, increased factor VII, vWF & PGI, increased fibrinogen, CRP, PAI-1 and neovascularisation. The major metabolic changes occurring at the cellular level are: increased aldose reductase activity (polyol-sorbitol pathway), diacylglycerol – protein kinase C activation [6] (myo-inositol pathway), the hexoseamine activation pathway and formation of advanced glycation end products (AGEs).

**Screening and Diagnosis of Diabetes Mellitus**

Current recommendations suggest screening for diabetes mellitus in:

- Asymptomatic men >45 years.
- Asymptomatic women >55 years.
- The screening of the Pacific and the Indo-Asian population should begin for men at 35 years and for women at 45 years.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>100</td>
<td>70.6930</td>
<td>5.85236</td>
<td>0.58524</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>88.4560</td>
<td>4.58353</td>
<td>0.45835</td>
</tr>
<tr>
<td>HbA1c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>100</td>
<td>6.8950</td>
<td>0.55748</td>
<td>0.05575</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>5.3560</td>
<td>0.28544</td>
<td>0.02854</td>
</tr>
</tbody>
</table>

**Table 2. HbA1c Levels with Respect to MCV**

**Table 3. Pearson Correlation**

<table>
<thead>
<tr>
<th>MCV</th>
<th>Pearson Correlation</th>
<th>Sig. (2-tailed)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>0.0001</td>
<td>200</td>
</tr>
<tr>
<td>HbA1c</td>
<td>-0.770**</td>
<td>0.903**</td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

**Figure 2. Bar Diagram showing the Mean HbA1c levels of Cases and Controls**

**Figure 3. Scatter Plot showing the Correlation between MCV and HbA1c**

It is evident from the above diagram that an inverse correlation exists between MCV and HbA1c.
Screening should be done once in 3–5 years depending on the risk. Screening for diabetes should begin at 25 years of age in people with the following risk factors:
- Ischaemic heart disease.
- Cerebrovascular disease.
- Peripheral arterial disease.
- Long term treatment with steroids.
- Long term antipsychotic use.
- BMI ≥ 30 (≥27 for Indo–Asian population).
- Family history of type 2 diabetes at an early age of onset in more than one first degree relative.
- Past personal history of GDM.

**Additional Risk Factors Include**
- Central obesity.
- Impaired glucose tolerance on previous assessment.
- Adverse lipid profile.
- High blood pressure.
- Polycystic ovary syndrome (PCOS).
- Current smoker or has quit smoking within the last 12 months.
- Children and young adults with BMI ≥ 30 (≥27 for the Indo–Asian population) should be screened for diabetes if.
- Family history of type 2 diabetes at an early age of onset.
- Pacific or Indo–Asian ethnicity.

ADA recommends the following criteria for the diagnosis of diabetes mellitus: Fasting plasma glucose (FPG) ≥126 mg/dL (7.0 mmol/L) or 2-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during an OGTT Or HbA1c ≥6.5% Or Classic diabetes symptoms + random plasma glucose ≥200 mg/dL (11.1 mmol/L).

**HbA1c, the Golden Tool**

HbA1c is commonly used to assess the long-term blood sugar control in diabetic patients. The results of the Diabetes Control and Complications Trial (DCCT) which was published in 1993,[9] and the U.K. Prospective Diabetes Study, published in 1998 showed that the relationship between HbA1c levels and the risk for development of diabetic complications in patients with type 1 and type 2 diabetes respectively. Studies have shown that, increase in HbA1c of 1% corresponds to a 20–30% increase in mortality or cardiovascular events. Thus, HbA1c resembles blood pressure or cholesterol in terms of the continuous relation with cardiovascular risk.

Chronic hyperglycaemic states tend to elevate the values of HbA1c and it correlates positively with the metabolic control. According to the guidelines published by the American Diabetes Association (ADA), HbA1c should be kept < 7% in patients with diabetes.[8] The values > 7% indicate an increased probability of diabetes – related complications. HbA1c serves as a reliable indicator of diabetes control over the past 90 days.

**HbA1c Levels and their Interpretation**

<table>
<thead>
<tr>
<th>Category</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic range</td>
<td>4.5–5.8%</td>
</tr>
<tr>
<td>Prediabetic range</td>
<td>5.8–6.5%</td>
</tr>
<tr>
<td>Diabetic range</td>
<td>&gt;6.5%</td>
</tr>
<tr>
<td>Risk of hypoglycaemia</td>
<td>&lt;4.5%</td>
</tr>
</tbody>
</table>

The relationship between HbA1c and plasma glucose level is complex. HbA1c is HbA1 which is being glycated at the N-terminal amino group of the β chain of haemoglobin (βN-1-deoxyfructosyl-haemoglobin). Non-enzymatic glycation of proteins occurring as a result of hyperglycaemia, has pronounced effects on the structure and functions of proteins. The two factors which can affect protein glycation are: the concentration of blood glucose and the half-life of the protein, the latter being constant, as it is genetically determined.[10,11] Thus, the quantity of HbA1c is determined solely by the blood glucose concentration.

The rate of the glycation reaction is proportional to the haemoglobin concentration of the blood. And also, the accessibility of the side chain amino groups of haemoglobin for glucose is constant and so is the lifetime of the red blood cells. So, only the concentration of glucose should influence the concentration of HbA1c. Accordingly, HbA1c would be a perfect proxy for blood glucose concentration over the lifespan of an erythrocyte.[12,13]

Erythrocyte life span averages 120 days. The level of HbA1c in the blood is contributed by all the circulating erythrocytes, from the oldest (120 days old) to the youngest. However, recent plasma glucose levels over the recent 3–4 weeks earlier contribute more to the level of HbA1c than does the long past plasma glucose levels (3–4 months earlier). Plasma glucose levels in the preceding 30 days contribute to about 50% to the final HbA1c result, and plasma glucose levels from 90–120 days earlier contribute to just only 10% of the final value of HbA1c.

Therefore, HbA1c is thus, just an average of blood glucose levels during the preceding 120 days. Thus, it is evident that it does not take 120 days to detect an appreciable change in HbA1c after a change in mean plasma glucose. This implies that HbA1c is a marker for chronic and not acute hyperglycaemia.

Fasting and post–meal glucose measurements when used, should be used with great caution as a measure of longterm glycaemia. Fasting plasma glucose tends to progressively underestimate HbA1c (and seven-point mean plasma glucose) at increasing levels of plasma glucose. Compared with the seven-point glucose profiles, post-breakfast blood glucose levels markedly overestimate the HbA1c levels, whereas post-lunch glucose levels show a relationship with HbA1c levels that is very much similar to that of mean plasma glucose.[14] Studies have also showed that, in patients with type 2 diabetes, post-lunch plasma glucose levels is a better indicator of glycaemic control than is FPG.[15,16]

Elevation of HbA1c levels are encountered in conditions where there is an increase in the RBC lifespan like, iron[17,18,19,20] and/or vitamin B12 deficiency, chronic renal failure, splenomegaly, hyperbilirubinaemia, alcoholism, large doses of aspirin.

Any condition that shortens the lifespan of RBC is likely to decrease the HbA1c levels. They include, administration of erythropoietin, iron &vitamin B12, reticulocytosis, chronic liver disease, hypertriglyceridaemia, haemoglobinopathies[21], splenomegaly, rheumatoid arthritis, ribavirin, dapsone, anti-retrovirals and many other drugs.

There is also evidence that fluctuations can occur in HbA1c levels between individuals which is not related to glycaemic status of the individual, suggesting that there are “low glycators” and “high glycators”.[22] This explains why a
fixed value of HbA1c can't be generalised for the diagnosis of diabetes mellitus in the general population.

From the above findings, HbA1c may appear to be the best tool available for the diagnosis of diabetes, but newer techniques will be required in the future, which would allow accurate diagnosis of diabetes, considering individual variations.

CONCLUSION
Our study thus concludes that an inverse correlation exists between MCV values and HbA1c levels. So microcytic anaemia, if present, must be evaluated and corrected before any decision, whether diagnostic or therapeutic, is made based on the HbA1c levels in non-diabetic patients.

REFERENCES