EFFECT OF MICROCYTIC ANAEMIA ON GLYCATED HAEMOGLOBIN (HbA_{1c}) IN NON-DIABETIC ADULTS

Gokhularaj Baskar¹, Sundaramurthy Ganesan², Karthigeyan 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 (HbA_{1c}) is used as a gold standard for monitoring glycaemic control of patients and is used as a predictor of diabetes-related complications. The HbA_{1c} 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 HbA_{1c} levels.

The aim of this study was to determine the effect of microcytic anaemia on the HbA_{1c} levels in non-diabetic patients, so as to analyse whether microcytic anaemia influences the HbA_{1c} 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 HbA_{1c} with MCV was calculated using Chi square test. And also, HbA_{1c} levels were compared between both the groups and analysed using independent t-test and its correlation with microcytic anaemia was calculated.

RESULTS

The mean HbA_{1c} 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 HbA_{1c} levels. In patients with microcytic anaemia, as the MCV decreases, the values of HbA_{1c} 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 (HbA_{1c}), Microcytic Anaemia, Diabetes Mellitus.

HOW TO CITE THIS ARTICLE: Baskar G, Ganesan S, Sethuraman KT, et al. Effect of microcytic anaemia on glycated haemoglobin (HbA_{1c}) in non-diabetic adults. J. Evolution Med. Dent. Sci. 2017;6(69):4942-4946, DOI: 10.14260/Jemds/2017/1072

BACKGROUND

Glycated haemoglobin (HbA_{1c}) 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. HbA_{1c} 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 HbA_{1c} levels was conducted by Brooks et al. Assessment of HbA_{1c} values was done before and after treatment with iron.

The observation was that HbA_{1c} levels in iron deficiency anaemia patients were higher and the HbA_{1c} 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 (HbA_{1c}) within an erythrocyte was an irreversible process. Hence, the concentration of HbA_{1c} within a single erythrocyte would linearly increase with the age of the red cell.^[2] Later, Hansen et al also demonstrated that HbA_{1c} levels tend to decrease upon treatment of the anaemia,^[3] adding evidence to the study done by Sluiter et al.

El-Agouza et al and Cogan et al argued that elevated HbA_{1c} 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

Financial or Other, Competing Interest: None.

Submission 09-07-2017, Peer Review 14-08-2017,

Acceptance 21-08-2017, Published 28-08-2017.

Corresponding Author:

Dr. Gokhularaj Baskar,

S/o. Dr. Baskar Govindharaj,

#34, R. K. V. Nagar,

Karungalpalayam (P. O.),

Erode-638003, Tamilnadu.

E-mail: gokhularaj@gmail.com

DOI: 10.14260/Jemds/2017/1072

whether microcytic anaemia affects HbA_{1c} 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.
4. Chronic kidney disease.
5. Chronic liver disease.
6. Pregnancy.
7. Established diabetes.
8. Impaired fasting glucose.
9. Impaired glucose tolerance.
10. Family H/O diabetes.
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).
4. Post Prandial Blood Glucose (PPBG).
5. Oral Glucose Tolerance Test (OGTT).
6. Glycated haemoglobin (HbA_{1c}).

HbA_{1c} 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 HbA_{1c} was calculated using Chi Square test, and 'p'

values were calculated. Statistical significance was considered if the 'p' value was below 0.05. HbA_{1c} values were compared between cases and controls using independent t test. Pearson's coefficient of correlation was also calculated between HbA_{1c} 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.

MCV (fl)	HbA _{1c} (%)			
	<6.5		>6.5	
	Count	% of Total	Count	% of Total
50 – 60	0	0.0%	6	7.0%
61 – 70	9	7.9%	38	44.2%
71 – 80	6	5.3%	40	46.5%
81 – 90	70	61.4%	1	1.2%
91 – 100	29	25.4%	1	1.2%
Total	114	100%	86	100%

Table 1. MCV and HbA_{1c} 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 HbA_{1c} values > 6.5% and 46.5% of the patients with MCV values ranging between 71 – 80 fl had HbA_{1c} values > 6.5%. And also, 61.4% of the patients with MCV values ranging between 81 – 90 fl had HbA_{1c} values < 6.5% and 25.4% of the patients with MCV values 90-100 fl had an HbA_{1c} values < 6.5%.

Thus, putting it together, 90.7% of the patients with HbA_{1c} levels >6.5% had an MCV 61 – 80 fl. 86.8% of the patients with HbA_{1c} levels <6.5% had an MCV 81 – 100 fl. The effect of MCV values on HbA_{1c} 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 HbA_{1c} Levels of Patients across the Various Ranges of MCV

	Group	N	Mean	Std. Deviation	Std. Error Mean
MCV	Case	100	70.6930	5.85236	0.58524
	Control	100	88.4560	4.58353	0.45835
HbA _{1c}	Case	100	6.8950	0.55748	0.05575
	Control	100	5.3560	0.28544	0.02854

Table 2. HbA_{1c} Levels with Respect to 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 HbA_{1c} 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 HbA_{1c} levels of the controls was 5.35 % with a standard deviation of 0.28 and a standard error of mean of 0.028. The mean HbA_{1c} levels between both the groups were compared using independent t test.

Figure 2. Bar Diagram showing the Mean HbA_{1c} levels of Cases and Controls

		MCV	HbA _{1c}	Hb
MCV	Pearson Correlation	1	-0.770**	0.903**
	Sig. (2-tailed)		0.0001	0.0001
	N	200	200	200

Table 3. Pearson Correlation

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

The Pearson correlation between MCV and HbA_{1c} is “-0.770” with a ‘p’ value of <0.01, which is statistically significant, thus signifying an inverse correlation.

Figure 3. Scatter Plot showing the Correlation between MCV and HbA_{1c}

It is evident from the above diagram that an inverse correlation exists between MCV and HbA_{1c}.

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^[5] 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-I 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 hexosamine activation pathway and formation of advanced glycation end products (AGEs).

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.

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.

Screening should be done once in 3–5 years depending on the risk.^[7] 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 HbA_{1c} $\geq 6.5\%$ Or Classic diabetes symptoms + random plasma glucose ≥ 200 mg/dL (11.1 mmol/L).^[8]

HbA_{1c}, the Golden Tool

HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} of 1% corresponds to a 20–30% increase in mortality or cardiovascular events. Thus, HbA_{1c} resembles blood pressure or cholesterol in terms of the continuous relation with cardiovascular risk.

Chronic hyperglycaemic states tend to elevate the values of HbA_{1c} and it correlates positively with the metabolic control. According to the guidelines published by the American Diabetes Association (ADA), HbA_{1c} should be kept < 7% in patients with diabetes.^[8] The values > 7% indicate an increased probability of diabetes – related complications. HbA_{1c} serves as a reliable indicator of diabetes control over the past 90 days.

HbA_{1c} Levels and their Interpretation

Non-diabetic range	4.5-5.8%
Pre-diabetic range	5.8-6.5%
Diabetic range	>6.5%
Risk of hypoglycaemia	<4.5%

The relationship between HbA_{1c} and plasma glucose level is complex. HbA_{1c} is HbA₁, that is being glycated at the N-terminal amino group of the β chain of haemoglobin (β N-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 HbA_{1c} 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 HbA_{1c}. Accordingly, HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} result, and plasma glucose levels from 90–120 days earlier contribute to just only 10% of the final value of HbA_{1c}.

Therefore, HbA_{1c} 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 HbA_{1c} after a change in mean plasma glucose. This implies that HbA_{1c} 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 HbA_{1c} (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 HbA_{1c} levels, whereas post-lunch glucose levels show a relationship with HbA_{1c} 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 HbA_{1c} 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, splenectomy, hyperbilirubinaemia, alcoholism, large doses of aspirin.

Any condition that shortens the lifespan of RBC is likely to decrease the HbA_{1c} 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 HbA_{1c} levels between individuals which is not related to glycaemic status of the individual, suggesting that there are “low glycaters” and “high glycaters”.^[22] This explains why a

fixed value of HbA_{1c} can't be generalised for the diagnosis of diabetes mellitus in the general population.

From the above findings, HbA_{1c} 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 HbA_{1c} levels. So microcytic anaemia, if present, must be evaluated and corrected before any decision, whether diagnostic or therapeutic, is made based on the HbA_{1c} levels in non-diabetic patients.

REFERENCES

- [1] Brooks AP, Metcalfe J, Day JL, ET AL. Iron deficiency and glycosylated hemoglobin A. *Lancet* 1980;2(8186):141.
- [2] Sluiter WJ, van Essen LH, Reitsma WD, et al. Glycosylated hemoglobin and iron deficiency. *Lancet* 1980;2(8193):531-2.
- [3] Gram-Hansen P, Eriksen J, Mourits-Andersen T, et al. Glycosylated haemoglobin (HbA_{1c}) in iron- and vitamin B12 deficiency. *J Intern Med* 1990;227(2):133-6.
- [4] El-Agouza L, Shahla AA, Sirdah M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. *Clin Lab Haematol* 2002;24(5):285-9.
- [5] Skyler JS. Diabetic complications. The importance of glucose control. *Endocrinol Metab Clin North Am* 1996;25(2):243-54.
- [6] Chang EY, Szallasi Z, Acs P, et al. Functional effects of over expression of protein kinase C - alpha, - beta, - delta, - epsilon, and - eta in the mast cell line RBL - 2H3. *J Immunol* 1997;159(6):2624-32.
- [7] Kilpatrick ES, Maylor PW, Keevil BG. Biological variation of glycated haemoglobin. Implications for diabetes screening and monitoring. *Diabetes Care* 1998;21(2):261-4.
- [8] American Diabetes Association. Standards of medical care in diabetes-2014. *Diabetes Care* 2014;37(Suppl 1):S14-S80.
- [9] The Diabetes Control and Complications Trial Research Group, Nathan DM, Genuth S, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993;329(14):977-86.
- [10] Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. *Acta Haematol* 2004;112(3):126-8.
- [11] Van Heyningen C, Dalton RG. Glycosylated haemoglobin in iron deficiency anemia. *Lancet* 1985;1(8433):874.
- [12] Stettler C, Mueller B, Diem P. What you always wanted to know about HbA_{1c}. *Schweiz Med Wochenschr* 2000;130(26):993-1005.
- [13] De Rosaa MC, Sannab MT, Messanaa I, et al. Glycated human haemoglobin (HbA_{1c}): functional characteristics and molecular modeling studies. *Biophys Chem* 1998;72(3):323-35.
- [14] McCance DR, Hanson RL, Charles MA, et al. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. *Br Med J* 1994;308(6940):1323-8.
- [15] Avignon A, Radauceanu A, Monnier L. Nonfasting plasma glucose is a better marker of diabetic control than fasting plasma glucose in type 2 diabetes. *Diabetes Care* 1997;20(12):1822-6.
- [16] Engelgau MM, Thompson TJ, Herman WH, et al. Comparison of fasting and 2 -hour glucose and HbA_{1c} levels for diagnosing diabetes. Diagnostic criteria and performance revisited. *Diabetes Care* 1997;20(5):785-91.
- [17] Rai KB, Pattabiraman TN. Glycosylated haemoglobin in iron deficiency anaemia. *Indian Journal of Med Res* 1986;83:40-1.
- [18] 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(4):797-802.
- [19] Mitchell TR, Anderson D, Shepperd J. Iron deficiency, haemochromatosis and glycosylated haemoglobin. *Lancet* 1980;2(8197):747.
- [20] Ford ES, Cowie CC, Li C, et al. Iron deficiency anemia, non-iron deficiency anemia and HbA_{1c} among adults in the US. *Journal of Diabetes* 2011;3(1):67-73.
- [21] Mohanty D, Colah RB, Gorakshakar AC, et al. Prevalence of β -thalassemia and other haemoglobinopathies in six cities in India: a multicentre study. *J Community Genet* 2013;4(1):33-42.
- [22] Yudkin JS, Forrest RD, Jackson CA, et al. Unexplained variability of glycated hemoglobin in non-diabetic subjects not related to glycemia. *Diabetologia* 1990;33(4):208-15.