HBA1C AND MICROCYTIC ANAEMIA, IS THERE A LINK?

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ABSTRACT

Protein glycation is a spontaneous reaction that happens as a function of the circulating blood sugar levels. The major form of protein glycation with a clinical consideration is glycated haemoglobin (HbA1c). The HbA1c fraction being abnormally elevated in chronic hyperglycaemic diabetic patients is used as a marker of glycaemic control. However, altered HbA1c levels have been documented in anaemic patients without any history of diabetes and the reports on the effects of iron deficiency anaemia on HbA1c levels are inconsistent. This study was henceforth conducted to establish the effect of microcytic hypochromic anaemia on HbA1c levels in non-diabetic individuals.

AIM

The aim of this study was to determine the effect of microcytic anaemia on the HbA1c levels in non-diabetic patients, so as to consider anaemia as an important factor which influences the HbA1c levels while monitoring the glycaemic status of diabetics.

METHODOLOGY

Fifty non-diabetic, anaemic patients and 50 age-matched healthy subjects were enrolled in this study. The patients who had glucose tolerance abnormalities (Impaired glucose tolerance or diabetes mellitus), haemoglobinopathies, haemolytic anaemia, infestation, chronic alcohol ingestion and chronic renal failure were excluded from the study. Haematologic investigations were done and the fasting and postprandial glucose and HbA1c levels were measured in all the subjects.

RESULTS

The mean HbA1c ($5.32 \pm 0.3\%$) level in the patients with IDA was higher than that in the control group (5.14 ± 0.5) ($p < 0.05$). There were no differences in the levels of fasting and postprandial glucose between the IDA and the control groups ($p > 0.05$). The haemoglobin, fasting and postprandial glucose and the HbA1c levels were normal in the control group ($p > 0.05$).

CONCLUSION

HbA1c is not affected by the blood sugar levels alone, and there are various confounding factors when HbA1c is measured, especially that of microcytic anaemia, probably due to iron deficiency which is very common in this part of the world. It is hence prudent to rule out anaemia, especially microcytic anaemia before making a therapeutic decision based on the HbA1c levels.

KEYWORDS

Microcytic Anaemia, Haemoglobin A1c, HbA1c, Glycated Haemoglobin, Diabetes.

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INTRODUCTION

Diabetes mellitus, a disorder of chronic hyperglycaemia is caused by either deficient production or inefficient utilization of insulin. According to World Health Organization (WHO), Diabetes will be the 7th leading cause of mortality by 2030.¹ India with around 62 million people diabetics is rightly named "the diabetes capital of the world" by Indian Heart Association.² Hence, a great amount of enthusiasm has been

involved in easing the diagnosis and monitoring of diabetes. HbA1c (Glycated haemoglobin) is one such entity, which has secured a pivotal place in the diagnostic criteria for diabetes. It was introduced as a marker of the glycaemic control status over the past 2-3 months and has come a long way in being used as a diagnostic modality for diabetes approved by the World Health Organisation (WHO) and American Diabetes Association (ADA). The recent identification of various pitfalls and confounding factors in the measurement of HbA1c has necessitated the review of its role. It is advised to be used with caution in certain population groups such as children, pregnant women, patients with haemolytic anaemia or haemoglobinopathies. One such confounding factor has been identified as anaemia and the iron stores (ferritin) of the individual.³ The exact role of anaemia on HbA1c estimation is yet to be established as the studies conducted so far have given conflicting results. This study has been henceforth

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conducted to establish the effect of microcytic hypochromic anaemia on HbA1c levels if any.

METHODOLOGY AND DATA COLLECTION

A detailed history regarding age, sex, occupation, diet, menorrhagia and anthropometrical measurements made. After obtaining their consent, blood was drawn and the following parameters done:

- Haemoglobin Levels (Hb):
- Mean Corpuscular Volume (MCV):
- Mean Corpuscular Haemoglobin (MCH):
- Mean Corpuscular Haemoglobin Concentration (MCHC):
- Red Cell Distribution Width (RDW):
- Platelets:
- Erythrocytes:
- White Blood Cell Count:
- Serum Fasting and Postprandial Glucose Levels: Serum Creatinine:
- HBA1C:
- Absolute HBA1C: Glycated Haemoglobin (%) * haemoglobin (g/dL)/100 Laboratory Methods:

Evaluation of the Haemogram was done using Sysmex automated cytology analyser. HbA1c was analysed using High Performance Liquid Chromatography (HPLC). Blood glucose estimation was done using glucose oxidase method. Serum creatinine was estimated using Jaffe's kinetic method.

Definitions.^{4,5}:

Microcytic hypochromic anaemia was defined as Haemoglobin less than 13 g/dL in males; less than 12 g/dL in females; RBC count less than 4,50,000 in males; less than 3,50,000 in females; Mean Corpuscular Haemoglobin (MCH) less than 27 pg. and Mean Corpuscular Haemoglobin Concentration (MCHC) less than 33 g/dL and Mean Corpuscular Volume (MCV) less than 76 fL having microcytic hypochromic anaemia in peripheral smear study. Healthy controls were defined as non-anaemic population with normal red cell indices and normal peripheral smear study.

Statistical Analysis

The results obtained were analysed using SPSS software. T-test was applied in comparative analysis results between different groups and to find significant (P) value. Mean values, Standard Deviation (S.D.), prevalence was assessed wherever relevant.

RESULTS

For all practical purposes, anaemia defined in the analysis and future discussion is to be considered as microcytic hypochromic anaemia as defined above.

Of the 100 participants, 63% (27 cases and 36 controls) are in the age group of 12-20 years, 20% (13 cases and 7 controls) in the age group of 21-40 years, 12% (7 cases and 5 controls) in 41-60 years' age group and 5% (3 cases and 2 controls) above 60 years. The majority of the anaemic (27%) were in the 12-20 years' age group and least in the group above 60 years.

There were 76 females and 24 males in the study population. Out of the 24 male participants 10 (41.7%) were anaemic and out of the 76 female participants 40 (52.6) were anaemic. The increased number of female participants can be

explained by the increased prevalence of anaemia among women due to various reasons; 10 male anaemic patients make up 20.0% of the total anaemic population, while 40 female anaemic patients make up the rest.

No stratification of severity of anaemia was attempted. Hence, the prevalence of severe anaemia could not be studied.

Majority of the participants of the study are students (63%). Out of these 63 students, 29 (46%) had anaemia. The next major group involved was employed people (27%) with 14 (51.9%) being anaemic. The anaemic patients of these two groups constitute a total of 43 (86%) cases of anaemic patients in the study. These two groups having a higher percentage of anaemia reflect the burden of anaemia on the work force of the society.

Only 25 (53.2%) of the 47 women with menorrhagia had anaemia, while the rest 22 (46.8%) are healthy. But out of 29 participants who did not have menorrhagia, 15 (51.7%) are anaemic. The association between menorrhagia and anaemia was not statistically significant.

Out of the 87 non-vegetarians 42 (48.3%) were anaemic, while 45 (51.7%) were non-anaemic. Out of the 13 vegetarians, 8 (61.5%) were anaemic. The association between diet habits and anaemia was not statistically significant.

The mean BMI in the anaemic population was 19.91 with S.D. 3.7, while it was 22.51 with S.D. 3.0 in healthy controls. The lower mean BMI in the anaemic group can be explained, as the patients had moderate-to-severe anaemia (<10 g% Hb) and the cause and effect of anaemia on BMI could be taken as the probable explanation.

The mean Red Blood Corpuscles (RBC) count in anaemic population was: male - 3.01 million/mm³ and female - 2.81 million/mm³.

The mean Red Blood Corpuscles (RBC) count in healthy male and female population were 4.75 million/mm³ 4.03 million/mm³.

The mean value of Hb in anaemic male and female population were 7.72 g/dL and 7.26 g/dL respectively, while the mean value of Hb in healthy male and female population were 14.21 g/dL and 13.51 g/dL respectively. The mean value of Hb in anaemic population was lower when compared to the mean Hb values in the study of Shanthi et al, which was conducted in the same city representing a similar population.

The possible explanations include involving people from lower socioeconomic status and the study population involving Inpatients who are probably severely anaemic.

The mean value of Mean Corpuscular Volume (MCV) in anaemic population was 69.92 fL and in healthy population it was 91.20 fL.

The mean value of Mean Corpuscular Haemoglobin (MCH) in anaemic population was 26.7 pg and in healthy population was 30.5 pg.

The mean value of Mean Corpuscular Haemoglobin concentration (MCHC) in anaemic population was 33.5 g/dL and in healthy population it was 38.5 g/dL.

The mean value of Red Cell Distribution width (RDW) in anaemic population was 19.65 and in healthy population it was 13.21.

The difference in MCV, MCH, MCHC and RDW between the groups were statistically significant (P<0.001).

The mean platelets count in anaemic population was 3.22

lakhs/mm³, which was not statistically significant from that of the mean platelets of 2.95 lakhs/mm³ in healthy population.

The mean White Blood Cell (WBC) count in anaemic population of 6,950 cells/mm³ and was not differing significantly from that of the healthy population's value of 6,796 cells/mm³.

The mean fasting blood sugar in the anaemic population was 78.72 mg/dL and healthy population was 81.14 mg/dL. (P>0.05).

The mean post-prandial blood sugar in the anaemic population was 124.68 mg/dL and the healthy population was 126.32 mg/dL. (P>0.05).

The mean HbA1c in anaemic population was 5.32%±0.32, while the mean HbA1c in healthy non-anaemic population was 5.14%±0.53. There was a statistically significant difference between two mean values at P value of 0.04.

The mean absolute HbA1c in anaemic population was 0.63±0.0, while the mean absolute HbA1c in healthy non-anaemic population was 0.70±0.07. The difference was not statistically significant (p>0.05).

	Anaemia		Total	P Value
	Anaemic	Healthy		
12-20	27 (42.9%)	36 (57.1%)	63 (63%)	≥0.05
21-40	13 (65%)	7 (35%)	20 (20%)	
41-60	7 (58.3%)	5 (41.7%)	12 (12%)	
Above 60	3 (60%)	2 (40%)	5 (5%)	

Table 1: Age Wise Distribution of Participants

Sex	Anaemic	Healthy	Total
Male	10 (41.7%)	14 (58.3%)	24 (24%)
Female	40 (52.6%)	36 (47.4%)	76 (76%)

Table 2: Sex Distribution and Microcytic Hypochromic Anaemia

	Anaemia	Mean±S.D.	P value
BMI	Anaemic	19.91±3.7	< 0.001
	Healthy	22.51±3.0	

Table 3: BMI and Anaemia

Sex	Anaemia			
	Anaemic		Healthy	
	Count	Mean (millions/cu. mm)	Count	Mean (millions/cu. mm)
Male	9	3.01	15	4.75
Female	20	2.81	56	4.03

Sex	Hb			
	Anaemic		Healthy	
	Count	Mean(g/dL)	Count	Mean (g/dL)
Male	10	7.72	14	14.21
Female	40	7.26	36	13.51

		Mean±S.D.	P VALUE
MCV (fL)	Anaemic (n=50)	69.62±3.17	P<0.001
	Healthy (n=50)	91.20±3.67	
MCH (pg)	Anaemic (n=50)	26.77±5.562	P<0.001
	Healthy (n=50)	30.50±2.71	
MCHC (g/dL)	Anaemic (n=50)	38.50±7.93	P<0.001
	Healthy (n=50)	33.50±3.19	
RDW	Anaemic (n=50)	19.65±2.88	P<0.001
	Healthy (n=50)	13.21±0.68	

Table 4: Red Cell Parameters and Anaemia

	Anaemia	N	Mean	S.D.	P value
Fasting Blood Sugar	Anaemic	50	78.72	10.14	>0.05
(FBS) (mg/dL)	Healthy	50	81.14	7.81	
Post-Prandial Blood	Anaemic	50	124.68	8.96	
Sugar (PPBS) (mg/dL)	Healthy	50	126.32	8.43	

Table 5: Fasting, Post-Prandial Blood Sugar and Anaemia

	Anaemia	N	Mean	S.D	P Value
HbA1C (%)	Anaemic	50	5.3248	.32798	0.043
	Healthy	50	5.1440	.53190	

Table 6: Anaemia and HbA1c

	Anaemia	N	Mean	S.D	P Value
Absolute HbA1C	Anaemic	50	.6372	.09474	>0.05
	Healthy	50	.7042	.07299	

Table 7: Anaemia and Absolute HbA1c

DISCUSSION

The discovery that proteins like Haemoglobin (Hb) were non-enzymatically glycosylated in vivo depending on the sugar values gave a new dimension to the field of monitoring the glycaemic control of diabetic patients. Apart from routine monitoring of fasting and post-prandial values, glycosylated haemoglobins gave a good index of glycaemic control over the past few weeks or months.

HbA1c, varyingly called as the glucohaemoglobin, glycosylated haemoglobin makes the most of the glycosylated haemoglobin. It has a stable adduct of glucose, which is linked by covalent bonds to the N-terminal valine of the β chain. Because life span of erythrocytes in the blood is approximately 120 days, HbA1c represents the overall picture of the glucose concentration over the preceding 8–12 weeks. The ease of being done at any time of the day with the fasting status of the patient having no bearing on the values measured made HbA1c a vital tool in the diagnosis, monitoring and prognostication of the microvascular complications of diabetes from 1990s.⁶ Later, as HbA1c values were found to be affected by the lifespan and other features of erythrocytes, much debate was started into the validation of HbA1c in special population with anaemia, haemoglobinopathies, haemolytic conditions and conditions like chronic kidney disease which alter the lifespan of RBCs.⁷ Many studies were conducted analysing the effect of microcytic anaemia on HbA1c measurements. These studies have come up with conflicting results. Some have shown a positive correlation, while some have shown a negative correlation.

Kim et al⁸ study on the effect of iron deficiency on HbA1c

distribution among non-diabetic adults of the National Health and Nutrition Examination Survey (NHANES) concluded that the HbA1c were elevated in iron-deficient individuals.

Ford et al⁹ also evaluated 1999–2002 NHANES data and identified a positive correlation between low Hb and HbA1c and an inverse correlation between iron levels and HbA1c. They inferred that caution should be exercised when using HbA1c values near diagnostic cut points in the setting of anaemia. Hb values were expected to change HbA1c a value by approximately 0.2% (2.2 mmol/mol). This change is especially more noticeable at the two ends of the spectrum of Hb levels.

In Hardikar et al¹⁰ study, a comparison between HbA1c and OGTT determined diabetes rates in Pune Children's Study cohort was done. In this study, people had anaemia of different aetiologies like folate and vitamin B12 deficiency were included. More number of people was diagnosed with diabetes or prediabetes when an HbA1c criterion was used than by the gold standard OGTT. Thus, the author proposed that there was an increased potential for misdiagnosis of diabetes and prediabetes in iron-deficient populations when HbA1c values alone are used. The haematological parameters that were relevant to higher HbA1c levels were erythrocyte microcytosis, low MCH, low MCHC and high RDW. Also, the results showed that mixed nutrient deficiencies can have various and unpredictable influences on HbA1c values.

Shanthi¹¹ and others studied the difference between HbA1c values in 50 non-diabetic individuals with iron deficiency anaemia and non-anaemic healthy controls. HbA1c values were significantly higher in the IDA group when compared with the control group ($P < 0.001$).

Coban and others¹² study comprised 100 participants (50 having Iron Deficiency Anaemia (IDA) with 50 healthy controls matched for age and sex. Patients with Iron Deficiency Anaemia (IDA) were given oral supplementation of ferrous sulphate 100 mg a day for 3 months. HbA1c values dropped significantly after iron treatment ($p < 0.001$). This study suggests that iron deficiency must be corrected before using HbA1c for making a diagnostic or therapeutic decision.

Sinha et al¹³ came up with a study which contradicted the other results, in that HbA1c and absolute HbA1c levels increased after correction of anaemia. This study included administering iron supplementation to 50 participants with iron deficiency anaemia and serial monitoring of the erythrocytic indices, while the erythrocytic indices were measured only once in controls. Mean HbA1c after 2 months ($5.9 \pm 0.6\%$) had significantly raised from the baseline ($p < 0.01$). A significant increase was observed in the absolute HbA1c levels at 2 months after treatment (0.29 g/dL vs. 0.73 g/dL, $p < 0.01$).

Having conflicting results from previous studies, this study was principally conducted to establish the role of microcytic hypochromic anaemia on HbA1c levels in non-diabetic individuals if any. The difference in the mean HbA1c values between anaemic and healthy population was statistically significant (P value 0.043). This is in concordance with the observation by Shanthi et al, Hardikar et al. They had all observed a modest increase of HbA1c in anaemic population. They postulated that iron deficiency causes increased glycation leading to spuriously high values of HbA1c. This is different from the observation by Sinha et al and Ford et al who have shown that mean HbA1c is decreased in anaemic population.

Absolute HbA1c values were not significantly different

between anaemic and healthy participants. This is in concordance with the observation by Sinha et al. It was the first and only study, which has correlated absolute HbA1c and anaemia and that has been confirmed by the present study.

CONCLUSION

Does it make Real Sense for General Population?

At this juncture, answer will be a definite yes. There is an increase in the number of people with young onset obesity, many among them are menstruating females at risk for iron deficiency anaemia. These people are expected to have high HbA1c values due to obesity related insulin resistance and other conditions.

Thus, a speculated increase due to iron deficiency though modest might shift the HbA1c value from pre-diabetes to diabetes.

Does it Make Real Sense for Diabetics?

The answer might not be a definite yes, as the expected change is not very high. The following might be the situations where this finding may have a bearing:

1. A search for abnormalities of erythrocyte indices when dealing with discordant glucose and HbA1c values.
2. HbA1c needs to be used with caution in menstruating women at risk for iron deficiency anaemia.
3. If there is an abnormality with erythrocyte indices or anaemia, they need to be corrected before considering using HbA1c for monitoring diabetes.

Lacunae and Future Research Prospects

Though there are hypothesis for spuriously elevated HbA1c levels like iron deficiency augmenting glycation, reduced lifespan of erythrocytes, the exact mechanism for the alteration in the value of HbA1c is not known. Also the effect of other types of anaemia and effect of mixed nutritional deficiencies needs to be determined.

LIMITATIONS OF THE STUDY

- The sample size was small.
- Majority of the individuals involved in the study was younger population. A study involving equal distribution of participants in various age groups would have thrown light upon other factors operating in the elderly individuals.
- A confirmatory test for iron deficiency like serum ferritin was not applied in this study. A broad label of microcytic hypochromic anaemia is used.
- The study was a case control study. No effort was made at correcting anaemia and henceforth the changes in the trend were not observed.
- Further studies involving a larger population where the limitations of this study are duly taken into consideration are needed.

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