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Original Research Article

TO ESTIMATE THE HBA1C LEVELS IN PATIENTS WITH IRON DEFICIENCY ANEMIA AND TO COMPARE THIS WITH HEALTHY INDIVIDUALS

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Abstract

Background: Hemoglobin A1C (HbA1c) is a measure of a patient's blood sugar levels over the last 3 months. Prior research has shown that iron deficiency may increase A1C levels, regardless of blood sugar levels. The aim is to estimate the HbA1c levels in patients with iron deficiency anemia and to compare this with healthy individuals. **Materials and Methods:** Study subjects were divided into two Groups, GROUP 1 included 50 patients with iron deficiency anaemia and GROUP 2 included 50 age and sex matched healthy individuals. A detailed clinical history was taken considering age, sex, duration of illness, history of blood loss, addictions, diabetes mellitus, systemic hypertension. Blood pressure, pulse rate and respiratory rate were recorded. Individuals of both sexes of age >20 yrs attending inpatient and outpatient units of medicine department of Government medical college; Calicut with following parameters- Hb <13 g/dl(men) & <12 g/dl (non-pregnant women), MCV < 90 fl, MCH <30 pg and S.Ferritin <15 microg/l were included in this study. Age and sex matched healthy individuals from bystanders of other patients, medical-paramedical staff and others willing to be a part of the study as the control group. **Result:** P value between control group and study group was less than 0.001 which is highly significant. It conveys that MCV was significantly lower in anemia group. P value between control group and study group was less than 0.001 which is highly significant. It conveys that MCH was significantly lower in anemia group. P value between control group and study group was less than 0.001 which is highly significant. It conveys that serum ferritin was significantly lower in anemia group. Mean HbA1c of iron deficiency anemia patients (4.67 ± 0.39) was significantly lower than control population (5.42 ± 0.29). p value of HbA1c distribution between control group and study group was less than 0.001 which is highly significant. It reveals that HbA1c was lower in anemia group. In the study group, Hb & HbA1C showed positive, weak correlation (r = 0.29) which was statistically very significant (p = 0.04). In the study group, MCV & HbA1c showed positive moderate correlation (r=0.42) which was statistically highly significant (p = 0.003). In the control group, Only S.Ferritin showed statistically highly significant positive correlation. **Conclusion:** The prevalence of iron deficiency anemia is more common in females of age group 21-30. HbA1c was significantly lower in patients with iron deficiency anemia compared to healthy control group. Hemoglobin and HbA1c showed statistically significant positive correlation in patients with iron deficiency anemia. MCV & HbA1c showed statistically significant positive correlation in patients with iron deficiency anemia. S.Ferritin & HbA1C showed statistically significant positive correlation in patients with iron deficiency anemia.

INTRODUCTION

Anaemia is defined as a low blood haemoglobin (Hb) concentration. It has been shown to be a public health problem that affects low, middle and high-income countries and has significant adverse health consequences, as well as adverse impacts on social and economic development. Blood haemoglobin concentration is the most reliable indicator of anaemia at the population level. But measurements of this concentration alone do not determine the cause of anaemia. Iron deficiency anaemia comprises 50% cases of anemia worldwide. Micronutrient deficiencies like folate, riboflavin, vitamins A and B12, acute and chronic infections, and inherited or acquired disorders that affect haemoglobin synthesis, red blood cell production or red blood cell survival are some other causes of anaemia.[1] The World Health Organisation (WHO) defines anaemia as Hb <130 g/L in men older than 15 years, 110 g/L in pregnant women, and <120 g/L in non-pregnant women older than age 15 years.[2]

Iron deficiency refers to the reduction of iron stores that precedes overt iron deficiency anemia or persists without progression. In developing countries, iron deficiency and iron-deficiency anemia typically result from insufficient dietary intake, loss of blood due to intestinal worm colonization, or both. In high-income countries, certain eating habits (e.g., a vegetarian diet or no intake of red meat) and pathologic conditions (e.g., chronic blood loss or malabsorption) are the most common causes. Serum ferritin level is the most sensitive and specific test used for the identification of iron deficiency. However, in determining iron status, it is important to consider the whole picture rather than relying on single test results.[3] A systematic overview of the diagnostic values used in the evaluation of iron deficiency anemia showed that serum ferritin was by far the most powerful test for the diagnosis of iron deficiency, outperforming red cell protoporphyrin, transferrin saturation, mean cell volume, or red cell distribution.[4]

Glycohemoglobin is produced by a ketoamine reaction between glucose and the N-terminal amino acid of both ß-chains of the hemoglobin molecule. The major form of glycohemoglobin is hemoglobin A1c (HbA1c).[5] Measurement of glycated hemoglobin is the standard method of assessing long-term glycemic control. When plasma glucose is consistently elevated, nonenzymatic glycation of hemoglobin increases; this alteration reflects the glycemic history over the previous 2–3 months, since erythrocytes have an average lifespan of 120 days. The HbA1c fraction is abnormally elevated in patients with chronic hyperglycemic diabetes mellitus and correlates positively with metabolic control.[6]

According to the recent American Association Guidelines, HbA1c levels are taken into consideration for both diagnosis and follow-up test for diabetes. Also as per the recent recommendations by the International Diabetes Federation (IDF) and American Association of Clinical Endicrinologists (AACE), the optimum level at which HbA1c should be maintained in diabetics has been brought down to the target of 6.5%. HbA1c levels are not affected by blood glucose alone.[7] They are also altered in haemolytic anaemias, haemoglobinopathies, acute and chronic blood loss, pregnancy and Vitamin B12, folate and iron deficiency anaemia.

Recently, researchers have become interested in studying HbA1c levels in more commonly encountered anemias like iron deficiency anemia (IDA). A recent review in this topic presented the controversies about this issue and highlighted the need for further studies in this field to confirm and elucidate the role of anaemia on HbA1c results.[8] A recent meta-analysis showed that iron deficiency Diabetes Mellitus(DM), but with a large confidence interval, and no statistical significance. As a result of the high heterogeneity among the available studies, the effects of IDA and/or ID remained inconclusive.[9]

MATERIALS AND METHODS

This is a cross- sectional study which was carried out in Government Medical College, Kozhikode for 1 year. Study subjects are divided into two Groups, GROUP 1 included 50 patients with iron deficiency anaemia and GROUP 2 included 50 age and sex matched healthy individuals. Study was conducted in 100 subjects of either sex with prior informed consent. A detailed clinical history was taken considering age, sex, duration of illness, history of blood loss, addictions, diabetes mellitus, systemic hypertension. Blood pressure, pulse rate and respiratory rate were recorded. Individuals of both sexes of age >20 yrs attending inpatient and outpatient units of medicine department of Government medical college; Calicut with following parameters- Hb <13 g/dl(men) & <12 g/dl (non-pregnant women), MCV < 90 fl, MCH <30 pg and S.Ferritin <15 microg/l were included in this study. Patients with a history of acute blood loss, haemolytic anaemia, hemoglobinopathies, kidney disease, pregnancy, established diabetes, impaired fasting glucose were excluded from the study. Persons not given written consent and subjects not matched to age group were excluded from the control group. Age and sex matched healthy individuals from bystanders of other patients, medical-paramedical staff and others willing to be a part of the study as the control group.
Informed consent was taken from both cases and controls.

Methodology

The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood. This method uses TTAB as the detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). Sample pretreatment to remove labile HbA1c is not necessary. All haemoglobin variants

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which are glycated at the β-chain N- terminus and which have antibody recognizable regions identical to that of HbA1c are measured by this assay. Haemoglobin, serum ferritin, MCV, MCH were measured with standard methods.

Stastical Analysis

Statistical Package for Social Sciences [SPSS] for windows, version 25.0, was used to perform statistical analysis. Descriptive analysis of all the explanatory variables was done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables. Chi-square test was used to compare the age and gender distribution among 02 groups. Independent student test was used to compare the mean values of different study variables between two groups. Pearson correlation test was used to assess the relationship between HbA1c levels and other study variables in each groups. Simple linear regression analysis was used to predict the HbA1c levels using significant contributing variables in test and control groups. The level of significance (p value) was set at $p < 0.05$.

* - Significant - $p < 0.05$

** - Very significant - $p < 0.01$

*** - Highly significant - $p < 0.001$.

RESULTS

The cross-sectional study consists of 100 individuals out of which 50 were patients with iron deficiency anemia and 50 were controls. The demographic details of the subjects were analyzed in terms of sex and age are presented in the [Table 1].

Majority of the study subjects were from age group 21-30 years. p value was 0.77 which is not significant i.e. the age distribution among the control and study group were equal. Majority of the study subjects were females (80%) while the remaining 20% were males. It confirms the fact that iron deficiency anemia is more common in females. p value was 0.35 which is not significant i.e. sex distribution among the control group and study group were equal.

p value was less than 0.001 which is highly significant i.e. mean hemoglobin level in study group was significantly lower than the control group as expected. p value between control group and study group was less than 0.001 which is highly significant. It conveys that MCV was significantly lower in anemia group. p value between control group and study group was less than 0.001 which is highly significant. It conveys that MCH was significantly lower in anemia group. P value between control group and study group was less than 0.001 which is highly significant. It conveys that serum ferritin was significantly lower in anemia group. Mean HbA1c of iron deficiency anemia patients (4.67 ± 0.39) was significantly lower than control population (5.42 ± 0.29). p value of HbA1c distribution between control group and study group was less than 0.001 which is highly significant. It reveals that HbA1c was lower in anemia group.

In the study group, Hb & HbA1C showed positive, weak correlation (r = 0.29) which was statistically very significant (p = 0.04). In the study group, MCV & HbA1c showed positive moderate correlation (r=0.42) which was statistically highly significant (p = 0.003).

In the control group, Only S.Ferritin showed statistically highly significant positive correlation.

Table 1: Age wise comparison of study subjects between 2 groups.

Category	Study n (%)	Control n (%)	χ2 Value	p-Value
Age				
21-30 yrs	24 (48)	29 (58)	1.146	0.77
31-40 yrs	15 (30)	11 (22)		
41-50 yrs	9 (18)	8 (16)		
51-60 yrs	2 (4)	2 (4)		
Gender				
Males	10 (20%)	14 (28%)	0.877	0.35
Females	40 (80%)	36 (72%)		

Table 2: Comparison of mean values of Hb, MCH between 2 groups

Variables	Groups	Mean (SD)	Mean Diff	t	p-Value
Hb	Study	6.77 (1.61)	-6.51	-27.742	<0.001***
	Control	13.28 (0.39)			
MCV	Study	65.05 (7.71)	-26.80	-23.145	<0.001***
	Control	91.86 (2.75)			
MCH	Study	19.24 (3.04)	-9.11	-20.104	<0.001***
	Control	28.35 (1.03)			
S. FERRITIN	Study	7.41 (2.18)	-219.11	-40.250	<0.001***
	Control	226.52 (38.43)			
HbA1C	Study	4.67 (0.39)	-0.75	-10.954	<0.001
	Control	5.42 (0.29)			

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Table 3: Relationship between HbA1c and other study variables in study group

	R	p-value
Hb	0.29	0.04*
MCV	0.42	0.003**
MCH	0.23	0.10
S.Ferritin	0.34	0.02*

Table 4: Relationship between HbA1c and other study variables in control group

	R	p-value
Hb	-0.21	0.14
MCV	0.11	0.43
MCH	-0.20	0.18
S.Ferritin	0.43	0.002**

DISCUSSION

Iron deficiency anemia is most common form of anemia observed in our country. HbA1c is one of the glycated haemoglobins which is used to assess the glycemic status of an individual over last 2 to 3 months and is mostly being used in diabetics and in those with impaired glucose tolerance. Certain studies have been done which showed that HbA1c levels are affected in iron deficiency anemias. Earlier studies on effect of iron deficiency anemia on glycated haemoglobin showed an inverse relation between HbA1c and IDA. Brooks et al.108 showed higher HbA1c concentrations in non- diabetic anemic individuals which decreased on treatment. They proposed that the quaternary structure of haemoglobin gets altered and that, glycation of beta globin chain occurs more readily in the relative absence of iron. Sluiter et al,[10] later gave a different explanation to the above findings. They postulated that the formation of glycated hemoglobin is an irreversible process and hence, the concentration of HbA1c in one erythrocyte will increase linearly with the cell's age. In patients with normal blood glucose values but with red cells that are younger than usual, as after treatment of iron deficiency anaemia, HbA1c concentration falls. However, if the iron deficiency has been persisting for a long time, the red cell production rate falls, leading not only to anemia but also to a higher than normal average age of circulating erythrocytes and, therefore, of increased HbA1c.

Heyningen et al,[11] demonstrated that when affinity gel separation is used for HbA1c estimation, iron deficiency anemia did not produce high glycosylated haemoglobin values and speculated that the differences observed previously could be due to difference in methods used for calculating HbA1c. But Rai et al,[12] reported that there was no significant difference in HbA1c values calculated by colorimetry, ion exchange chromatography and affinity chromatography. Hansen et al,[13] showed that there was no difference in HbA1c values at baseline between iron deficiency anaemia patients and controls but demonstrated a decrease in HbA1c levels to subnormal levels after treatment. They explained by stating that it was due to increase in the number of immature erythrocytes.

Further studies by El-Agouza et al,[14] and Coban et al,[6] gave another explantion for increased HbA1c in iron deficiency anemia. They postulated that, if serum glucose is accepted to remain constant, a decrease in the hemoglobin concentration might lead to an increase in the glycated fraction but the exact mechanism still remains elusive.

Although these reports have shown positive effect of IDA on HbA1c results, only a few of them revealed a clinical significant effect.[9] The explanations provided in the studies quoted above are merely speculations.

Grossman et al,[15] in studying association of glycated hemoglobin with hemoglobin levels in elderly nondiabetic subjects demonstrated that in elderly nondiabetic subjects, HbA1c levels are not correlated with hemoglobin level or nutritional factors associated with anemia and may be interpreted without consideration of these factors.

Koga et al,[16] in studying association of erythrocyte indices with glycated hemoglobin in pre-menopausal women demonstrated that RBC count is positively, but Hb, MCH and MCV negatively associated with HbA1c in premenopausal women, but none of them showed any association with HbA1c in post-menopausal women.

Ford et al,[17] observed influence of iron-deficiency anemia and non-iron-deficiency anemia on HbA1c levels among adults in the US by using cross-sectional data from 8296 adults who participated in NHANES 1999–2002 and found that there was a significant positive correlation between Hb concentrations and HbA1c concentrations after adjusting for age, gender, and race or ethnicity, with HbA1c rising from a mean of 5.28% among participants with Hb < 100 g/L to 5.72% among participants with Hb > 170 g/L. They also found that the adjusted mean concentrations of HbA1c were 5.56% and 5.46% among participants with and without iron deficiency, respectively ($p = 0.095$). They observed that erythrocyte size and Hb have a negative correlation with HbA1c, which was compatible with the hypothesis that iron deficiency increases Hb glycation, whereas ferritin had a positive correlation with HbA1c, which perhaps was related to an association with insulin resistance. So, in contrast with several previous studies, they suggested that IDA had little population effect on concentrations of HbA1c or on diabetes prevalence.

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The difference in concentrations of HbA1c between extremes of concentrations of Hb being the order of an absolute 0.2%. They were thus of the opinion that only people with anemia who are close to the diagnostic threshold may require retesting of HbA1c or the use of another diagnostic method for DM.

Due to the variation in results obtained from these multiple studies, we were prompted to conduct our own study to investigate the affects of iron deficiency anemia on HbA1c levels. The present study revealed that HbA1c (%) is significantly lower in IDA group (4.67 $\pm$ 0.39) compared to the control group (5.42 $\pm$ 0.29) ($p < 0.001$). This is supported by studies done by Sinha et al. in 2012,[18] Cavagnolli et al in 2015, Kalasker et al. in 2014,[19] and Solomon et al in 2019.[20] They all stated that HbA1c concentration tends to be lower in the presence of iron deficiency anemia. In the study group, Hb & HbA1c showed positive, weak correlation ($r = 0.29$) which was statistically very significant ($p = 0.04$). In the study group, MCV & HbA1c showed positive moderate correlation ($r=0.42$) which was statistically highly significant ($p = 0.003$). In the study group, S.Ferritin & HbA1c showed positive, weak correlation ($r = 0.26$) which was statistically very significant ($p < 0.05$). In the control group, only S.Ferritin showed statistically highly significant positive correlation. The positive correlation demonstrated between HbA1c and the other study variables Hb, MCV, MCH, S.Ferritin is strikingly different from the other studies Nitin Sinha et al,[21] showed that values of HbA1c decreased with fall in haemoglobin values and with treatment these values increased in the next 2 months. The probable reason for these observations given was that the population in study was generally from a lower socio economic strata. So in them, cause of iron deficiency is not only bleeding and malabsorption but also nutritional deficiency which may be playing an important role in the etiology of iron deficiency. These factors (or other unknown variables) may have a bearing on such results. Similar to this study, in 2014 a study was conducted by Vishal Kalasker et al,[19] on the effect of iron deficiency anemia on glycosylated hemoglobin levels in non- diabetic Indian adults. They postulated that Hb concentrations are positively correlated with HbA1c concentration and that HbA1c concentration tended to be lower in the presence of iron deficiency anemia. But they concluded that iron deficiency anemia is unlikely to be a major concern in diagnosing diabetes using concentration of HbA1c.

CONCLUSION

The prevalence of iron deficiency anemia is more common in females of age group 21-30. HbA1c was significantly lower in patients with iron deficiency anemia compared to healthy control group. Hemoglobin and HbA1c showed statistically significant positive correlation in patients with iron deficiency anemia. MCV & HbA1c showed statistically significant positive correlation in patients with iron deficiency anemia. S.Ferritin & HbA1C showed statistically significant positive correlation in patients with iron deficiency anemia.

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