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Original Article

Biochemistry Section

Correlation of Haemoglobin Percentage and Glycated Haemoglobin Level among Iron Deficient versus Non-Iron Deficient Patients without Diabetes: An Observational Study in a Tertiary Care Centre of Bihar, India

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ABSTRACT

Introduction: Iron Deficiency Anaemia (IDA) and diabetes are extremely common in Indian settings. Glycated haemoglobin (HbA1c) indicates patient's glycaemic status over past 8 to 12 weeks. Formation of HbA1c is irreversible and its concentration in the blood depends on life span of Red Blood Cell (RBC), blood glucose concentration, Haemoglobin (Hb) percentage (Hb%), serum iron, etc. Some studies suggest that iron depletion is associated with increased glycation of Hb leading to falsely high values of HbA1c independent of glycaemia.

Aim: To compare and correlate Hb% and HbA1c level among iron deficient (non-diabetic) and non-iron deficient (non-diabetic) patients.

Materials and Methods: This was an observational, cross-sectional study conducted on 200 non-diabetic subjects which were divided into group A (n=100, IDA but non-diabetic) and group B (n=100, normal healthy subjects without IDA and without diabetes). Investigations like Complete Blood Count (CBC), Fasting and Postprandial (PP) blood sugar, HbA1c, blood urea and serum creatinine and iron profile were estimated. Data was analysed using Graph Pad Instat software by student's t-test (unpaired t-test) and Hb% and HbA1c were correlated by using correlation coefficient (r).

Results: Mean Hb%, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC), serum iron and ferritin were significantly higher in group B in comparison with group A (p-value <0.0001). Mean serum Total Iron Binding Capacity (TIBC) was significantly higher in group A subjects in comparison to group B. Mean HbA1c in group A was significantly on higher side as compared to group B. It showed that iron deficient patients had higher value of HbA1c as compared to non-iron deficient subjects. Value of Correlation Coefficient (r)= -0.4064 between Hb% and HbA1c in group A showed moderate strength of linear relationship in opposite direction (p-value <0.0001). Value of Correlation Coefficient (r)= 0.07228 between Hb% and HbA1c in group B shows no/extremely weak linear relationship in positive direction. Two tailed p-value 0.4748, was considered not significant.

Conclusion: This study concluded that iron deficiency (decreased Hb%) leads to increase in HbA1c.So,using HbA1c as a common diagnostic tool for diabetes one must keep in mind the IDA status for better endocrinological profile and medication of patients.

Keywords: Increased mean HbA1c, Non-diabetic patients, Reduced mean Hb%, Serum ferritin, Serum iron, Serum total iron binding capacity

INTRODUCTION

Anaemia is a worldwide health problem affecting both developing and developed countries and has a great impact on human health, social and economic development [1]. It is characterised by reduction in the number of RBCs or Hb concentration due to incomplete Hb synthesis that results in microcytic and hypochromic RBCs [2]. According to World Health Organisation, criteria for anaemia is Hb value <12 g/dL in women and <13 g/dL in men, although others have proposed lower age-adjusted values for Hb to define anaemia [3,4]. Causes of anaemia in developing countries are multi-factorial which include nutritional deficiencies (iron, folate, and vitamin B12 deficiencies), infections (such as malaria and intestinal parasitic infection) and chronic illness [5]. Dietary lack is rare in developed countries (abundance of meat in their food). Other causes may be chronic blood loss (most common), impaired absorption like sprue, fat malabsorption (steatorrhea) chronic diarrhoea, gastrectomy and external haemorrhage or bleeding into the gastrointestinal, urinary, or genital tracts. In growing infants, children,premenopausal women, during pregnancy there is a high requirement of iron, hence can lead to it's deficiency [6].

Diabetes Mellitus (DM) is defined as a metabolic disorder with heterogeneous aetiologies characterised by chronic hyperglycaemia. HbA1c is one such entity which has a pivotal role in the diagnostic criteria for diabetes. HbA1c can be used as an indicator of a patient's glycaemic status over the past 8 to 12 weeks. Formation of HbA1c is essentially irreversible. Its concentration in the blood depends on both the life span of the RBC (average life span is 120 days) and the blood glucose concentration. Values of HbA1c are unaffected by day-to-day glucose fluctuations, recent exercise or food ingestion [7]. Interpretation of HbA1c depends on RBCs having a normal lifespan. Patients with haemolytic disease or other conditions with shortened RBC survival exhibit a substantial reduction in HbA1c [8]. Similarly, individuals with recent significant blood loss have falsely low values owing to a higher fraction of young erythrocytes. Some studies have reported iron depletion is related to increased glycation of Hb resulting in false hig values of HbA1c [9].

The exact role of IDA on HbA1c estimation is yet to be established as the studies conducted so far have given conflicting results [10-13]. This study has been henceforth conducted to establish the effect of Hb% and HbA1c level among iron deficient and non-iron deficient patients.

MATERIALS AND METHODS

This was an observational, cross-sectional and comparative study, conducted in the Department of Biochemistry, Indira Gandhi Institute of Medical Sciences, Sheikhpura, Patna, Bihar, India, for 18 months from February 2018 to July 2019. Study was approved from the Institutional Ethics Committee of IGIMS, Patna (vide letter number. 52/ Acad./ Dated-22-01-2018). Study was started after getting informed consent from the study subjects. In 18 months approximately 185000 samples were processed in the Department of Biochemistry (approx 500 samples per day, excluding holidays). Out of these only 40% of samples were for CBC (including Haemogram), Blood Sugar (PP and Fasting), HbA1c (Glycated Hb) and Serum iron profile. Hence taking 95% as confidence level, 6.79% as margin of error, 40% population proportion and 185000 population size, the size of consecutive samples taken was 200 [14]. Out of which 100 cases of IDA without diabetes (Group A) and 100 healthy subjects as control without IDA and without diabetes (Group B) were consecutively selected from OPD and IPD of Department of Medicine and Department of Obstetrics and Gynaecology.

Inclusion Criteria

1. Both male and females of age 18 to 65 years were included as case as well as control.
2. Non-diabetic patients with confirmed diagnosis of IDA were included as cases.
3. Healthy individuals without IDA, diabetes and/or renal disease were included as control.
4. Cut-off value of Hb% (gm/dl) for inclusion as cases was <12 for female and <13 for male.

Exclusion Criteria

Following patients were not included in this study either as case or control.

1. Age less than 18 and more than 65.
2. Diabetic patient irrespective of blood glucose level.
3. Renal disease of any type.
4. Anaemia due to acute and chronic blood loss and haemoglobinopathies.
5. Pregnant women.
6. Blood transfusion in recent three months.

Laboratory Investigations

Blood sample was collected from each of the study subject and following investigations were performed only for once because it was an observational and cross-sectional study.

1. Haemogram (CBC)- White Blood Cell (WBC) count, Total leucocyte count, Differential leucocyte count, RBCs, Platelet count, Packed Cell Volume (PCV), Red Cell Distribution Width (RDW), Hb%, MCH, MCV, MCHC.
2. Estimation of Blood sugar- Fasting and Postprandial (PP)
3. Estimation of HbA1c
4. Estimation of blood urea and serum creatinine
5. Estimation of Iron profile- Serum iron, Serum ferritin, TIBC

Procedures

A 10 ml of blood was collected from both groups in overnight fasting condition in the morning hours. The antecubital vein of the arm was preferred for sample collection. Blood was collected in plain vial, EDTA and Fluoride vial. The filled tube was properly mixed but not shaken because vigorous mixing can cause haemolysis. The tubes were appropriately labeled and transferred to the laboratory.

Processing of the Blood Sample

Blood was collected in plain vial and Fluoride vial was allowed to clot by placing the vacutainer tube with red and lavender cap, respectively in a rack at the room temperature for at least 30 minutes. Then vials were centrifuged at 2000 rpm for 10 minutes and serum was separated. The clear serum was analysed within eight hours. Grossly haemolysed samples were avoided for analysis. After proper mixing of EDTA blood haemogram (CBC) was done on Advia 2120 Hematology System by Siemens Healthineers India [15]. Serum iron [16], Serum TIBC [17], glycated haemoglobin [18], fasting and PP blood glucose [19] and kidney function test [20,21] was done on AU 5800 chemistry analyser by Beckman Coulter. Serum ferritin [22] was estimated by Chemiluminescence Immunoassay (CLIA) method in Beckman Coulter Access2 instrument by Beckman Coulter. Internal and external quality control of all parameters was regularly maintained by controls provided by Bio-Rad and CMC Vellore.

STATISTICAL ANALYSIS

The mean and the Standard Deviation (SD) for all the variables of both the groups were analysed using Graph Pad Instat software (Version 3.06, 32 bit for Windows) and Microsoft Excel (Microsoft Office Home and Student 2016). Statistical analysis was done by student's t-test (unpaired t-test) after testing for homogeneity of variance and p-value was calculated (p-value <0.05 significant). The correlations between IDA (Hb%, Serum iron, Serum ferritin, TIBC) with HbA1c were presented by correlation coefficient (r) for each pair for both the study groups. Results were interpreted at level of 95% confidence interval.

RESULTS

In group A, 27 subjects were male (27%) and 73 (73%) were female. In group B, 55 subjects were male (55%) and 45 were female (45%). Mean age of group A was 40.57 years and mean age for group B was 36.87 years [Table/Fig-1].

Variables	Group A (Case, n=100)	Group B (Control, n=100)
Male	27	55
Female	73	45
Age (years) (Mean±SD)	40.57±15.68	36.87±16.03

[Table/Fig-1]: Demography (gender and age).

The mean of Hb%, MCV, MCH and MCHC were higher in group B in comparison with group A (p-value <0.0001-Extremely significant) because control group (group B) was taken as non-iron deficient patients [Table/Fig-2]. Mean serum iron and mean serum ferritin was higher in group B as compared to group A and this difference was extremely significant. But mean serum TIBC was more in group A subjects in comparison to group B and the difference was extremely significant [Table/Fig-3]. Mean HbA1c in group A was in higher side as compared to group B and this difference was extremely significant. It shows that iron deficient patients had higher value of HbA1c as compared to non-iron deficient subjects [Table/Fig-4].

Variables	Group A (Case)		Group B (Control)				
	Mean±SD	SEM	Mean±SD	SEM	Mean difference	t value	p-value
Hb% (gm/dL)	7.85±2.39	0.239	13.72±0.97	0.097	5.869	22.787	<0.0001- ES
MCV (fl)	70.22±10.57	1.057	87.78±6.28	0.628	17.555	14.283	<0.0001- ES
MCH (pgm/cell)	24.05±4.22	0.421	29.23±1.95	0.195	5.176	11.146	<0.0001- ES
MCHC (gmHb/dL)	29.44±3.11	0.311	33.28±1.56	0.156	3.841	11.045	<0.0001- ES

[Table/Fig-2]: Comparison of haemogram in Group A (n=100) and Group B (n=100):
Student's "t" test; MCV-Mean corpuscular volume; MCH-Mean Corpuscular haemoglobin; MCHC-Mean Corpuscular haemoglobin concentration; ES-Extremely significant

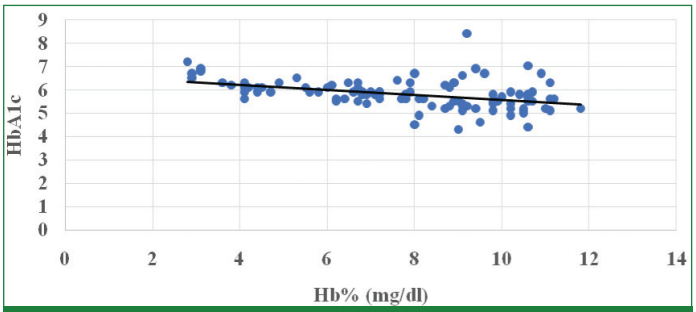
Variables	Group A (Case)		Group B (Control)				
	Mean±SD	SEM	Mean±SD	SEM	Mean difference	t-value	p-value
Serum Iron (µgm/dL)	31.40±18.21	1.821	73.19±22.26	2.226	41.790	14.530	<0.0001- ES
Serum Ferritin (µgm/dL)	17.81±29.11	2.911	63.06±48.18	4.818	45.252	8.039	<0.0001- ES
Serum TIBC (µgm/dL)	383.80±134.50	13.45	263.44±83.49	8.349	-120.36	7.603	<0.0001- ES

[Table/Fig-3]: Comparison of iron profile in Group A and Group B.

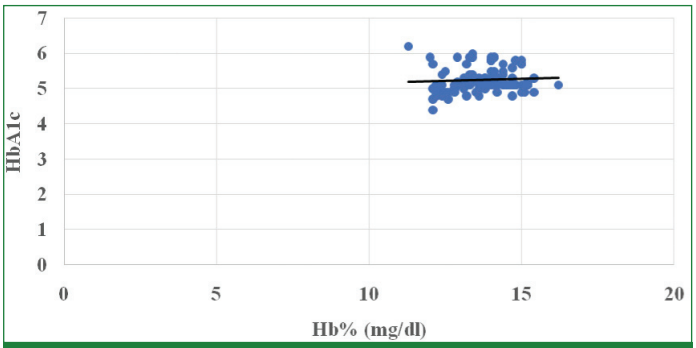
	Group A (Case)		Group B (Control)				
	Mean±SD	SEM	Mean±SD	SEM	Mean difference	t value	p-value
Glycated Haemoglobin (HbA1c) (gm Hb%)	5.82±0.63	0.063	5.25±0.35	0.035	-0.566	7.89	<0.0001- ES

[Table/Fig-4]: Comparison of HbA1c level in group A (n=100) and group B (n=100).
Student's "t" test

Value of Correlation Coefficient (r)= -0.4064 between Hb% and HbA1c in group A shows moderate strength of linear relationship in opposite direction. Two tailed p-value was <0.0001, considered extremely significant [Table/Fig-5]. Value of Correlation Coefficient (r)= 0.07228 between Hb% and HbA1c in group B shows no/extremely weak linear relationship in positive direction. Two tailed p-value was 0.4748, considered not significant [Table/Fig-6].



[Table/Fig-5]: Correlation between Hb% and HbA1c in group A.



[Table/Fig-6]: Correlation between Hb% and HbA1c in group B.

The mean age of group A and group B was 40.57±15.68 years and 36.87±16.03 years, respectively. The age of study subjects ≤40 and >40 years in group A (case, n=100) and in group B (control, n=100) were 52% and 48% and 61% and 39%, respectively. In group A, 32, 33 and 35 cases were in 18-30, 31-45 and 46-65 years of age group. In group A (IDA) and B (Non-iron), female and male cases were 73% and 27% and 45% and 55%, respectively.

DISCUSSION

Like this, other studies had been conducted in past to correlate or compare Hb% level and HbA1c level in iron deficient and non-iron deficient patients who were non-diabetic. Compared to results obtained from this study, results of some studies were in support and some other were against.

Previous studies which supported present study (i.e., higher HbA1c level compared to Hb% in non-diabetic iron deficient patients)

Shanthi B et al., found that the mean HbA1c value in the patients with IDA was more than the control group (p <0.001) [9]. Kim C et al., reported an increase in HbA1c in iron-deficient people and lead to an upward shift of HbA1c distribution. In the present study similar results were obtained but the HbA1c level was in normal range [23]. Parlapally RP et al., found that the mean HbA1c level in the patients with IDA (6.13%±0.57%) was higher than that in the control group (5.12%±0.30%) (p<0.001) [24]. Horton BF and Huisman TH, found that out of four cases, two had increased level of HbA1c and two had normal level of HbA1c in IDA patients. Similar results were obtained in present study [25]. Hashimoto K et al., found that HbA1c levels were significantly increased in the third trimester compared with early pregnancy, but serum glycated albumin did not change; HbA1c was negatively correlated with serum ferritin and transferrin saturation, suggesting that HbA1c was influenced by iron stores rather than by glucose control. Results of Hashimoto K et al., was in concordance with results from present study in terms of Hb%. Although pregnant women were not included in this study. HbA1c had weak but positive correlation with serum ferritin in group A IDA patients and this result was opposite to the result obtained by Hashimoto K et al., [26]. Rajagopal L et al., found in their study that mean HbA1C in anaemic group (6.84±0.07%) was higher than the non-anaemic group (5.12±0.04%) and this difference was statistically significant (p< 0.05). HbA1C level was high when severity of anaemia was worst. Results of this study supported the result obtained from present study i.e., negative and moderate strength of correlation between HbA1c and Hb% [10].

Previous studies which supported present study but also compared HbA1c level and Hb% after giving iron therapy to iron deficient non-diabetic patients

Tarim O et al., reported that, HbA1c reduced from 7.6±2.6 to 6.2±1.4% in iron-deficient patients after iron therapy (p<0.05), inspite of similar glucose levels [27]. Coban E et al., found that among non-diabetic adults with IDA, the HbA1C was 7.4±0.3% before treatment and 6.2±0.6% after treatment [28]. Brooks AP et al., reported an increase in HbA1c concentration in iron-deficient non-diabetic adults, which became normal fter iron replacement. In present study higher HbA1c levels were found in iron-deficient non-diabetic subjects. But no effect of iron replacement therapy was observed on HbA1c level as present study was a cross-sectional and observational [29]. Hansen PG et al., depicted normal HbA1c concentrations in IDA patient, which lead to subnormal levels after iron supplementation. Similar results were obtained in this study in which HbA1c level was in normal range but on higher side in non-diabetic IDA subjects [30]. Bhardwaj K et al., found in their study that the mean baseline HbA1c level in anaemic patients (6.60) was significantly higher than the controls (5.48). But, decline from 6.60 to 5.74 (significant) was seen in HbA1c levels after three months of treatment [11].

Previous studies whose results were against the present study (i.e., HbA1c level was normal or below normal compared to Hb% in non-diabetic iron deficient patients)

Adeoye S et al., found that non-diabetic anaemic patients had lower mean of HbA1c (5.3 vs 5.7). Result of Adeoye S et al., was different from present study [31]. Sinha N et al., found that mean baseline HbA1c level in anaemic patients was significantly lower than that

the control group (p<0.05). A significant increase was observed in the patients' absolute HbA1c levels at two months after treatment (0.29 g/dL vs. 0.73 g/dL, p<0.01). However, this study was different from present study [12]. Heyningen V and Dalton RG, concluded no differences in HbA1c concentrations between non-diabetic patients with IDA before and after iron treatment and healthy controls. The differences in the laboratory methods used for measuring HbA1c could result in different HbA1c concentrations before and after iron supplementaion [13].

Hansen PG et al., reported no significant differences in HbA1c concentrations in iron deficient patients, vitamin B12-deficient patients, and healthy controls. They reported that in IDA, the erythrocyte survival rate was normal, while in vitamin B12 deficiency, the red cell survival rate reduces, but the haemolytic component is small and affects both mature and immature erythrocytes [30].

The mechanisms leading to increased HbA1c levels are not clear but few studies had proposed some explanations

Brooks AP et al., reported, in iron deficiency, there was alteration of quaternary structure of Hb molecule and glycation of the globin chain was seen in the absence of iron [29]. Sluiter WJ et al, concluded that HbA1c concentration in erythrocyte increases linearly with age of the cell, and the HbA1c formation is an irreversible process. He also said that, HbA1c concentration was reduced in subjects with normal blood glucose levels with young red cells. However, if the iron deficiency was prolonged, the red cell production rate will decrease. But, if iron deficiency has stayed on for a long time, the red cell production rate would decrease, resulting in anaemia and a higher-than-normal average age of circulating erythrocytes and increased HbA1c levels [32]. El-Agouza I et al., and Coban E et al., said that elevated HbA1c levels in IDA could be if serum glucose remains constant, a fall in the Hb concentration could result in an increase in the glycated fraction [33,28]. As proven by the above studies, some were in support and some were against the results obtained from this study and this study shows that the level of HbA1c level increases when level of Hb% decreases. The precise manner in which IDA affects HbA1c levels is not very clear. The justifications provided in the studies above are only speculated.

Limitation(s)

There were certain limitations in this study as pregnant women were not included. Pregnancy causes IDA as there is increased requirement. We have not seen effect of iron replacement therapy on the level of HbA1c as this study was a cross-sectional and observational study. In this study patients follow-up were not done, it could be a limitation. Sample size could be a limitation as due to various reason like patients who should be enrolled for this study did not turned up or given consent.

CONCLUSION(S)

This study concluded that iron deficiency (decreased Hb%) leads to increase in HbA1c. In Indian setting IDA and diabetes are extremely common. Using HbA1c as a common diagnostic tool for diabetes one must keep in mind the IDA status for better endocrinological profile and medication of patients. HbA1c should always be interpreted carefully in anaemic patients. Exact mechanism of high HbA1c level in IDA is not clear and various theories exist to explain this. However more large-scale studies are required to find out the proper mechanism underlying this correlation.

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