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Study of HbA1c in iron deficiency anemic non diabetic subjects

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Abstract--To study the effect of iron deficiency anemia on glycated hemoglobin (HbA1c) in non- diabetic Indian subjects. The present observational study was performed on minimum 100 patients attending General Medicine outpatient department. All patients presenting to General Medicine outpatient department fulfilled the inclusion and exclusion criteria enrolled in the study. An informed written consent was obtained from the patients. The detailed history was taken and necessary clinical examination was carried out in all patients. In the current study, patients with iron deficiency anaemia had a mean HbA1c of 5.86. The mean serum iron level in patients with Hb1Ac levels ranging from 4-5.6 was 24.25. The corresponding mean serum iron for HbA1c range 5.7-6.4 was 22.25. The corresponding mean serum iron for HbA1c levels greater than 6.5 was 21.96. According to the findings, there is an inverse relationship between HbA1c and mean serum iron levels, and this relationship is statistically significant (p0.05), i.e. mean serum iron decreases as HbA1c level increases. The present study concluded that the levels of HbA1c were increased significantly among patients with iron deficiency anemia. So, Iron deficiency anemia has to be kept in mind before using the HbA1c to diagnose diabetes.

Keywords---iron deficiency, anemia, glycated hemoglobin (HbA1c).

Introduction

Diabetes mellitus is a life-threatening medical condition. Diabetes mellitus affects an estimated 422 million people worldwide, according to the World Health Organization's (WHO) most recent 2016 data. Diabetes type 2 accounts for 85-90 percent of all cases.¹ Many of the long-term complications of both Type 1 and Type 2 diabetes are related to the increased risk of macrovascular complications like coronary artery disease and strokes. However, the most serious health issue associated with Type 2 diabetes is an increased risk of peripheral arterial disease. Diabetes must be diagnosed early in its progression because effective diabetes care can dramatically lower the likelihood of problems.² Both the World Health Organization (WHO) and the American Diabetes Association (ADA) have recommended that HbA1c to be used in addition to other criteria to diagnose type 2 diabetes (at a value >6.5 percent). The HbA1c test evaluates average glycemia over three months rather than instantaneous blood glucose readings. HbA1c has been used as an objective measure of glycemic management for many years and it is now widely used in diabetic patient monitoring. It is used to make important management decisions, such as starting insulin therapy.³

HbA1c is influenced by a number of factors other than diabetes and blood sugar control. Iron deficiency anaemia (IDA), a common co-morbidity in the Indian population, affects a person's HbA1c level and necessitates treatment to restore it to normal. As a result, before making any decisions or following any guidelines based on HbA1c values, it is critical to demonstrate the impact of iron deficiency anaemia on HbA1c levels. All previous studies on this topic have yielded contradictory results, and the specific mechanism, as well as the relationship between iron deficiency anaemia and HbA1c levels, remains unknown. Because of this lack of matching evidence and the lack of conclusive studies on this topic, we were encouraged to conduct the current study to evaluate the effect of Iron Deficiency Anemia on HbA1c levels among non-diabetics in an Indian setting.

Materials and Methods

The present observational study was performed on minimum 100 patients attending General Medicine outpatient department of Medicine, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pune, Maharashtra.

Inclusion criteria

- Anemia with haemoglobin < 13.5 g/dl in males and <12.0 g/dl in females and ferritin less than 10 g /dl.
- Age ≥ 14 years
- Both sexes included

Exclusion criteria

- Pregnancy

- Haemolytic anemia
- Diabetes Mellitus
- Impaired glucose tolerance or fasting blood sugar >100mg/dl.
- Haemoglobinopathies
- End stage kidney disease.
- End stage liver disease.
- Confirmed case of malignancies

Methodology

All patients presenting to General Medicine outpatient department fulfilled the inclusion and exclusion criteria enrolled in the study. An informed written consent was obtained from the patients. The detailed history was taken and necessary clinical examination was carried out in all patients.

Laboratory investigations

- CBC by Automated Cell Counter.
- Peripheral Blood Film.
- Fasting blood Glucose by blood glucose meter.
- Renal Function Test
 - Blood Urea Nitrogen by Urease/Glutamate dehydrogenase coupled enzymatic technique.
 - Serum Creatinine by Kinetic Jaffe's method.
- Iron Studies
 - Serum Iron Ferrizyme method on Imola fully automatic analyser.
 - Serum ferritin Immuno turbid metric method.
 - TIBC by Colorimetric method on Imola fully automatic analyser.
- HbA1c Chromatographic method: The chromatographic assay uses an HPLC instrument and ion exchange or affinity column to separate HbA1c molecules from other hemoglobin molecules. The HbA1c content is calculated based on the ratio of HbA1c peak area to the total hemoglobin peak. Interpretation of HbA1c
 - 4-5.6: Non Diabetic
 - 5.7 - 6.4: Pre Diabetic Stage
 - >6.5: Diabetic
 - 6.5 -7.0: Well Controlled Diabetes
 - 7.1 -8.0: Unsatisfactory Control
 - >8.0: Poor Control

Statistical analysis

The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2010) and then exported to data editor page of SPSS version 20 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics included computation of percentages, means and standard deviations were calculated. The statistical tests applied for the analysis were Pearson's chi-square test, t-test, One-way Analysis

of Variance. For all tests, confidence interval and p-value were set at 95% and ≤ 0.05 respectively.

Table 1
Distribution of type of anaemia (Hb<10 g/dl)

Variables	Number of patients (n)	Percentage (%)
Microcytic Hypochromic Anemia	120	60.0
Normocytic Normochromic Anemia	35	17.5
MegaloblasticAnemia	45	22.5
Total	200	100.0

Among 200 patients who were diagnosed anaemic; 120 (60.0%) were diagnosed with microcytic hypochromic anaemia, normocytic normochromic anemia 35 (17.5) and megaloblasticanemia found among 45 (22.5%) patients.

Table 2
Gender wise comparison of various biochemical parameters

Variables	Gender	Number of patients	Mean	Std. Deviation
Hb	Male	36	8.06	1.31
	Female	64	7.59	1.94
MCV	Male	36	67.92	7.75
	Female	64	65.50	6.98
Serum Iron	Male	36	22.91	7.70
	Female	64	21.92	6.07
Serum Ferritin	Male	36	5.08	2.09
	Female	64	4.10	1.70
TIBC	Male	36	424.30	112.27
	Female	64	421.29	80.67
HbA1c	Male	36	5.99	0.79
	Female	64	5.92	0.77

The mean values of various biochemical parameters levels between male and female. Hb (8.06, 7.59), MCV (67.92, 65.50). Serum Iron (22.91, 21.92), Serum Ferritin (5.08, 4.10), TIBC (424.30, 421.29) and Hb1Ac (5.99, 5.92).

Table 3
Distribution of clinical findings

Clinical Findings	Number of patients (N)	Percent
Pallor	91	91.0

Clinical Findings	Number of patients (N)	Percent
Pallor	91	91.0
Fatigue	47	47.0
Dyspnea	34	34.0
Nail Changes	18	18.0
PICA	17	17.0
Glossitis	7	7.0

Clinical finding as, Palor 91% followed by Fatigue 47%, Dyspnea 34%, Nail changes 18%, PICA 17% and Glossitis 7%

Table 4
Association between Mean Serum Iron levels & HbA1c

HbA1c	N	Serum Iron	Serum Ferritin	TIBC
		Mean±SD	Mean±SD	Mean±SD
4-5.6	46	24.25±6.85	6.24±1.81	409.13±84.69
5.7-6.4	31	22.15±6.39	4.58±1.32	423.82±102.07
>6.5	23	21.96±6.75	3.66±1.72	437.34±84.39
p-value	0.039*		0.048*	0.552 (NS)

Test applied: One-way ANOVA. *Indicates statistical significance.

On analysis inverse association was observed between HbA1c and mean levels of serum iron store (p=0.039) and serum Ferritin levels (p=0.048). No significant association observed between Mean TIBC levels & HbA1c (p=0.552).

Discussion

The most frequent type of anemia is iron deficiency anemia. Iron deficiency is responsible for roughly half of all anemia cases in India. The most vulnerable people are children and mothers. The causes that cause iron deficiency anemia range depending on the population. HbA1c is glycated haemoglobin that can be used to assess a diabetic's glycemic status during the previous three months. Haemolytic anemias, hemoglobinopathies, acute and chronic blood loss, pregnancy, and uremia have all been linked to changes in HbA1c levels.⁴⁻⁹ HbA1c levels have long been used to monitor diabetic glycemic management. The American Diabetic Association and an international expert council have authorized HbA1c for diabetes diagnosis. A 6.5 percent HbA1c diagnostic cutoff limit has been proposed. Other than plasma glucose levels, a variety of factors can influence HbA1c levels. A variety of factors can cause artificially lower or higher results. HbA1c assays are influenced by hemolytic anemia, iron deficiency anemia, hemoglobinopathies, uremia, and chronic blood loss. HbA1c levels in more regularly encountered anemia's, such as iron deficiency anemia, have recently piqued the interest of researchers.

Despite the lengthy life span of erythrocytes, the concentration of glycosylated haemoglobin has been found to be higher in anemic patients. The increase in glycosylated haemoglobin levels in anemic individuals has been attributed to a number of processes. It has been suggested that with iron deficiency, the quaternary structure of the haemoglobin molecule is changed, allowing for increased glycosylation of the beta-globin chains. The chromatographic assay used in this work separates HbA1c molecules from other haemoglobin molecules using an HPLC apparatus and an ion exchange or affinity column. The ratio of HbA1c peak area to total haemoglobin peak is used to calculate HbA1c content. Rai KB¹⁰ evaluated the various methods for determining HbA1c and found no differences between colorimetric methods, ion-exchange chromatography, and affinity chromatography.

In the current study, out of a total of 200 anemic patients (Hb10g/dl), 120 (60%) were diagnosed with microcytic hypochromic anemia. Serum iron, serum ferritin, and TIBC testing were performed on all 120 patients. Iron deficiency was found in 108 (90%) of the patients, resulting in iron deficiency anaemia in 54% of the patients. Mondal S et al.,(2017) discovered that out of 150 patients diagnosed with microcytic hypochromic anaemia, 120 (80 percent) were iron deficient.¹¹ Iron deficiency anaemia was found in 64% of females and 36% of males in the current study. The majority of patients, or 51 percent of all patients, were between the ages of 48 and 60. In a recent study conducted in the rural populations of Punjab's major cities, Kaur and Kaur discovered that 98 percent of female and 56 percent of male subjects were anaemic.¹² It was also suggested that poor nutrition and menstrual loss in women are positively correlated with haemoglobin levels. Similar findings were found in another study by Sinha et al. who discovered that Iron Deficiency Anaemia was more prevalent in females (68%) than males (32%).¹³ In a similar study done by Christy et al. iron deficiency anaemia was reported in 58.3 percent of females and 41.7 percent of males.¹⁴

The mean Hb in this study was 7.76. Males had a mean Hb of 8.06, while females had a mean Hb of 7.59, indicating moderate anaemia. In our study, 62 percent of participants had moderate anaemia, while 18 percent had severe anaemia. In a study conducted by Gupta VK et al. out of 90 percent of cases diagnosed with iron deficiency anaemia, they discovered that moderate anaemia was observed in approximately 50% of the patients and severe anaemia was observed in only 2% of the patients.¹⁵ In the current study, patients with iron deficiency anaemia had a mean HbA1c of 5.86. The mean serum iron level in patients with Hb1Ac levels ranging from 4-5.6 was 24.25. The corresponding mean serum iron for HbA1c range 5.7-6.4 was 22.25. The corresponding mean serum iron for HbA1c levels greater than 6.5 was 21.96. According to the findings, there is an inverse relationship between HbA1c and mean serum iron levels, and this relationship is statistically significant ($p=0.05$), i.e. mean serum iron decreases as HbA1c level increases. This is consistent with the findings of a case control study conducted by Kalasker V et al. who discovered that the mean HbA1c of cases was 5.91 while it was 6.54 in healthy controls; a statistically significant difference.¹⁶ Serum ferritin and HbA1c were found to have a similar relationship in the current study. The mean serum ferritin level for Hb1Ac levels ranging from 4-5.6 was 6.24. The corresponding mean serum ferritin was 4.58 for HbA1c levels ranging from 5.7 to

6.4. The corresponding mean serum ferritin for HbA1c levels greater than 6.5 was 3.66.

Similarly, in a study conducted by Shanthi B et al. the mean HbA1c of cases were 7.6, compared to 5.5 in healthy controls. The HbA1c difference between the case and control groups was statistically significant. They hypothesised that serum iron and ferritin levels are inversely related to HbA1c levels, and that HbA1c levels tend to be higher in the presence of iron deficiency anemia.¹⁷ In contrast, Christy et al. found that the mean HbA1c of cases was 6.87, while it was 5.65 in healthy controls. They hypothesized that serum iron and ferritin levels were related to higher HbA1c levels.¹⁴ The mean TIBC level in our study for Hb1Ac ranges 4-5.6 was 409.12. The corresponding mean TIBC for HbA1c levels ranging from 5.7 to 6.4 was 423.82. The corresponding mean TIBC for HbA1c levels greater than 6.5 was 437.34. From the above values, a direct association between HbA1c and mean levels of TIBC was observed, but it was found to be statistically non-significant ($p > 0.05$).

We were unable to compare the association of Hb1Ac with TIBC in this study due to a lack of data. Pallor accounted for 91% of clinical symptoms observed in this study, followed by Fatigue (47%), Dyspnea (34%), Nail changes (18%), PICA (17%), and Glossitis (7%). In a similar study, Bharadwaj et al. found that 96 percent of patients complained of weakness and malaise, while 38 percent reported dyspnoea and 60 percent reported disinterest in work.¹⁸ Another study, conducted by Fourn L et al. found pallor in 50% of the study subjects, as well as glossitis and nail changes in 15% and 25%, respectively.¹⁹ Bager P found fatigue in 44 percent of his patients in his study.²⁰ In their study, Wu YC et al. discovered glossitis in 27% of the patients with iron deficiency who were referred to them.²¹

The study population's Fasting Blood Sugar (FBS) level (SD) was 95.66 in this study (6.25). Out of a total of 100 subjects, 74% had FBS levels greater than 90, while the remaining 26% had levels less than 90. Kim C et al. discovered that iron deficiency increased HbA1c slightly regardless of fasting glucose level.²² In the current study, the significant mean MCV level (66.33) was much lower than the normal value. Similar levels of MCV were found in an iron deficient patient study conducted by Piplaniet al.²³

Limitations with Recommendation

The study's limitations included a small sample size and the inability to track the response to iron therapy due to the cross-sectional design. Large-scale population studies are needed to assess the difference between the severity and effects of IDA on HbA1c levels. In either case, clinical data is insufficient, and more research is needed to determine the role of erythrocyte indices in HbA1c level modulation.

Conclusion

Iron deficiency anemia is the commonest nutritional deficiency worldwide but it was observed that the prevalence is higher in developing countries, and most vulnerable groups to IDA are women, children and adolescents. The present study concluded that the levels of HbA1c were increased significantly among patients

with iron deficiency anemia. So, Iron deficiency anemia has to be kept in mind before using the HbA1c to diagnose diabetes. Large scale trials over longer durations may give accurate information about the influence of iron deficiency anemia over HbA1c levels. This will increase the reliability of HbA1c in diagnosing diabetes.

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