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# INFLUENCE OF IRON DEFICIENCY ANAEMIA ON HAEMOGLOBIN A1C LEVELS IN DIABETIC INDIVIDUALS WITH CONTROLLED PLASMA GLUCOSE LEVELS

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**ABSTRACT** This case control study was done in the Department of Biochemistry, K.P.C. Medical College, Jadavpur, Kolkata. A total of 140 patients of more than 18 years were studied. All participants were controlled diabetic patients. 140 patients were divided into Case & Control group depending on presence of Iron Deficiency Anaemia. Each group consists of 70 patients. A comprehensive history of Anaemia, Blood loss, duration of diabetes, familial disorders were taken from the selected patients.

This study showed that mean HbA1C was remarkably higher in cases than controls and found to be of statistical significance ($p<0.001$). In both male and female participants mean HbA1C levels are higher in IDA patients than in non-IDA. Here, we significantly demonstrated that iron deficiency anemia not only increases A1C levels in controlled diabetic individuals but also it can interfere with its ability to determine glycemic status of diabetic individuals.In patients with iron deficiency anaemia, negative correlation was found between haemoglobin and HbA1C but of no statistical significance ($r=-0.243, p=0.089$).

This finding shows no significant linear association between haemoglobin & HbA1C. Though IDA had significant linkage with elevation of HbA1C. However, Hb played no predominant role in elevating A1C. These two findings are paradoxical.

**KEYWORDS :** Iron Deficiency anaemia, HbA1c, Diabetic and Ferritin.

## INTRODUCTION

Anaemia is one of the most under-diagnosed conditions and if left untreated, can have many serious implications such as cardiovascular disease and compromised immune functions. According to WHO estimates, India is one of the countries in the world that has highest prevalence of anaemia1. The bliss of motherhood thrives under the looming presence of anaemia in India.

Anaemia is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status. Malaria, HIV/AIDS, hookworm infestation, schistosomiasis, and other infections such as tuberculosis are particularly important factors contributing to the high prevalence of anaemia in some areas. In developing countries every second pregnant woman and about 40% of preschool children are estimated to be anaemic.[1]

Iron deficiency is the commonest nutritional deficiency worldwide, affecting; 50% of the world population. The prevalence is higher in low- and middle income countries compared with high income countries, and women, children, and adolescents are the most susceptible. It is the only nutrient deficiency which is also significantly prevalent in industrialized countries. The numbers are staggering: 2 billion people – over 30% of the world's population1 – are anaemic, many due to iron deficiency, and in resource-poor areas, this is frequently exacerbated by infectious diseases. Iron Deficiency Anaemia can cause reduced work capacity in adults and impact motor and mental development in children and adolescents. There is some evidence that iron deficiency without anaemia affects cognition in adolescent girls and causes fatigue in adult women.[2] Iron Deficiency Anaemia may affect visual and auditory functioning and is weakly associated with poor cognitive development in children. Although the etiology of Iron Deficiency Anaemia is multifaceted, it generally results when the iron demands by the body are not met by iron absorption, regardless of the reason. Individuals with Iron Deficiency Anaemia have inadequate intake, impaired absorption or transport, physiologic losses associated with chronological or reproductive age, or chronic blood loss secondary to disease. In adults, Iron Deficiency Anaemia can result in a wide variety of adverse outcomes including diminished work or exercise capacity, impaired thermoregulation, immune dysfunction, GI disturbances, and neurocognitive impairment.[3]

Haemoglobin A1c (HbA1c) is a glycated haemoglobin that can be used as an indicator of a patient's glycemic status over the previous 3 months.[4] According to the American Diabetes Association Guidelines published in 2007, HbA1c levels should be maintained below 7% in all diabetic patients in order to prevent the development of microvascular complications.[5] HbA1c levels are not affected by blood glucose levels alone. They are also altered in haemolytic anemias,[6] hemoglobinopathies,[7] acute and chronic blood loss,[8,9] pregnancy[10-12], and uraemia[13-15].Vitamin B12, folate, and iron deficiency anemias have also been shown to affect HbA1c levels. Conditions that affect erythrocyte turnover affect HbA1c concentrations. Although many forms of anaemia are associated with lowering of HbA1c, iron deficiency tends to increase HbA1c. In addition to ambient glycemia, factors affecting erythrocyte life span affect HbA1c concentrations. For a comparable glycemic exposure, conditions that shorten erythrocyte life span reduce HbA1c concentrations (haemolytic anemias, infections, blood loss, hypersplenism, malaria, and pregnancy). On the other hand, prolongation of erythrocyte survival (iron deficiency, splenectomy, aplastic anaemia, and certain hemoglobinopathies) elevates HbA1c concentrations[16].Kidney and liver disease have complex effects on HbA1c concentrations. In addition, individual differences in erythrocyte permeability to glucose and intracellular concentrations of its metabolites have been shown to influence rate of glycation and could explain some of the variance [17,18]. In addition to increasing erythrocyte survival, iron deficiency could alter these parameters. It is also suggested that iron deficiency may alter the quaternary structure of the haemoglobin molecule and facilitate glycation of the b-globin chain[19]. Finally, as yet un-investigated genetic and environmental factors, which may influence erythrocyte dynamics, including inflammation, could also contribute to the remaining variance. [20,21]

Studies showed that HbA1c levels were higher in patients with iron deficiency anaemia and decreased significantly upon treatment with iron [16-18].The results of these studies are conflicting, and the exact mechanism underlying the effects of iron deficiency anaemia on HbA1c levels is not yet known. Since there is scarcity of similar studies conducted on the diabetic population, moreover in a setting where both the conditions i.e. T2 DM and Iron deficiency anaemia are widely prevalent; this study was planned to investigate the effects of iron deficiency anaemia on HbA1c levels in diabetic patients.

HbA1c is expected to be elevated in Diabetic individuals having Iron Deficiency Anaemia with controlled plasma glucose level in comparison to controlled diabetic population; therefore creating

confusion regarding decision making for management of diabetes. Based on this hypothesis, the aim of the present study was to assess the influence of Iron Deficiency Anaemia on Haemoglobin A1C Levels in Diabetic Individuals with Controlled Plasma Glucose Levels.

We find out the relation between Haemoglobin and HbA1c, whether Haemoglobin percentage is independent of HbA1c level in patients of Iron Deficiency Anemia and to identify any association between HbA1c and Ferritin.

## MATERIALS AND METHODS

This Case-control study was conducted at Hospital based K.P.C. Medical College & Hospital, Jadavpur, Kolkata from May 2017- April 2018.

**Sample size:**

**a) Case:** Seventy (70) patients of Iron Deficiency Anaemia (IDA) with controlled diabetes were taken.Adult patients of Iron Deficiency Anemia with controlled diabetes irrespective of sex, nutritional status, socio-economic status & duration of diabetes as per ADA 2016 Guideline.

**b) Control:** Seventy (70) patients of controlled diabetes without IDA were included in this study. Adult controlled Diabetic patients matched for Age, Sex and duration of diabetes

**Inclusion criteria for cases:**

1) Patients suffering from Iron Deficiency Anemia having low hemoglobin levels (<12 g% in males and <11 g% in female), predominantly microcytic indices (MCV <76 fL), and hypochromic indices (MCH <27 pg/cell) and low serum ferritin levels (<29 and <20 ng/ml in males and females, respectively).
2) Diabetic patients plasma glucose levels in control (FPG <126 mg/dl) since last 3 months

**Inclusion criteria for controls:**

Diabetic patients plasma glucose levels in control (FPG <126 mg/dl) since last 3 months

**Exclusion criteria for cases:**

Patients with hemoglobinopathies, hemolytic anemia or other causes of anaemia

**Exclusion criteria for both cases and controls:**

1) Hypothyroidism
2) Pregnancy
3) Patients having abnormal renal function test (serum urea, creatinine, and estimated glomerular filtration rate)
4) Severely ill or moribund patient
5) Those who did not give consent for the study

**Study tools:**

- Pre-designed and pre-tested schedule for collecting relevant information
- Existing medical records
- Weighing machines, sphygmomanometer, measuring tape, stethoscope
- Centrifuge machine
- Auto analyser
- Respective reagent kit
- HPLC machine

**Study techniques**

- Interview for taking socio-demographic information and disease history
- Clinical examination
- Laboratory investigation

a) Biochemical investigation
b) Hematological investigation

- Review of records

**Statistical Methods**

Continuous variables are expressed as Mean, Median and Standard Deviation and compared across groups using and Mann-Whitney U test/Kruskal Wallis Test as appropriate.

Association between Continuous variables are captured using Spearman's Rank correlation coefficient. The statistical software SPSS version 20 will be used for the analysis.

An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it will be considered as significant. The data were presented as mean ± SD. A student's t-test was applied for comparison of group means. Pearson's coefficient of correlation was calculated to determine the correlation between the two variables. Categorical data was analyzed by $\chi 2$ test.

## RESULTS

Data of HbA1c, peripheral smear, haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), serum ferritin, and plasma glucose levels was also collected from the non-anemic controls (matched for sex and plasma glucose levels).

**Selection of cases and controls**

The subjects with microcytic hypochromic peripheral smear, low haemoglobin levels (<12 g% in males and <11 g% in female), predominantly microcytic indices (MCV <76 fL), and hypochromic indices (MCH <27 pg/cell) were considered to have iron deficiency anemia, which was confirmed by low serum ferritin levels (<29 and <20 ng/ml in males and females, respectively). Subjects having FPG >126 mg/dl were excluded from the study. Patients with hemoglobinopathies, hemolytic anemia, hypothyroidism, pregnant patients, and patients having abnormal renal function test (serum urea, creatinine, and estimated glomerular filtration rate were also excluded from the study.

HbA1c was measured by HPLC method using Bio-Rad D-10 analyzer. Method of estimation and the analyzer used to perform HbA1c analysis were the same throughout the study period. Hemoglobin, MCV, MCH, and MCHC estimation was carried out by (Beckman Coulter) BC-5380 hematology cell counter, and serum ferritin estimation was performed by electrochemiluminescence method using Roche/ Hitachi Cobas e411 analyzer. Also, plasma glucose was estimated by glucose oxidase/peroxidase method using BS-390 Biochemistry analyse r(Mindray Company).

Majority of the participants in total (37.1%) and also cases (40.0%) and controls (34.3%) aged between 51-55yrs; followed by 21.4% and 25.7% of total participants and cases respectively in age group 46-50yrs, and 22.9% of controls in 56-60yrs. None of the cases were below 46yrs and more than 65 yrs.

More than half of the selected patients were female. The sex distribution of cases and controls were same (57.1% females and 42.9% males) as the participants were matched by sex.

The mean age of total participants, cases and controls were 54.5yrs, 54.39yrs and 54.7yrs respectively. Females were more than male. Mean fasting plasma glucose values were 94.22 gm/dl, 95.81 gm/dl and 92.63 gm/dl. No significant differences in age and Fasting Plasma Glucose levels between cases and controls were found; which establishes the adequacy of matching.

Mean Hb was significantly lower in cases than controls. Hb value ranged between 8.8 to 10.5 for cases and between 12.5 to 15.5 for controls. The median values were 9.4 and 13.4 respectively.

Multiple bar diagram shows that for both cases and controls mean Hb levels were higher in male than in females. Mean Hb of males with iron deficiency anaemia was 9.65gm/dl compared to 9.42 gm/dl in case of females with IDA. For non anemic individuals the mean Hb for males were 14.71gm/dl compared to 12.97 for females.

Mean MCV was significantly lower in cases than controls. The observed values of MCV ranged between 59-79 for cases and between 81-91 for controls. The median values were 70 and 85 respectively.

Multiple bar diagram compares the MCV levels in males and females for both cases and controls. Mean MCV levels were higher in females than in males for the patients with IDA however this picture was otherwise in case of controls. Mean MCV of females with iron deficiency anaemia was 67.73 compared to 72.48 in case of males with IDA. For non anemic patients the mean MCV for males were 87.33 compared to 83.95 for females.

Mean ferritin was significantly lower in cases than controls. The

observed values ranged between 5-11 for cases and between 32-151 for controls. The median values were 8 and 84 respectively.

For both cases and controls mean Ferritin levels were higher in male than in females. Mean Ferritin of males with iron deficiency anaemia was 9.18ng/ml compared to 8.1ng/ml in case of females with IDA. For non anemic individuals the mean Ferritin for males were 82.2ng/ml compared to 81.97ng/ml for females.

The differences in mean values of serum iron levels in cases and controls. Mean serum iron was significantly lower in cases than controls. The observed values ranged between 10-53 for cases and between 48-110 for controls. The median values were 22 and 69 respectively.

For both cases and controls mean Serum Iron levels were higher in male than in females. Mean Serum Iron of males with iron deficiency anaemia was 36.03mcg/dl compared to 18.33mcg/dl in case of females with IDA. For non anemic individuals the mean Serum Iron for males were 80.6mcg/dl compared to 62.33mcg/dl for females.

Mean TIBC was significantly higher in cases than controls. The observed values ranged between 417-528 for cases and between 315-379 for controls. The median values were 447 and 351 respectively. The direction of differences corroborated with other haematological parameters.

For both cases and controls mean TIBC levels were higher in male than in females. Mean TIBC of males with iron deficiency anaemia was 461.4mcg/dl compared to 451.15mcg/dl in case of females with IDA. For non anemic individuals the mean TIBC for males were 352.27mcg/dl compared to 349.53mcg/dl for females.

Mean MCH was significantly lower in cases than controls. The observed values were between 16 to 29 for cases and between 26 to 31 for controls. The median values were 22 and 28 respectively. For both cases and controls mean MCH levels were higher in male than in females. Mean MCH of males with iron deficiency anaemia was 22.78pg/cell compared to 21.03pg/cell in case of females with IDA. For non anemic individuals the mean MCH for males were 28.3pg/cell compared to 27.83pg/cell for females.

Mean MCHC was significantly lower in cases than controls. The observed values ranged between 21 to 29 for cases and between 33 to 36 for controls. The median values were 25.5 and 34 respectively.

For both cases and controls mean MCHC levels were higher in male than in females. Mean MCHC of males with iron deficiency anaemia was 27.33g/dl compared to 24.7g/dl in case of females with IDA. For non anemic individuals the mean MCHC for males were 34.73g/dl compared to 33.4g/dl for females. Mean RDW was significantly lower in cases than controls. The observed values between 15 to 18 for cases and between 11 to 14 for controls. The median values were 16.8 and 12.5 respectively.

For both cases and controls mean RDW levels were higher in female than in males. Mean RDW of females with iron deficiency anaemia was 16.98% compared to 16.7% in case of males with IDA. For non anemic individuals the mean RDW for females were 12.58% compared to 12.7% for males.

Mean HbA1C was significantly higher in cases than controls. The observed values between 6.61 to 8.16 for cases and between 5.40 to 6.25 for controls. The median values were 7.21 and 5.87 respectively. This finding is also present when we stratify the data in genders, as shown in the following figure.

For both cases and controls mean HbA1C levels were higher in male than in females. Mean HbA1C of females with iron deficiency anaemia was 7.42% compared to 6.97% in case of males with IDA. For non anemic individuals the mean HbA1C for females were 5.91% compared to 5.79 for males. In both male and female participants mean HbA1C levels are higher in IDA than non-IDA.

## DISCUSSION

This was a comparative study done in the department of Biochemistry, KPC Medical College &Hospital, Jadavpur, Kolkata. In this study, 70 patients having IRON deficiency Anaemia with controlled diabetes mellitus were studied. 70 age and sex matched Non Iron deficiency Anaemia with controlled Diabetes mellitus was also studied in controlled group.

In this study, patients above 18 years of age were included. Patients aged between 51-60 years were maximum in number (37.1%) combining both case & control groups. There was no significant difference in age and gender between two group. Both groups were matched in terms of age and gender. Majority of the participants in case group (40.0%) and controls (34.3%) aged between 51-55yrs. None of the cases were below 46yrs and more than 65 yrs. The present study had female predominance among case. The male to female ratio [M : F] was 42.9 : 57.1. Pie diagram shows that more than half of the selected patients were female. The sex distribution of cases and controls were same (57.1% females and 42.9% males) as the participants were matched by sex. The mean ages of participants in case and control groups were 54.39 yrs and 54.7yrs respectively. Statistically non-significant correlation was found between case and control groups (p=0.736). Patients in both groups were age matched. Average ages of total participants were 54.5 years.

In this study, mean fasting plasma glucose values for case, control and combined were 95.81 mg/dl, 92.63 mg/dl and 94.22 mg/dl respectively. Fasting Plasma Glucose levels had no significant association between cases and controls groups (p=0.082).

Our study shows mean Haemoglobin level was significantly lower in case group compared to controls where p<0.001. Hb values were ranged between 8.8 g/dl to 10.5 g/dl for cases. In control group, Haemoglobin ranged from 12.5 g/dl to 15.5 g/dl. The median values for both case and control were 9.4 g/dl and 13.4 g/dl respectively. Combining both case and control groups, we observed that mean Hb levels were higher in males. Male patients with iron deficiency anaemia had mean Hb value of 9.65gm/dl in contrast to female IDA patients, where we found mean Hb level of 9.42 gm/dl. Further, we found that in non anaemic individuals, mean Hb levels for males and females were 14.71 g/dl and 12.97g/dl respectively.

This study shows Mean MCV value was prominently lower in cases than controls. The correlation was found to be of statistical significance (p<0.001). The observed values of MCV ranged between 59-79 fL for cases and 81-91 fL for controls. The median values were 70 fL and 85 fL respectively. Mean MCV levels in IDA patients were higher in females compared to males. In case group, Mean MCV of males was 67.73 fL and females was 72.48 fL. For non anemic patients the mean MCV for males and females were 87.33 and 83.95 respectively.

Statistically significant association was found between Case and Control group in respect to MCH (p<0.001). The mean MCH values were reportedly lower in IDA patients compared to control group. The values of MCH varied between 16 to 29 pg/ml for cases and 26 to 31 pg/ml for controls. The median values for case and control group were 22 pg/ml and 28 pg/ml respectively. Our study shows that for both cases and controls mean MCH levels were higher in male than in females. Mean MCH of males with iron deficiency anaemia was 22.78pg/cell compared to 21.03pg/cell in case of females with IDA. For non anemic individuals the mean MCH for males were 28.3pg/cell compared to 27.83pg/cell for females

In this study, we assessed MCHC levels in patients of both groups. Statistically significant association was found. Mean MCHC levels were comparatively lower in cases than controls (p<0.001). The MCHC values were ranged between 21 to 29 g/dl for cases and 33 to 36 g/dl for controls. The median values were 25.5 g/dl and 34 g/dl for IDA & non – IDA group respectively. Our study shows that for both cases and controls mean MCHC levels were higher in male than in females. Mean MCHC of males with iron deficiency anaemia was 27.33g/dl compared to 24.7g/dl in case of females with IDA. For non anemic individuals the mean MCHC for males were 34.73g/dl compared to 33.4g/dl for females

Moreover, it was found that mean RDW was significantly lower in cases than controls (p<0.001). The results varied from 15 to 18 % for IDA patients and 11 to 14 % in non IDA patients. The median values were 16.8% and 12.5% for case & control group respectively. Our study shows that for both cases and controls mean RDW levels were higher in female than in males. Mean RDW of females with iron deficiency anaemia was 16.98% compared to 16.7% in case of males

with IDA. For non anaemic individuals the mean RDW for females were 12.58% compared to 12.7% for males

Here, we found that mean ferritin levels were markedly lower in cases than controls (p<0.001). The observed ferritin values ranged between 5-11ng/ml for cases and between 32-151ng/ml for controls. The median values were 8ng/ml and 84ng/ml respectively. The aforementioned findings conclude that there is statistically significant correlation between ferritin & IDA. Our study shows that for both cases and controls mean Ferritin levels were higher in male than in females. Mean Ferritin of males with iron deficiency anaemia was 9.18ng/ml compared to 8.1ng/ml in case of females with IDA. For non anaemic individuals the mean Ferritin for males were 82.2ng/ml compared to 81.97ng/ml for females

We also observed that mean serum iron was significantly lower in case group than controls (p<0.001). The values ranged between 10-53 mcg/dl for cases and between 48-110 mcg/dl for controls. The median values of serum Iron were 22mcg/dl and 69mcg/dl for case and control group respectively. These finding suggest that in IDA, serum Iron levels markedly diminishes. Our study shows that for both cases and controls mean Serum Iron levels were higher in male than in females. Mean Serum Iron of males with iron deficiency anaemia was 36.03mcg/dl compared to 18.33mcg/dl in case of females with IDA. For non anaemic individuals the mean Serum Iron for males were 80.6mcg/dl compared to 62.33mcg/dl for females

As per our study, mean TIBC was remarkably higher in IDA patients which is of statistical significance where p<0.01). The values generated were of interval between 417-528 mcg/dl for cases and 315-379 mcg/dl for controls. The median values were 447 mcg/dl and 351 mcg/dl for IDA & non –IDA participants respectively. The direction of differences corroborated with other haematological parameters. Our study shows that for both cases and controls mean TIBC levels were higher in male than in females. Mean TIBC of males with iron deficiency anaemia was 461.4mcg/dl compared to 451.15mcg/dl in case of females with IDA. For non anaemic individuals the mean TIBC for males were 352.27mcg/dl compared to 349.53mcg/dl for females
Mean HbA1C levels in our study were significantly higher in IDA patients compared to control group (p<0.001). The recorded values varied between 6.61 to 8.16 for cases and 5.40 to 6.25 for non IDA patients. Similarly, median values were markedly higher in IDA patients. 7.21 and 5.87 were the HbA1C median values in case and control groups respectively. This finding is also present when we stratify the data in genders. Our study shows that for both cases and controls mean HbA1C levels were higher in male than in females. Mean HbA1C of females with iron deficiency anaemia was 7.42% compared to 6.97% in case of males with IDA. For non-anaemic individuals the mean HbA1C for females were 5.91% compared to 5.79 for males. In both male and female participants, mean HbA1C levels were higher in IDA. Therefore, iron deficiency anaemia not only increases HbA1C levels in non-diabetic individuals but also it can interfere with its ability to determine glycaemic status of diabetic individuals.

**Relation between haemoglobin and HbA1c:**

Similarly, in our study Hb was negatively correlated with HbA1c, but no significant correlation was found(r=-0.243, p=0.089). Koga et al. [22] found that HbA1C and haemoglobin were associated negatively in non-diabetic premenopausal women. In addition, post-menopausal women did not show any significant association [22]. This study shows higher levels of A1C in females both in pre and postmenopausal groups, but the probability of having an A1C above 6.5 was low and statistically non-significant. A1C was more in postmenopausal compared to premenopausal women. In a study by Dasgupta et al. [23], no significant difference in HbA1c levels were noted in postmenopausal and premenopausal women irrespective of anemia [23]. These findings suggest that anaemia has a predominant role in elevating HbA1C. Observations of our study in context to relation between Hb & HbA1C were very much in concordance to other studies. Most of the authors found similar outcomes, where Hb has negative correlation with HbA1 but, statistically non-significant.

**Correlation between HbA1c and Ferritin:**

In patients with iron deficiency anaemia, ferritin and HbA1C were found to be negatively correlated. Whereas in non-IDA patients, positive correlation was found in this study. However, none of the correlations were found to be statistically significant(r=-0.132,p=0.360). This study could not explain the lack of correlation of serum ferritin levels with HbA1c. As explained previously, in iron deficiency anemia, ferritin is decreased with increase in the red cell life span, and increased red cell life span is associated with increased HbA1c. However, one of the studies did not show any significant correlation of serum ferritin levels and red cell life span [24], indicating the lack of significant correlation between ferritin and HbA1c in our study. Various studies have shown elevated ferritin in diabetic population, though its mechanism is still debatable. In a study by Raj and Rajan [25], ferritin showed positive correlation with HbA1c in diabetic individuals. In addition, Canturk et al. [26] found that serum ferritin was elevated as long as glycemic status was not achieved, thus they found normal ferritin levels in diabetic individuals. Sharifi and Sazandeh [27] did not find any significant correlation between HbA1c and ferritin in diabetic population. Our findings are partially similar to other studies.

**Effect of Iron Deficiency Anemia on HbA1c levels in Diabetic population having plasma glucose levels in control:**

The effect of Iron deficiency on HbA1C was found to be statistically significance (p<0.001). There was marked difference in mean HbA1C levels between case (IDA) and control group (Non-IDA). Here, we observed that 84.3% of the IDA patients had HbA1c levels higher than the standard cut-off range (i.e. >6.5). However, none of the participants in control group i.e. patients without IDA had HbA1C values above standard cut-off. Initial studies conducted by Brooks et al. , Gram-Hansen et al. , and Coban et al. showed effects of iron therapy on glycated haemoglobin and found a significant reduction in HbA1c levels after iron therapy in non-diabetic population [28-31]. According to the explanation provided by Sluiter et al. [32], hemoglobin glycation is an irreversible process. Hence, HbA1 levels in erythrocyte will be increased with cell age. In iron deficiency, red cell production decreases, consequently an increased average age of circulating red cells ultimately leads to elevated HbA1 levels. According to some workers, the changes in HbA1c levels were due to different laboratory methods used to analyze it. Goldstein et al. [33] demonstrated that HbA1c measured by HPLC was increased two hours after a standard breakfast and incubating the red cell in 0.9% saline at 37°C for five hours eliminated this increment [33], which was explained by presence of labile HbA1c. This effect was eliminated by reagents used in newer enzymatic kits. Rai and Pattabiraman conducted a study to evaluate different methods used to analyze HbA1c and found no significant difference between them [34]. However, in a study by one study, the results were inconclusive with some subjects showing elevation in A1C, while some showed no elevation. In a study carried out by Hashimoto etal. [35],A1C levels were elevated in pregnant diabetic women. Pregnancy is another condition which can cause spurious A1C elevation. Pregnancy is mostly associated with iron deficiency anemia. The study showed that it was iron deficiency anemia which caused elevated A1C, and not pregnancy itself. Hence, Hashimoto and co-workers [35] concluded that it should not be used as a marker of glycemic control, especially in later half of pregnancy.

In our study, we found negative correlation between HbA1C with MCV ®= -0.155 p=0.283), Ferritin (r= -0.132 p=0.360), TIBC (r= -0.072 p=0.621), MCH (r= -0.024 p=0.870), MCHC (r=-0.099 p=0.494). None of the above parameters had statistically significant relation with HbA1C. This study also found that, HbA1C had negative correlation but statistically significant relationship with Iron (r=-0.294 p=0.038). Positive correlation was found between HbA1C and RDW, though of no statistical significance (r=0.111 p=0.442). However, positive correlation of statistical significance was observed between HbA1C and Haemoglobin. Two previous studies demonstrated that a correlation between HbA1c and Hb, MCV, and MCH levels exists. [36,37] Starting from data of the National Health and Nutrition Examination Surveys, Ford et al. observed that among all participants HbA1c increased progressively. Using a linear regression analysis, the difference between adjusted mean concentrations of HbA1c among individuals with IDA (5.56%) and those without (5.46%) was only 0.1% (p=0.095). Using a generalised linear model, a negative correlation between HbA1c and MCHC was observed. In addition, the correlation with MCV was also negative. More recently, in a retrospective study, Grossman et al. found that the correlation between HbA1c and Hb, haematocrit, and nutritional factor causing anaemia in elderly subjects, was inconsistent. [38] Similar to our findings, no statistical association was found between HbA1C & Haematological

indices like MCV, MCH & MCHC.

In patients with iron deficiency anaemia, ferritin and HbA1C were found to be negatively correlated whereas in non-IDA patients these were positively correlated. However, none of the correlations were found to be statistically significant(r=-0.132, p=0.360). This study could not explain the lack of correlation of serum ferritin levels with HbA1c. This study found statistical correlation between HbA1C and iron deficiency anaemia patients. Most (84.3%) of the patients with iron deficiency anaemia had HbA1C values higher than the standard cut-off.

## CONCLUSION

While concluding, this study reinforces the observation that there is strong association between Iron deficiencies with HbA1C. Before altering the treatment regimen for diabetes, iron deficiency anemia should be considered. Therefore HbA1C cannot be the sole parameter for monitoring glycaemic status of a diabetic patient.

As our topic of study is socially sensitive, before validating the observations, this requires population based research rather than a hospital based study. Once verified, this can potentially improve the quality and standard of monitoring and management.

**Table-1: Baseline Information And Biochemical Parameters**

| Variables | | Total [n=140] | Cases (IDA) [n=70] | Controls (Non-IDA) [n=70] | Significance |
|---|---|---|---|---|---|
| Age (years) [mean (SD)] | | 54.54(5.49) | 54.39 (4.8) | 54.70 (6.1) | 0.736a |
| Sex [No. (%)] | Male | 60 (42.9) | 30 (42.9) | 30 (42.9) | NA |
| | Female | 80 (57.1) | 40 (57.1) | 40 (57.1) | |
| FPG (mg/dl) [mean (SD)] | | 94.22 (10.84) | 95.81 (11.4) | 92.63 (10.06) | 0.082a |
| Haemoglobin (gm/dl) Mean (SD) | | | 9.52 (0.52) | 13.72 (0.97) | <0.001* |
| MCV Mean (SD) | | | 70.44 (5.6) | 85.4 (2.96) | <0.001* |
| Ferritin Mean (SD) | | | 8.88 (1.77) | 83.76 (28.3) | <0.001* |
| Iron Mean (SD) | | | 25.9 (12.76) | 70.16 (19.26) | <0.001* |
| TIBC Mean (SD) | | | 455.54 (29.62) | 350.7 (14.18) | <0.01* |
| MCH Mean (SD) | | | 22.14 (3.12) | 28.91 (2.81) | <0.001* |
| MCHC Mean (SD) | | | 26.09 (3.6) | 34.69 (1.59) | <0.001* |
| RDW Mean (SD) | | | 16.43 (1.81) | 12.94 (1.18) | <0.001* |
| HbA1C % Mean (SD) | | | 7.33 (0.86) | 5.9 (0.34) | <0.001* |

**Table -2: Correlation Between Hba1c And Different Haematological Parameters In Patients With Ida**

| | | Hba1c |
|---|---|---|
| Hb | Correlation Coefficient | -0.243 |
| | p Value | 0.089 |
| MCV | Correlation Coefficient | -0.155 |
| | p Value | 0.283 |
| FPG | Correlation Coefficient | 0.941 |
| | p Value | <0.001 |
| FERRITIN | Correlation Coefficient | -0.132 |
| | p Value | 0.360 |
| IRON | Correlation Coefficient | -0.294 |
| | p Value | 0.038 |
| TIBC | Correlation Coefficient | -0.072 |
| | p Value | 0.621 |
| MCH | Correlation Coefficient | 0.024 |
| | p Value | 0.870 |
| MCHC | Correlation Coefficient | -0.099 |
| | p Value | 0.494 |
| RDW | Correlation Coefficient | 0.111 |
| | p Value | 0.442 |

**Table 3: Association of HbA1C values with Iron deficiency status**

| Iron deficiency status | HbA1C values | | | p-value |
|---|---|---|---|---|
| | High (>6.5%) | Normal (≤ 6.5%) | Total | <0.001* |
| Cases (IDA) | 32 (45.71%) | 38 (54.29%) | 70 (100.0%) | |
| Controls (Non-IDA) | 0 (0.0%) | 70 (100.0%) | 70 (100.0%) | |
| Total | 32 (22.86%) | 108 (77.14%) | 140 (100.0%) | |

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