IRON DEFICIENCY ANEMIA; CHALLENGES THE DIAGNOSTIC AND PROGNOSTIC EFFICACY OF HBA1C IN DIABETES MELLITUS

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ABSTRACT

Objective: To find out the effect of iron deficiency anemia HbA1c levels in female patients with and without diabetes by comparing its values in groups.

Study Design: Comparative cross-sectional study

Place and Duration of Study: Physiology department, Al-Nafees Medical College and Pakistan Institute of Medical Sciences, Islamabad, from Jan 2017 to Dec 2018.

Methodology: Study Participants were divided into four groups. Control group A, had females without diabetes and iron deficiency anemia whereas group B had anemic females without diabetes. Study subjects of groups C were having diabetes with anemia where group D females were with diabetes and without iron deficiency anemia. Values of HbA1c were compared in anemic and non-anemic groups, in females with and without diabetes by applying ANOVA and Post Hoc Tukey's Test. Haemoglobin levels, HbA1c, erythrocyte indices and fasting blood glucose (FBG) were measured.

Results: One hundred and forty adult females with and without diabetes were selected in the study. Significant p values were obtained when HbA1c levels of patients with anemia were compared with those without anemia in nondiabetics (*p*-value <0.00) as well as in diabetic group (*p*-value=0.048). Pearson correlation also showed significant negative correlation (r= -0.66, *p*-value <0.00) of haemoglobin levels with HbA1c.

Conclusion: Iron deficiency anemia was found to be associated with an increase in HbA1c values in females with and without diabetes and hence can affect screening, diagnostic and prognostic efficacy of HbA1c for diabetes mellitus.

Keywords: Diabetes mellitus, Diagnostic marker, HbA1c, Iron deficiency anemia.

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INTRODUCTION

Diabetes mellitus is a chronic progressive metabolic disorder associated with hyperglycemia due to abnormal insulin secretion, action, or both.[1] Drastic raise in number of patients of this disease is the result of a shift of people towards urbanization which is an essential consequence of modernization.[2] Constant and persistent rapid rise in this disease, its chronic course and high incidence of associated yet preventable severe complications demand an early diagnosis of diabetes. HbA1c is the latest addition to diagnostic criteria and researches on HbA1c now recommend it with a cut off of over 6.5% to be a better diagnostic test for diabetes mellitus.[3] Although the methods of estimation of HbA1c have been standardized worldwide and benefits associated with its blood glucose estimation greatly prove its superiority over fasting blood sugar (FBS) and oral glucose tolerance test (OGTT),[4] there are confounders which still keep the debate on its use as a diagnostic and screening tool alive.[5] Anemia being most prevalent of the confounders of HbA1c has recently gained a lot of attention, especially in developing countries.[6]

In Pakistan, prevalence of iron deficiency anemia (IDA) is especially high among females of reproductive age ranging as high as to 50%.[7] Therefore in our country where there is a high incidence of both diabetes and IDA; the effect of IDA on this diagnostic test of diabetes must be evaluated. Studies show that IDA is associated with a change HbA1c values, the correlation of these two however remains a controversy.[8,9]

Owing to the high prevalence of diabetes and Iron deficiency anemia in our population, rationale of this study was to investigate the effect of iron deficiency anemia on HbA1c and use of this test for diagnosing and screening diabetes especially in areas with high prevalence of IDA.

METHODOLOGY

This comparative cross sectional study was carried out at Al Nafees Medical College and Hospital Islamabad in alliance with Pakistan Institute of Medical Sciences Islamabad, from January to Decem-

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ber 2017. WHO calculator was used to calculate the sample size of 35 in each group, using 5% level of significance, 95% confidence interval, 7.5% margin of error and 29% pooled prevalence of four study groups. [10-12]

Inclusion Criteria: Females over 18 years of age were included in the study. Groups A and B carried non diabetic females with normal and reduced haemoglobin levels respectively while groups C and D had patients of diabetes with and without IDA respectively.

Exclusion Criteria: Pregnant females and patients with uncontrolled diabetes or with renal complications (protein urea), cardiac failure and hemoglobinpathies were excluded from the study.

Ethical review committee of Al Nafees Medical College and Hospital, Islamabad evaluated and allowed to conduct the study (ltr no. F.2/IUIC-ANMC/EC-132/2016).

Study subjects which were divided into four group. Confirmation of cases of controlled diabetes and iron deficiency anemia was done on basis of laboratory tets. Non-probability consecutive sampling was carried out at the OPD and from the admitted patients. Blood samples were taken in CBC bottles as well as in serum gel bottles. Samples were saved and stored at 4-C to be analyzed for CBC and HbA1c. Serum ferritin levels were also checked to exclude cases of anemia other than IDA and FBG was taken by glucometer to exclude uncontrolled diabetes. Urine was tested for proteins by Dipstick to exclude cases of diabetic nephropathy.

Data were analyzed using Statistical Package for the social sciences (SPSS) version 23. Mean and SD were calculated followed by application of ANOVA and Post Hoc Tukey's test. Pearson correlation was applied to find degree of association between haemoglobin levels and HbA1c. The *p*-value of ≤0.05 was considered significant.

RESULTS

A total of 140 subjects (70 patients with diabetes and 70 without diabetes) participated in the study. Each group had further equal number of anemic and non-anemic individuals with 35 subjects in each group.

Comparison of HbA1c levels in groups A and B i.e. females without diabetes and anemia (3.97% ± 0.66) with anemic females without diabetes (5.98% ± 0.58) showed a markedly higher values with a highly significant *p*-value of 0.001. Patients without diabetes and anemia when compared for FBG with females having diabetes and anemia i.e. groups A and C respectively were found to have highly significant *p*-value of 0.001 as shown in Table-I.

In addition, mean HbA1c of controlled diabetics with and without anemia were 6.14% ± 0.88 and 5.62% ± 1.12, respectively inferring an increased value in anemic group with a significant *p*-value of 0.048. Whereas, FBG in patients with diabetes amongst anemic and non-anemic groups were not marked with much difference in values (93.5 g/dl ± 12 and 93.28 g/dl ± 11) in groups A and B and (101.20 g/dl ± 14 and 108.08 g/d l ± 15) in groups C and D. Insignificant *p*>0.05 values were obtained on comparison of FBG levels in both groups with values 1 and 0.148 respectively. There was an insignificant difference in comparison of HbA1c values of groups B (females without diabetes having anemia) and C (females with diabetes and anemia) *p*-value 0.084 and insignificant *p*-value of 0.283 was obtained when HbA1c of group B (females without diabetes having anemia) was compared with group D (females with diabetes and without anemia), depicted in Table-II.

Table-I: Comparison of HbA1c levels in the study groups (n=35).

Groups Variables	Group A (Non Diabetic Non-Anaemic)	Group B (Non Diabetic Anaemic)	Group C (Diabetic Anaemic)	Group D (Diabetic Non Anaemic)	*p*-value
Hemoglobin (g/dl)	13.24 ± 0.83	10.38 ± 0.95	10.25 ± 1.0	13.08 ± 0.8	0.001
HbA1c (%)	3.97 ± 0.66	5.98 ± 0.58	6.14 ± 0.88	5.62 ± 1.12	0.001
Fasting Blood Glucose (mg/dl)	93.5 ± 12	93.28 ± 0.11	108.08 ± 15	101.20 ± 14	0.001

Table-II: Post hoc Analysis of the study groups.

Groups	Group A (Non Diabetic Non-Anaemic)		Group B (Non Diabetic Anaemic)		Group C (Diabetic Anaemic)	
Variables	Group B (Non Diabetic Anaemic)	Group C (Diabetic Anaemic)	Group D (Diabetic Non Anaemic)	Group C (Diabetic Anaemic)	Group D (Diabetic Non Anaemic)	Group D (Diabetic Non Anaemic)
Hemoglobin (g/dl)	0.001	0.001	0.904	0.947	0.001	0.001
HbA1c (%)	0.001	0.001	0.001	0.084	0.283	0.048
Fasting Blood Glucose	1.000	0.001	0.085	0.001	0.072	0.148

The correlation of haemoglobin with HbA1c; Hb and HbA1c levels showed highly significant negative correlation with one another (r=-0.66. *p*-value <0.00) alue as shown in Table–III.

Table-III: Correlation of Hemoglobin levels.

Parameter	Comparison Parameter	*p*-value	Correlation Coefficient
Hemoglobin (g/dl)	HbA1c (%)	0.001	- 0.669¥
	FBG (mg/dl)	0.348	- 0.081¥

*Significant p≤0.05, **Highly significant p≤0.01, ¥ Negative Correlation ∑ Positive Correlation*

Haemoglobin levels when plotted against HbA1c for correlation showed a significant negative correlation between the two-para meters proving that a decrease of haemoglobin level was associated with decrease in HbA1c levels (Figure). Haemoglobin levels when correlated with FBG showed an insignificant negative correlation (r=-0.08, *p*-value=0.34). This result showed insignificant association of the two parameters and therefore was important in having the suggestibility that a change in haemoglobin levels will not essentially correlate with FBG levels which were therefore unaffected by anemia in the population (Table-III).

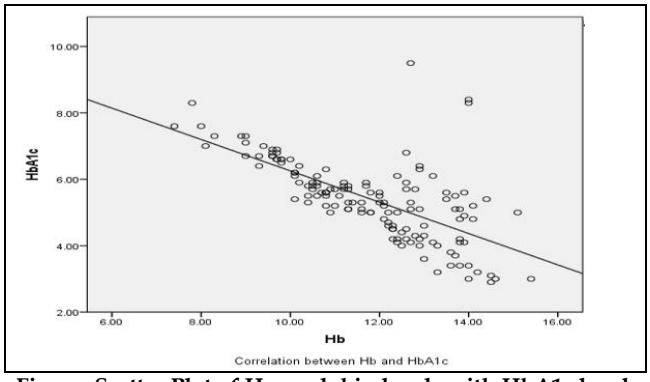

Figure: Scatter Plot of Hemoglobin levels with HbA1c levels.

DISCUSSION

HbA1c is the newer recommended diagnostic tool for diabetes mellitus which was included in diagnostic criteria of diabetes by American Diabetic Association (ADA) (2010) and World Health Organistaion (WHO) (2011).[14] The use of HbA1c has become a concern since its recommendation as a diagnostic and screening tool especially in developing countries as its confounders include one of highly prevailing IDA in third world countries.[15]

Results inferred a marked increase in HbA1c of anemics when compared with non-anemic population in both females with and without diabetes. These results were endorsed by a study done by Urrechaga *et al*.[8] who found a positive correlation between iron deficiency and increased HA1c levels and inferred that that the diagnostic reliability of HbA1c is limited in anemic patients.

Study done by Attard and Herring *et al*, contradicted this study and revealed that in women with IDA, there was lesser risk of being identified as diabetic while using HbA1c as a diagnostic tool, meaning thereby, that HbA1c was lower in anemic population.[9] Another study contradicting our results was done in India in which Sinha *et al* found similar association between iron deficiency and HbA1c. In this study a negative correlation was found between IDA and HbA1c while analyzing his results Sinha *et al*, took the position that factors other than Iron Deficiency, due to multiple deficiencies in the individuals, contributed to the decrease in HbA1c values.[16] Another recent contradiction to the results of current study is published by Soloman *et al*, who found HbA1c (%) to be significantly lower in their anemic group (6.18 ± 1.57) when compared to the group without anemia (7.74 ± 1.81) however the *p*-value was insignificant ($p \leq 0.05$).[17]

The negative correlation between haemoglobin and HbA1c inferring the possibility of an associated increase in HbA1c Value with a decrease in Hb levels in our results was supported by an Indian study by Parlapally *et al*, revealed that the mean of HbA1c in anemic patients (6.13% ± 0.6%) was more than non-anemic group (5.12% ± 0.5%), (p-value<0.001).[18] In anemic individuals increased HbA1c therefore may mimic a poor control in patients with diabetes and a pre diabetic tendency in individuals without diabetes.

A possible mechanism for the results shown by our study was suggested in literature by Tarim *et al*,[19] and Christy *et al*,[20] who explained the results on basis of postulation that hemoglobin gets irreversibly glycated in circulation. In iron deficiency anemia there is reduced red cell haemoglobin levels and consequently an increased average age of circulating red cells ultimately leads to elevated HbA1 levels. Hence, HbA1c levels in anemic erythrocytes will be increased.

In present study, both patients with and without diabetes were taken to compare and hence rule out the effect on HbA1c caused by presence of hyperglycemia in diabetic population. However, we found that the increase in HbA1c with IDA was seen in both diabetic and non-diabetic population. We enrolled controlled diabetics in our study to exclude the confounder effect of diabetes itself on HbA1c. The difference of HbA1c in anemic population was even more marked in non-diabetics as compared to the diabetics. This finding

poses a threat to misdiagnose an anemic patient with higher HbA1c as pre-diabetic where actual cause of rise in HbA1c may be his IDA. Soliman *et al*. who reviewed the work of various researchers on this subject and concluded in sustenance to our results that Iron replacement therapy decreases HbA1c in both diabetic and non-diabetic individuals and that it should be kept in mind while interpreting HbA1c results for diagnostic and prognostic purposes.[21] This conclusion was inferred by another research done by Madhu, who did this study on non-diabetic group and found that patients with IDA had mean HbA1c in pre-diabetic range. When they were treated for this anemia increase in HbA1c was also settled with in normal range.[22] A possible justification of false rise in HbA1c in cases with IDA came from the review in which he asserted that in Iron deficiency promotes peroxidation which increases terminal proline glycation due to change in the structure of hemoglobin. Increased irreversible glycation is responsible for falsely raised HbA1c Values seen in IDA.[23]

From this study it can be concluded that glycation of Hb may be affected in IDA, both in diabetic and non-diabetic populations. In females without diabetic group, study was aimed to analyze the influence of iron deficiency anemia on glycated hemoglobin in anemic and control group. The comparison of these groups showed a false increase in HbA1c in anemic group. This increase can be related to a possibility of its falsely overestimating pre-diabetes and diabetes in an anemic population if screened or diagnosed for the DM using HbA1c.

In diabetic group HbA1c was found to increase linearly with an increase in IDA in diabetic group, signifying that IDA might be associated with falsely raised HbA1c values and may mislead if this test is used as a prognostic indicator for diabetes in anemic individuals. Most probable cause of this increase is the increase in life span of iron deficient red cell, which get more time for irreversible glycosylation as compared to an average red blood cell.

CONCLUSION

HbA1c was significantly affected by change in hemoglobin levels. Iron deficiency anemia results in false positive increase in HbA1c values therefore, the effectiveness of HbA1c as a diagnostic and prognostic marker for Diabetes Mellitus is reduced in Iron deficiency anemia. IDA should be checked and corrected before any diagnostic or therapeutic decision is made based on the HbA1c level especially in setting of an area with high prevalence of IDA.

Conflict of Interest: None.

Authors' Contribution

MB: Conceived, designed and did statistical analysis & manuscript writing, MA: Proof reading and final approval, GML: Helped in topic selection and did proof reading and final approval, AS: Helped in stats analysis and write up, RA: Helped in stats analysis and data collection, AAK: Sample collection and stats analysis

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