THE RELATION OF GLYCOSYLATED HEMOGLOBIN LEVELS WITH HEMOGLOBIN LEVELS IN NON DIABETIC PATIENTS HAVING IRON DEFICIENCY ANEMIA

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ABSTRACT

Objective: To determine the relationship between levels of glycosylated hemoglobin and hemoglobin levels in non diabetic patients having iron deficiency anemia.

Material and Methods: This Cross-sectional descriptive study was conducted in Pathology department of Khyber Teaching Hospital,Peshawar-Pakistan from January 2019 to November 2019. Non diabetic cases with iron deficient anemia were included in study. The hemoglobin level of less than 13 gm /dl in males and less than 12 gm per dL for females was taken as cut off for anemia. The mean cell volume of less than 75 fl was taken as a cut off for iron deficiency anemia. The hematologist confirmed the cases to have iron deficiency anemia and analysed the blood counts. glycated hemoglobin A levels were detected in all the cases using Cobaschemillumniscent 411-E equipment by consultant chemical pathologist. Pearson correlation test and linear regression model were used to determine correlation between hemoglobin levels and HbA1c levels .

Results: Fourty eight non-diabetic cases with iron deficiency anemia were included in the study. Mean age of the population was 38±8.9 years (range :12-70 years). There were 17 (35.4%) males and 31(64.6%) females. HbA1C levels were high in 35 (72.9 %) cases, normal in13 (27.1 %). Pearson-r test showed a weak negative correlation (r=-.077, n=48, p=.604). Linear regression model was applied which showed that HbA1C levels can not be predicted for a given hemoglobin value (R2 =-.016, p value= .604)

Conclusion: There is no significant correlation between hemoglobin levels and HbA1c levels in non diabetics.

Keywords: Glycated, hemoglobin A, Iron deficiency, Anemia, hemoglobins.

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INTRODUCTION

Hemoglobin A1c (HbA1c) is the glycated form of hemoglobin molecule[1]. It is formed when the beta globin molecule is glycated at the valine residue near the the amino end of the molecule[1]. When the HbA1c was first discovered, it was thought it is an abnormal form of hemoglobin that is increased only in diabetic patients[1]. But later on, the researchers determined that there is a certain cut off levels for HbA1C in diabetics and non diabetic population[1]. It shows the spikes in blood glucose level in the previous three months which might have not been detected by random or fasting blood sugar levels[1]. Therefore, it is used in clinical setup to determine control of glucose level in the diabetic patients in the previous two to three months[1]. A rise in levels show that there has been a poor control of glucose level in the past two months. American Diabetes Association Guidelines (ADA) has approved to use it as a screening and diagnostic test for diagnosing diabetes mellitus[2]. According to the ADA, the levels of HbA1c should be less than 6.5% in diabetic patients and a level of more than 6.6% confirms the diagnosis of diabetes mellitus[3].

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It is now an established fact that it is not only the blood glucose level that effects the HbA1c levels. Its level is also found to be effected by iron deficiency anemia[1,2]. The iron deficiency anemia is common all over the world[1]. The prevalence is higher among those living in developing countries and those with low socioeconomic class[1]. The researchers have tried to explain the reason of changes in HbA1c levels in iron deficiency states by putting forth various possible suggestions[1]. Some of the suggestions are the increased glycosylation of hemoglobin molecule due to changes in the quartenry structure of hemoglobin induced by iron deficiency states and a prolonged survival of red blood cells in iron deficiency states that may result in increased levels of HbA1c levels[1]. Although different mechanisms are suggested for changes in HbA1c levels in iron deficiency anemia, yet the levels of HbA1c in iron deficient patients are still variable in different studies[1]. The results of different researchers are conflicting and the actual reason is still unknown[1]. The International Expert Committee has notified the physicians to be aware of conditions that effect the HbA1c levels other than diabetes mellitus[2,4]. Other conditions that effect the levels of HbA1c include high levels of serum triglycerides and urea, high serum bilirubin levels and alcohol intake[4]. Other common conditions include long term intake of vitamin C supplements and salicylates, pregnancy and opiode addiction[4,5]. All these cause raised levels of HbA1c[4]. The literature suggests that anemias that cause a rapid turn over of the red blood cells lead to a decreased level of HbA1c[4,6]. On the other hand, anemias that slows turn over of red cells result in an elevated levels of HbA1c[4,6,7]. Sluiter proposes that as the age of the red blood cells increase, the level of HbA1c increases in them[8]. A recent discovery is made that in iron deficiency anemia, the levels of malondialdehyde increases, which in turn causes increased glycosylation of the hemoglobin molecule and thus a raised HbA1c[9,10]. As the studies done so far give conflicting results about relation of HbA1c levels and hemoglobin levels in iron deficiency anemia. Therefore, this study was done to find relations ship between levels of HbA1c and hemoglobin levels in cases of iron deficiency, so that physicians and post graduate trainees may be aware of interpreting the results of HbA1c in cases of iron deficiency status in patients.

MATERIAL AND METHODS

It was a Cross-sectional analytical study. It was conducted in Pathology department of Khyber Teaching Hospital, Peshawar, from January 2019 to November 2019 (11 month duration). Ethical approval was obtained from the ethical board. Non probability purposive sampling was done. Patients of all ages and both genders who were non diabetics and had iron deficiency anemia were included in the study. Cases whose blood samples were insufficient were excluded from the study.

The hemoglobin level of less than 13 gm /dl in males and less than 12 gm per dL was taken as cut off for anemia as per WHO recommendations[1,11]. The mean cell volume of less than 75 femtolitre was taken as a cut off for iron deficiency states. HbA1C levels were detected in all the cases. The biggest confounder was cases with thalassemia trait. This was minimized by excluding the cases that had higher red cell count, lower hemoglobin, ratio of mean cell volume to red cell cout below 13, all of which are indications to thalassemia trait.

The HbA1c level was determined by Cobas by immunoturbidity principle under the supervision of consultant chemical pathologist. The hematologist confirmed the cases to have iron deficiency anemia and analysed the blood counts. The percentage of HbA1C was determined by using ratio of concentration of HbA1C to total hemoglobin level. The data regarding age, gender, and glycosyalated hemoglobin level was analyzed. Mean and standard deviations were applied for quantitative data while frequency was used for qualitative data. SPSS version 16 was used to do data analysis. In order to determine the normality of the data, histograms were used visually and Shapiro Wilk test was done. Correlation between hemoglobin and HbA1C was done by Pearson Correlation for parametric data. Linear regression model was applied inorder to determine if value of HbA1c can be predicted for a given value of hemoglobin. A scatter plot was drawn for the relationship between the variables. Durbin Watsin test was applied followed by moderation regression analysis to find the effect of gender on the HbA1c. The moderation effect of gender on HbA1c was shown in scatter box.

RESULT

About 48 non-diabetic cases having iron deficiency anemia were included in the study. Mean age of the population was 38±8.9 years, with range of 12 years to 70 years. Gender distribution in study population is shown in figure 1. The changes in HbA1c levels are shown in table 1 and figure 2. Pearson r data analysis showed a weak negative correlation between the variables (r= -.077, n=48, p= .604). Linear regression model was applied which showed that HbA1C can not be predicted from Hb value (R^2=-.006, p value= .604). Moderation analysis for gender showed a slight yet statistically significant improvement in correlatioin coefficient from .077 to .307 (p <.005) in the presence of gender as a moderator. The correlation in female gender was comparatively better than as compared to male gender (r= .204 for female gender versus r=.144 for male gender).

Table 1: Value of HbA1C in non diabetic iron deficient cases (n=48)

Hemoglobin A1C (%)	Range	Mean ±SD
	12.4 - 4.6	2.3±9.1

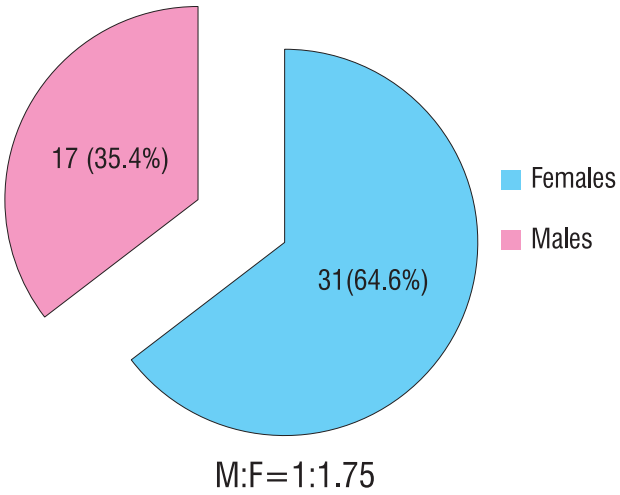

Figure 1: Gender distribution of the study population (n=48)

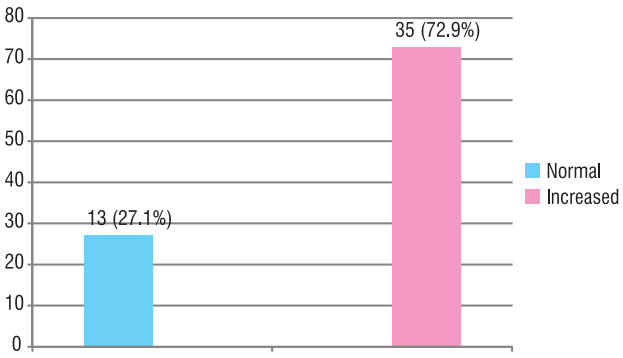

Figure 2: Pattern of changes in HbA1C in study population (n=48)

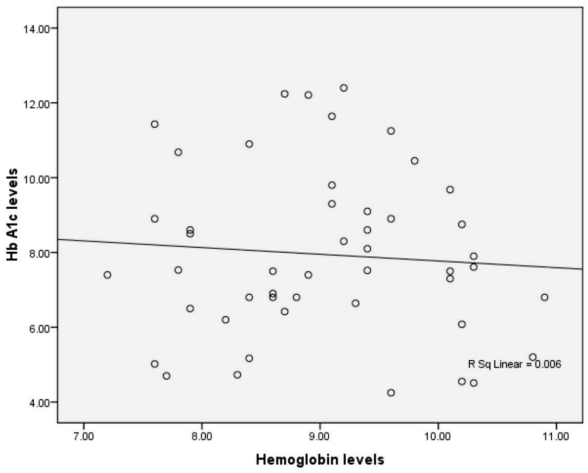

Figure 3: Scatter plot using linear regression model showing effect of hemoglobin on HbA1c (R2=.006).

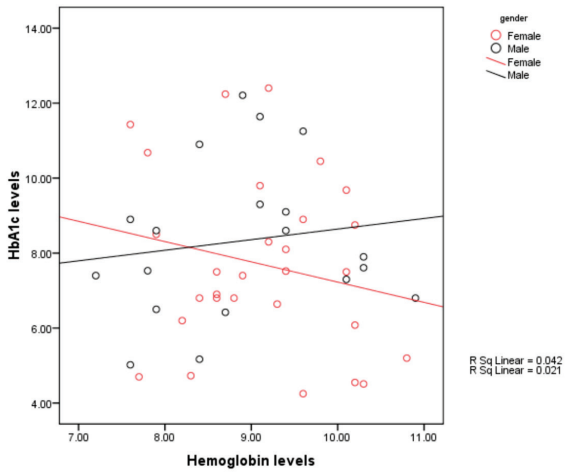

Figure 4: Scatter plot usingmoderation regression model showing effect of gender on relationship between Hemoglobin levels and HbA1c levels.

DISCUSSION

According to the ADA, the levels of glycated hemoglobin can be used to monitor control of blood sugar in diabetics and also to diagnose diabetes mellitus[1]. In 2009, the HbA1c was recommended to diagnose diabetes mellitus, despite acknowledging the fact that the levels may be effected by conditions that effect the lifespan of erythrocytes[12,13]. Both the ADA and American Association of Clinical Endocrinologists suggest that anemia may cause false changes in levels of HbA1c[12,13]. Thus, using HbA1c levels to diagnose Diabetes in already anemic patients may cause falsely raised prevalence of Diabetes Mellitus in general population[13].

Literature suggests conflicting reports about changes in levels of HbA1c in iron deficiency anemia[1,14,15]. The association between iron deficiency anemia and HbA1c levels was first discovered in 1965 by Horton[14]. Horton proposed that in iron deficiency anemia, the levels of HbA1c are low[14]. But later on Brookes proposed a conflicting report in 1980 that the levels of HbA1c were rather increased in iron deficiency anemia[15]. So, the present study was done to detect changes of HbA1c in our setup.

In our study, mean age of the study population was 38 years. Female population was predominant as compared to males. Similar findings was reported by Kalairajan where female population was predominant[1]. This finding shows that iron deificiency anemia is more common in female population.

In our study, the HbA1C levels were elevated in 73% cases of iron deficiency anemia. However, the correlation was better in female gender where a high hemoglobin level predicted a lower HbA1c levels. So in females, the iron deficiency anemia is associated with higher HbA1c levels. Coban and Hardikar reported in their stud-

ies that levels of HbA1c are high in cases of iron deficiency anemia[16,17]. Kannan S and Silvia JF reported same findings[18,]. Kalairajan S showed that HbA1c levels were low in cases of iron deficiency anemia[1]. A meta analysis done by English et al reported that HBA1c levels are raised in cases of iron deficiency anemia[19]. Solomon A from Ethiopia also showed that HbA1c levels were lower in iron deficient diabetics[20]. On the other hand, Sinha N reported that regarding the levels of HbA1C, there is no difference between iron deficient and non irondeficient cases[21]. The conflicting findings in literature has created confusion in the minds of health care providers regarding using HbA1c levels for diagnosis of diabetes mellitus. Also, it is warranted to do more studies to find the underlying mechanism by which iron status effects HbA1c levels[22].

LIMITATIONS OF THE STUDY

The study was done in a single center. Also the number of patients was less. There is a need to do more studies in which larger study group is analysed.

CONCLUSION

There is a weak negative correlation between hemoglobin levels and HbA1c in non diabetic cases. The correlation is somewhat more in female gender as compared to males. So, interpretation of HbA1c levels in iron deficient women must be done carefully as not to mislabel them as diabetic.

RECOMMENDATIONS

Further studies should be done using large sample size and keeping in consideration the other markers of glycemic control as fructosamine and glycated albumin to determine effect of iron deficieny states on HBa1c levels.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Rahman S: Data Compilation, Main Idea

Khan MI: Write up, Result analysis, discussion.

Rahman S: Literature Review.

Waqar S: Critcal review, data analysis.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.