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ORIGINAL ARTICLE

Effect of Iron Supplementation on HbA1c levels in Type 1 Diabetic patients

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ABSTRACT

Aim: To evaluate the effect of iron supplementation on HbA1c levels in Anemic type 1 diabetes mellitus individuals and its association.
Methodology: A cross-sectional study was conducted on 90 Type 1 Diabetic patients of which 60 had anemia and 30 had normal Hemoglobin levels. Structured questionnaires were used to collect information on socio-demographics and other co-morbidities / clinical conditions. The Cell Dyn 1800 hematological analyzer was used to test the venous blood for a complete blood count (CBC). Alongside, electrochemiluminescence (ECL) E411 (Kit Roche) was used to perform serum ferritin, serum iron, and total iron-binding capacity. HbA1c levels were accessed by glyco-hemoglobin reagent kit.
Results: Patient's hemoglobin levels were 10.1g/dl at baseline and 11.1g/dl following therapy. Anemic patients had an average HbA1c level of 8.603% and after iron supplementation it was 7.608%. On the other hand the HbA1c in the control group was 8.1% previously and after three months was 8.3%. Hence, after three months of therapy, a significant decrease in HbA1c levels was seen. HbA1c levels are altered not only in diabetes but in many other instances including different forms of anemia. This study was aimed at finding the correlation between iron levels and corresponding changes in HbA1c levels. Understanding the role of iron levels on HbA1c values prevents physicians from misevaluating the intensity of diabetes as sometimes a high reading HbA1c may only be due to iron deficiency and not because of increasing severity of diabetes.
Conclusion: Anemia has an inverse relationship with HbA1c levels. When the level of hemoglobin lowers in anemic individuals, HbA1c levels rise in tandem. Furthermore, when anemia is corrected, their HbA1c levels drop to near-normal levels. Aside from blood glucose, a variety of additional variables influence the computed HbA1c quantification number, which should be considered before starting or changing a treatment regime. Since iron deficiency anemia is so widespread, it should ideally always be ruled out when high HbA1c levels are identified and addressed promptly, to reach optimal HbA1c values.
Key words: Anemia, HbA1c levels, Type 1 Diabetes Mellitus, Hemoglobin (Hb), serum Iron, BSR (Blood Sugar Random)

INTRODUCTION

Type 1 Diabetes Mellitus is an autoimmune disease, largely as a result of immunity mediated by the T-cell, which kills the insulin-producing Beta cells in the Islets of Langerhans in the pancreas[1]. The hormone Insulin is responsible for the conversion and storage of excess glucose in the blood to glycogen, which is its storage form within the liver and muscles[2]. It regulates blood glucose levels and facilitates their storage or utilization within the cells[3].

Although, Type 1 Diabetes is generally a chronic disease of adolescence and childhood, it can transpire at any time of life with a higher prevalence in males than females. The American Diabetic Association (ADA) and the American College of Endocrinology (ACE) recommends levels of HbA1c as the analytical criteria for Diabetes Mellitus and a level of more than 6.5% is considered as diagnostic for the disease, by the American Diabetes Association.[4,5] Not only does it have diagnostic value, but is also used by physicians to monitor therapy. HbA1c helps determine plasma glucose levels over the past 2-3 months[6]. The conversion of hemoglobin A to HbA1c occurs throughout the life of the red blood cell, and the pace of this reaction is faster in diabetics due to the higher prevalent glucose content, resulting in a higher HbA1c concentration[7].

Anemia is defined as a decrease in the blood's oxygen-carrying capacity, as measured by aHb (hemoglobin) level of less than 12.0 mg/dl in non-pregnant adult females and less than 13.0 mg/dl in adult males[8]. When glucose interacts with an amino group on a hemoglobin molecule to create ketamine, it forms glycosylated hemoglobin (HbA1c). The glucose molecules are connected to one or both of the N-terminals of normal adult hemoglobin's polypeptide chains[6]. Glycated hemoglobin is a parameter of choice that evaluates the patient's glycemic status and control over the past 3 months. Several variables can influence or interfere with HbA1c findings, depending on the methods employed to quantify it[8]. A glucose residue is linked to the terminal NH2 group (valine residue) of one or both HbA beta chains in glycosylated hemoglobin (HbA1c).

The HbA1c test is a reliable method for determining chronic hyperglycemic levels. It has a strong link to establish the risk of diabetic incidence and complications. It is suggested that HbA1c be used to diagnose diabetes[9]. Iron is a crucial cation in a variety of metabolic activities and physiological responses, including the tricarboxylic acid cycle. A rise in iron levels hinders glucose metabolism, resulting in an increase in blood glucose levels[10].

Anemia may either raise or reduce the HbA1c levels due to variations in half-life of Red Blood cells. Consequently, corresponding to the kind of anemia, it can be correlated with either a rapid turnover of erythrocytes, leading to lesser HbA1c levels, or a slow turnover of erythrocytes, leading to amplified glycosylation of Hb and therefore, increased HbA1c levels.As a result, the existence of anemia may cause HbA1c values to be misinterpreted. HbA1c values are lower in cases of hemolyticanemia and patients with substantial blood loss, but they are higher in instances that cause a spike in the erythrocyte age, such as iron deficiency anemia[10,11].

Previous studies suggest that HbA1c values are not only influenced in anemia due to iron deficiency but also in hemolyticanemia, hemoglobinopathies, acute blood loss, pregnancy, vitamin B12, and folate deficiency[12,13,14,15,16]. In addition, in pre-menopausal women who are not anemic, HbA1c levels are linked to erythrocyte indices such as total hemoglobin (Hb), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV), regardless of glucose levels[9]. One thousand and six hundred million people worldwide are affected by anemia, accounting for 24.8% of the world's population. Iron deficiency is a

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frequent cause of anemia in the general population, and it is especially common in diabetic patients.[4]There is a high prevalence of anemia worldwide and HbA1c has a pertinent role in diagnosing Diabetes Mellitus. This study is aimed to find an association between the anemia and HbA1c values in type 1 Diabetes Mellitus before and after treatment of anemia with iron supplementation.

METHODOLOGY

Research Design: This is a cross-sectional study
Population: Type 1 Diabetic Individuals
Sampling and Sample Size: Random Sampling was done and a sample size of 90 patients with Type 1 Diabetes Mellitus (60 with Anemia and 30 without Anemia) were selected. 30 non-anemic diabetic type 1 subjects were enrolled to serve as the control. Out of 90 type 1 diabetic patients, 60 anemic patients served as a case. Out of 60 cases, 30 were males and 30 were females. The control included 14 males and 16 females. Performa Questionnaires were used to gather Socio-demographic information, family history of diabetes and the clinical history details of study participants. Body mass index (BMI) matches were measured in this study to prevent variation in range. Anemic patients were screened on the basis of their hemoglobin levels and general physical appearance such as the presence of skin pallor, generalised weakness, shortness of breath, cold hands and feet etc. All subjects underwent a detailed general physical examination and their findings were classified along a spectrum.

The Cell Dyn 1800 hematological analyzer was used to test the venous blood for a complete blood count (CBC); serum ferritin, serum iron, and total iron-binding capacity were performed by using electrochemiluminescence (ECL) E411(Kit Roche) and HbA1c tests were performed by glyco-hemoglobin reagent kit. The levels of HbA1c, CBC, BSR, serum ferritin, TIBC, and serum iron were examined both at baseline, as well as after 90 days of iron supplementation. Values of HbA1c and BSR were also compared with individuals who were in the control group. The group of patients with anemia was given iron supplementation for three months and then tested for changes in several indicators. The control group on the other hand was not given any iron supplementation. SPSS version 21 was used to analyze the data. Independent t-tests, chi-square, and Pearson's correlation were computed.

RESULTS

Ninety type 1 diabetic patients were included in this study out of which 60 were anemic and served as the case group while the remaining 30 were non-anemic and were selected for the control group. As shown in Table 1, the 60 cases were equally divided into males and females whereas the Control group comprised of 14 males and 16 females

Table 1: Details of the participants (n=90)

Category	Total
Gender	
Male	30
Female	30
Control	30
Mean age	38.0 (range 18-45yr)
Mean BMI	22.67±2.06
Waist hip ratio	0.812

On the basis of hemoglobin levels, patients were categorized as having mild, moderate, or severe anemia. The degree of anemia amongst thediabetic patients was determined by calculating the frequency.

The case group was supplemented with iron for 3 months and evaluated after this duration for changes in various parameters including hemoglobin levels, HbA1c levels, serum iron levels, etc. It was observed that almost all variables underwent significant changes in values.

HbA1c which was the variable under study decreased significantly with iron supplementation in anemic individuals. The mean HbA1c levels in anemic patients was 8.603% and after iron supplementation came down to 7.608 % as shown in Table 4. Females were 9.43% at baseline and 8.09% after three months of supplementation.The mean HbA1c levels in males were 7.79% and 7.12% at baseline and after three months respectively. This gender-wise variation is shown in Fig 1.The observed difference was statistically significant (P<0.01).In the control group the initial HbA1c value was 8.1% and after three months was 8.3%. This slight increase in HbA1c levels in the control group was attributed to the absence of iron supplementation and other dietary variations.

Table 2: Grading of anemia according to the NCI classification.

Grade	Symptom Severity	Hemoglobin Values
0	Within normal limits	12.0 -16.0g/dl for women and 14.0 to 18.0g/dl for men
1	Mild	10g/dl to level within normal limits
2	Moderate	8.0-10.0g/dl
3	Serious/severe	6.5-7.9g/dl
4	Life threatening	<6.5g/dl

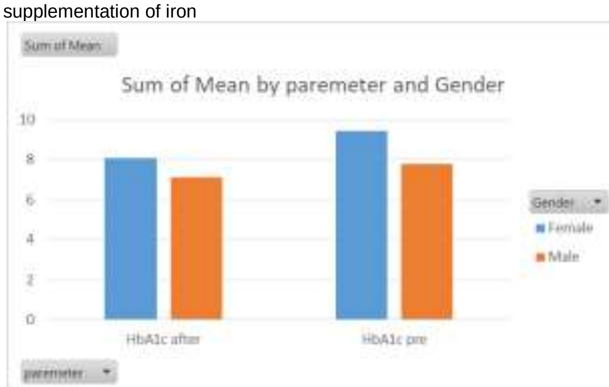
Table 3: Variables on examination

Severity of anemia	No. of patients (case group)	%age
Severe	18	30%
Moderate	20	33%
Mild	22	37%
Symptoms		
Generalized weakness	30	50%
Lack of interest in work	15	25%
Malaise	15	25%

Table 4: Measurement of HbA1c before and after supplementation in anemic type 1 diabetic patients

HbA1c	Mean	Std. deviation	Std.Error mean	Normal Range
Pre	8.608	2.4814	32.03	<5.7
Post	7.608	1.9988	25.80	<5.7

Fig 1: Gender-wise distribution of treatment of HbA1c (%) before and after supplementation of iron



As seen in Table 5, the mean hemoglobin level of patients at baseline was 10.1g/dl and after treatment was 11.1g/dl. The mean hemoglobin for females at baseline was 10.1g/dl and after 90 days of treatment was 11.02g/dl. As for males this value was 10.10g/dl and 11.02g/dl before and after treatment respectively, which was still lower than the normal value. This is shown in Table 6. The difference that was detected is statistically substantial (P<0.01). The hemoglobin levels of anemic individuals increased significantly (P<0.01) after 90 days of therapy. Although the results indicate that patients reacted well to therapy they were still unable to attain normal levels during the 90-day treatment period. On the other hand the mean hemoglobin level of the control group was 11.5g/dl, and after three months without iron replacement, the value was 11.3g/dl.

Table 5: Hemoglobin levels before and after supplementation

Hb	Mean	Std. deviation	Std. Errormean	Normal Range
Pre	10.110	2.1139	.2729	Male:13.8-17.2 Female:12.1-15.1
Post	11.137	1.7920	.2313	Male:13.8-17.2 Female:12.1-15.1

Table 6: Hemoglobin level in case group gender wise before and after iron supplementation

HB	Male	Std. Deviation	Female	Std. Deviation
Before	10.12	2.148	10.10	2.117
After	11.25	1.885	11.02	1.719

As shown in Table 7, mean blood glucose levels before treatment was found to be 112.54mg/dl (in females = 113.17 and in males =110.87) and after treatment the mean value was recorded as 108.5 mg/dl (in females = 104.7 and in males = 112.7) (P<0.01). Mean fasting glucose in control was found to be 103.44 mg/dl and after three months without any treatment 100.22 mg/dl. These results were not statistically significant.

Mean serum iron levels at baseline in study group was found to be 65.12 mg/dl while at 3 months after treatment these levels rose to 84.45mg/dl. This signifies that with supplementation, the iron levels increased markedly. At baseline, the mean serum ferritin concentrations in the study group were 90.95ng/ml, whereas those in controls were 127ng/ml. The difference between these values before the supplementation and after it is very substantial (p<0.01). The mean TIBC values in patients of the study group at baseline were 104.45 mcg/dl and after three months of ironsupplementation as 127.02. The difference between these values before the treatment and after it was also very substantial (p<0.01).

Table 7: Comparison of variables at pre and post level

Variables	Pre-level treatment	Post level treatment	Normal Range
BSR (mg/dl)	112.54	108.5	<125
Serum iron (mcg/dl)	65.12	84.45	60-170
Serum Ferritin (ng/ml)	90.95	127.63	Male: 12-300 Female: 12-150
TIBC (mcg/dl)	104.45	127.02	240-450

DISCUSSION

The present study analyzed 90 type 1 diabetic participants, 60 case subjects were anemic, and 30 control subjects were non-anemic. There was little difference between the mean age of the case and the control group. The mean age of 60 cases was 38.0 (range 18-45 yr) years, and that of 30 control was 39.0 (range21 - 45yr) years. BMI was matched appropriately between both groups. Participants in the study were selected with approximately the same BMIs *(*Table 1*).* Complete blood count (CBC), HbA1c, serum iron, serum ferritin, and BSR levels were measured at baseline and after three months.

According to their Hemoglobin levels, patients were divided into three groups: mild, moderate, and severe anemia. In the study, it was classified that severe anemia was found in 30% of the patients, moderate anemia 33% of the patients, and mild anemia in 37% of the patients. The majority of the cases complained of generalized weakness. Other symptoms found in patients, was lack of interest in work and malaise *(*Table 2, 3).

Based on their blood test results, the case group diabetic anemic patients were treated with iron supplements and after three months of treatment, their value of before treatment was compared with their value after treatment *(*Tables 4-7). Mean hemoglobin of the control group was 11.5g/dl and after 90 days without any iron treatment was 11.3g/dl. The hemoglobin level of anemic individuals increased significantly (P<0.01) after 90 days of therapy for anemia. Mean blood glucose levels in cases before treatment was found to be 112.54 mg/dl (female 113.17 and male 110.87) and after treatment 109.5 mg/dl (females= 104.7 and males= 113.7) (P<0.01). Mean fasting glucose in control was found to be 103.44 mg/dl and after three months without any treatment 100.22 mg/dl.

The research group's mean blood iron levels were 65.12 mg/dl at the start, but these levels jumped to 84.45 mg/dl ninety days later. This means that over the ninety days of anemia therapy, the iron levels had significantly increased. Anemic patient's serum iron levels were found to be significant (p >0.01). At baseline, the mean serum ferritin values in the study group were 90.95 ng/ml, while the controls had a value of 127. The difference between these values before the treatment and after it is very substantial (p<0.01).

The mean TIBC values in patients of the study group at baseline were 104.45 mcg/dl and that after three of treatment was 127.02 mcg/dl. The difference between these values before the treatment and after it is very substantial (p<0.01). Anemic patient's mean hemoglobin and mean serum ferritin levels were raised after ninety days of iron therapy.

According to the current study HbA1c considerably reduced in the Anemic group; females were 9.43% at baseline and 8.09% after three months of treatment as compared to the control group which had a baseline value of 8.1% and after three months HbA1c was 8.3%. This increase in HbA1c levels was unremarkable and was due to the fact that the control group was not given any iron supplementation.

Due to iron supplementation, HbA1c levels were lower in diabetic anemic patients as compared to the control group (as they were not given iron supplements). This is supported by studies done in 2019 by Solomon et al[17]**.** Furthermore, in 2015 by Cavagnolli et al demonstrated that HbA1c concentrations are likely to be lower in the presence of iron deficiency anemia.[4]Thus, the degree of anemia in the research participants, is explanatory for the lower HbA1c. All investigations show HbA1c levels and compare them with other parameters. HbA1c concentrations are influenced by erythrocyte turnover, and the International Expert Committee has cautioned physicians to be mindful of any diseases that may impact red blood cell turnover[17,18].

Although various types of anemia have been linked to reduce HbA1c, iron deficiency has been demonstrated to modestly raise it. Other factors that raise HbA1c should also be considered apart from diabetes and anemia, like obesity and sleep deprivation. Studies have shown that the development and control of diabetes are affected by both the quality and duration of sleep. There is also an increased occurrence of diabetes in patients with Obstructive Sleep Apnea Syndrome[19]**.** Others include gum disease, use of certain drugs, chronic inflammation etc. Furthermore, long-term H. pylori infection is significantly associated with high levels of HbA1c and decreased insulin secretion[20].

We conclude that anemia, specifically Iron deficiency Anemia, is related with greater HbA1c concentrations among type 1 DM patients with similar levels of glycemia. This means that if two patients with similar blood glucose levels are tested for HbA1c, the patient with iron deficiency anemia will have a higher value of HbA1c as compared to the patient with normal iron levels. Furthermore, iron replacement treatment lowers HbA1c levels in diabetes type 1 patients. When interpreting HbA1c values in type 1 diabetes, the patient's iron status must be taken into account.

CONCLUSION

It was established that anemia has a direct association with HbA1c levels, in an inverse relationship pattern. This means that when the level of hemoglobin lowers in anemic individuals, HbA1c levels rise in tandem. Furthermore, when anemia is corrected with iron supplementation, the HbA1c levels drop to near-normal levels. A decrease in HbA1c levels of all individuals supplemented with iron was seen, regardless of the type of anemia. Aside from blood glucose, a variety of additional variables influence the computed HbA1c number, which should be considered before starting a treatment plan. As anemiais so widespread and iron deficiency being the most common type, it should always be ruled out when high HbA1c levels are identified. It is vital when interpreting HbA1c values that the patient's iron status be taken into consideration. It is vital to be addressed timely so that optimal HbA1c values can be reached and attained.

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