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**Original article**

# Iron Deficiency Anemia in Children with Type 1 Diabetes Mellitus

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**Abstract**

**Background**: Because of the rising incidence of type1 diabetes (T1D), it remains a challenge. With time, systemic consequences such as anemia may develop in children with T1D. Anemia in T1D may have a complex, multifactorial background. **Aim**: we aimed to assess the iron deficiency anemia, correlate it with duration of diabetes mellitus in children with Type 1 DM in a group of Egyptian children. **Methods**: this case control study included 110 children, fifty-five of them with type 1 diabetes mellitus following up at the outpatient's pediatric endocrinology clinic, pediatric department, Beni-Suef university hospital and fifty-five healthy control children. All patients underwent complete history taking, full physical examination and investigations including complete blood count, serum ferritin, serum iron, HbA1C, fasting blood sugar and 2-hour postprandial blood sugar. **Results**: Anemia was significantly more prevalent among type 1 Diabetes than controls (29.1% vs 12.7%

respectively). The mean values of Hb, RBC, MCV, MCHC, MCH, serum iron, serum ferritin was significantly lower in diabetic children than controls. A significant difference was found regarding Hb and MCV mean values among children with type 1 diabetes in comparison with duration of diabetes as the lower mean values was among over 6 years diagnosed children. BMI was significantly lower among anaemic-diabetic children with serum ferritin ≤20 than non anaemic children with serum ferritin >20**.** HBA1c mean values was significantly higher among anaemic diabetic children (whose ferritin ≤20) than those non anaemic diabetic children (whose ferritin >20). There was a moderate negative correlation between HB and HBA1c. **Conclusion and recommendations:** In conclusion, our study demonstrated that anemia especially iron deficiency anemia is prevalent among children with type 1 diabetes with association with longer disease duration, poor control of diabetes and lower BMI. So, we recommend routine screening of children with type I DM, strict control of diabetes, and nutritional counseling of those children.

## 1. Introduction:

Diabetic mellitus is the word for A chronic hyperglycemia that results from insufficient insulin secretion, exercise, or both characterizes diabetes mellitus (DM), a complicated metabolic condition. This causes deviations from baseline blood glucose levels that have a significant effect on the metabolism of carbohydrates. It is one of the most well-known endocrine disorders affecting children worldwide and is a non-communicable illness that is spreading quickly everywhere **[1].**

There are two types of long-term diabetes complications: macrovascular (microangiopathy of micro vessels) and microvascular (microvascular disease) (macroangiopathy of macro vessels).

Microvascular complications can affect the kidneys (diabetic kidney disease), the eyes (retinopathy), which can cause visual impairment or even blindness, the nerves (neuropathy), which can cause diabetic foot, a condition caused by peripheral arterial disease that can result in severe ischemia and possibly amputation. Diabetic cardiomyopathy is one of the macrovascular consequences, along with an elevated risk of infections that might worsen peripheral artery disease, respiratory failure, and stroke. Anemia is not directly caused by diabetes, although it may be exacerbated by certain of its side effects, such as nephropathy and neuropathy.**[2].**

Nephropathy, which is often associated with diabetes, results in anemia because the kidneys secrete erythropoietin (EPO), which increases the production of red blood cells in the bone marrow. The kidneys of diabetics don't secrete enough EPO to meet the body's demands **[3].** In diabetics, autonomic neuropathy impairs the body's ability to properly stimulate the kidneys to produce more erythropoietin in response to anemia **[4].**

Reduced red blood cell mass (RBC) mass, which carries oxygen and carbon dioxide and impairs the body's ability to exchange gases, is a sign of iron deficiency **[5].** One of the possible factors contributing to the deterioration in kidney function is renal ischemia, which is brought on by a reduced oxygen supply as a result of lower hemoglobin levels and diabetic cardiomyopathy **[4]**

The most prevalent kind of anemia is iron deficiency, which may be efficiently treated with dietary modifications, parenteral or oral iron, and other supplements. The creation of hemoglobin (Hb), electron transport for cellular respiration, DNA synthesis, and other critical enzymatic activities all depend on iron.**[6].**

Since iron deficiency anemia affects cognitive function, exhaustion, and work capacity in individuals with type 1 diabetes, it has an impact on the psychosocial development of children with type 1 diabetes. Children's quality of life is thus improved by early identification and treatment of anemia associated with type 1 diabetes **[7].**

However, to the best of our knowledge there are few studies investigating anaemia and its related parameters in type1diabetes in children. Hence, we aimed to assess the iron deficiency anemia and correlate it with duration of diabetes mellitus in children with Type 1 DM in a group of Egyptian children.

## 2. Patients and Methods:

This case control study was conducted from April 2022 to November 2022 included 110 children, fifty-five of them were diagnosed with type 1 diabetes mellitus following up at the outpatient's pediatric endocrinology clinic, paediatric department, Beni-Suef university hospital and fifty-five were healthy control children.

The findings of a previous research that aimed to compare the hematological indices of diabetic children to those of healthy controls and to link these indices with hemoglobin A1c and blood glucose were used to calculate the sample size **[8].** As a result, sample size was determined using a priori analysis for the difference between two independent means using the G*Power (3.1.9.4) program (two groups) t-test for independent samples, based on an effect size 0.65, alpha error 0.05, a study power 95% and an allocation ratio 1 to 1. The sample was found to be 52 in each group. We assumed a10% dropout rate or non-response to be 55 in each group.

**Inclusion criteria**

We included pediatric patients with type1 diabetes mellitus aged from 3 years to 18 years of both genders.

**Exclusion criteria**

We excluded children on iron treatment, children with congenital or hemolytic anemia, children with co-morbid diseases such as, renal failure, cardiac diseases and chronic hepatitis, illnesses that may result in elevated blood ferritin levels include recent infection, inflammation, autoimmune diseases, malignancy and obese children.

**Patients and Methods:**

All individuals included in the study were subjected to history taking as personal history including age, sex, and special habits of medical importance. In addition to complaint, present history including duration of diabetes, insulin dose per day, occurrence of DKA/year, history of bleeding, history of parasitic infestation.

All participants underwent clinical examination including as general examination (height-weight…), chest, cardiac and abdominal examination. The laboratory investigations included complete blood count. For an accurate test result, the blood sample has to be collected from venous blood under completely aseptic conditions after being thoroughly mixed and anticoagulated with EDTA. Since prolonged storage might alter test results, the test was completed in less than six hours to get the blood specimen. The samples were then analyzed in an automated

counter utilizing an Abbott Diagnostics, USA Cell Dyne 1800 device.

The Serum iron, TIBC and Fasting blood sugar and 2-hour postprandial blood sugar were done also. Using sterile tubes that were left to clot undisturbed for an hour at room temperature, serum samples were taken from venous blood. Centrifugation was performed on tubes, followed by pipetting the supernatant serum to a different tube and centrifugation once more. After that, the supernatant serum was transferred to tubes so that the DTN-405-DIA LAB Austria spectrophotometric analyzer could test it. The serum ferritin evaluation done using kits for VIDAS (bioMerieux SA-France). We classified children into anaemic and non anaemic at a cut off 20 µg/L of ferritin **[9].**

In the assessment of HbA1C, it was measured nephelometrically by (Mispa-i2, AGAPPE) kits. Utilized with a particular protein analyzer (AGAPPE Diagnostic Switzerland GmbH). Two milliliters of whole blood were drawn into an EDTA tube from each patient and control group. The detection limit of this technique ranges from 3 to 13 percent.

**Statistical analysis:**

A SPSS for Windows version 23 was used to tabulate, code, and analyze the data that had been gathered. Categorical data were shown as numbers and percentages, while continuous variables were shown as mean values ± standard deviation (SD). The Fisher and Chi-squared tests were used to compare the qualitative data. When comparing groups of quantitative data, the independent sample t-test was used. Appropriate statistical tests of significance were used for further statistical analysis. One way ANOVA was used to compare between the subgroups of diabetic patients regarding the scale variables. P-values less than 0.05 were regarded as statistically significant inference.

**Ethical Considerations:**

All subjects' parents provided written informed permission before to participation in the study. The necessary administrative rules were followed. The ethical approval of the study was obtained from the faculty of medicine (REC) was obtained prior to the beginning of the work under number FMBSUREC/10042022/Mahmoud.

## 3. Results:

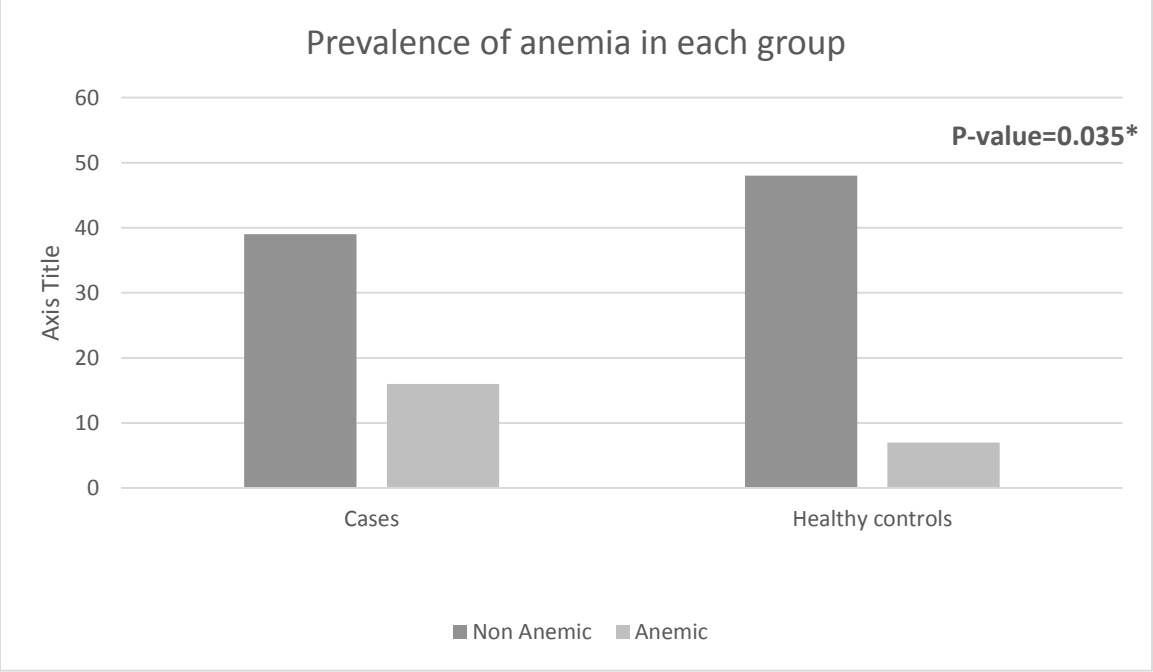
This case control study included110 children, fifty-five of them with type 1 diabetes mellitus following up at the outpatient's paediatric endocrinology clinic, paediatric department, Beni-Suef university hospital and fifty-five healthy control children.

**Table (1)** demonstrated age, BMI and sex distribution of the cases and the controls; mean of age among cases was 11.20±4.14 years and among controls was (11.38±3.91) without significant difference. Among cases, 34.5% were males and 65.5% were females, with the same distribution in controls. There was no statistically significant difference between the cases and the control groups regarding sex and BMI (p-value≥ 0.05).

**Table (1): Baseline characteristics of the studied groups:**

| Items | | N (%) | | p-value |
|---|---|---|---|---|
| | | **Patients N= 55** | **Healthy Controls N= 55** | |
| **Age (mean ±SD)** | | 11.20±4.14 | 11.38±3.91 | 0.805 |
| **BMI** | | ***17.73±2.96*** | 18.70±2.33 | 0.050 |
| **Sex** | **Male** | 19 (34.5) | 19 (34.5) | 1.00 |
| | **Female** | 36 (65.5) | 36 (65.5) | |

The prevalence of anemia among children with Type 1 Diabetes was 29.1% (no=16) while among healthy Controls was 12.7%(no=7) **(Figure 1).**




**P-value is significant*

**Figure (1): Prevalence of iron deficiency anemia among Children with Type 1 Diabetes and Healthy Controls**

There was a significant difference regarding difference of Iron-Deficiency Anaemia blood Parameters between children with type 1 diabetes and studied healthy controls as mean values of Hb, RBC, MCV, MCHC, MCH, Serum iron, serum ferritin was lower in diabetic children with significant difference as p value was 0.002, 0.0001, 0.014, 0.010, 0.04, 0.0001, respectively. there was a significant difference regarding HBA1c mean values among children with type 1 diabetes and healthy controls which was higher among diabetic children (10.27±2.08) **(Table 2).**

**Table (2): Comparison between Type 1 Diabetes and Healthy Controls regarding their labs:**

| Parameters | Patients N= 55 | Healthy Controls N= 55 | p-value |
|---|---|---|---|
| **Hb** | 11.25±1.43 | 12.33±1.56 | 0.002* |
| **RBC** | 255.90±113.36 | 424.34±122.27 | 0.0001* |
| **MCV, fL** | 74.01±9.75 | 78.71±9.94 | 0.014* |
| **MCHC** | 33.55±3.74 | 35.42±4.10 | 0.561 |
| **MCH, pg** | 23.67±3.24 | 24.10±4.39 | 0.010* |
| **Serum iron** | 68.97±28.28 | 80.55±25.98 | 0.040* |
| **Serum ferritin** | 30.61±18.74 | 48.45±20.95 | 0.0001* |
| **HbA1c** | 10.27±2.08 | 5.25±.706 | ٠.0001* |

*P-value is significant

Our result indicated that there was a significant difference regarding Hb and MCV mean values among children with type 1 diabetes in comparison with duration of diabetes as the lower mean values was among over 6 years diagnosed children (9.46±.79), (66.33±6.78) p value 0.016, 0.049 respectively. There was a non-significant difference regarding HbA1c mean values among children with type 1 diabetes in relation to duration of diabetes (P-value > 0.05) **(Table 3).**

**Table (3): Laboratory parameters in Children with Type 1 Diabetes in in relation to duration of diabetes (no=50):**

| Parameters | Group A: 0–1 y n=12 | Group B: 1–3 n=11 | Group C: 4–6 y n=24 | Group D > 6 y n=8 | p-value |
|---|---|---|---|---|---|
| **Hb** | 12.14±1.95a | 11.61±2.52a | 11.24±1.55a | *9.46±.79b* | ٠.016* |
| **RBC** | 293.91±144.83 | 277.36±121.00 | 233.54±105.27 | 236.50±59.16 | 0.415 |
| **MCV, fL** | 77.55±6.687a | 72.50±13.90a | 75.50±8.57a | *66.33±6.78b* | 0.049* |
| **MCHC** | 33.91±4.18 | 34.20±3.81 | 33.81±3.54 | 31.36±3.48 | ٠.357 |
| **MCH, pg** | 23.38±3.52 | 23.28±4.14 | 24.62±2.29 | 21.76±3.45 | 0.162 |
| **Serum iron** | 71.36±37.33 | 69.18±24.87 | 64.60±24.70 | 78.25±30.33 | ٠.689 |
| **Serum ferritin** | 39.75±25.01 | 20.90±12.58 | 29.87±17.61 | 32.50±13.56 | 0.113 |
| **HbA1c** | 10.28±2.19 | 10.95±3.27 | 10.21±1.59 | 9.51±1.019 | ٠.533 |

*P-value is significant

**Different letters denote statistically significant difference between subgroups (Bonferroni pairwise comparison)**

There was a significant difference regarding BMI as lower mean values was among anaemic-diabetic children (16.61±3.48) p value was 0.049 **(Table 4).**

**Table (4): Association between baseline characteristics and presence of anaemia in Children with Type 1 Diabetes:**

| Parameters | | Anemic Serum ferritin≤ 20 n=16 | Non-anemic Serum ferritin>20 n=39 | p-value |
|---|---|---|---|---|
| **Age** | | 9.81±3.86 | 11.76± 4.17 | 0.113 |
| **Sex n (%)** | **Male** | 5 (31.3) | 14 (35.9) | 0.742 |
| | **Female** | 11(68.8) | 25 (64.1) | |
| **BMI** | | 16.61±3.48 | 18.19±2.63 | 0.049* |
| **Age of onset of DM** | | 6.96±3.31 | 7.95±3.41 | 0.331 |
| **Duration of DM** | | 42.00±24.29 | 48.47±32.94 | 0.481 |
| **Family historyn (%)** | | 2 (12.5) | 12 (30.8) | 0.158 |
| **History of DKAn (%)** | | 10 (62.5) | 24 (61.5) | 0.980 |

*P-value is significant

**Figure 2** demonstrated that HBA1c mean values was higher among anaemic diabetic children (11.40±2.40) vs (9.81±1.78) in non anaemic with significant difference p value 0.0001.

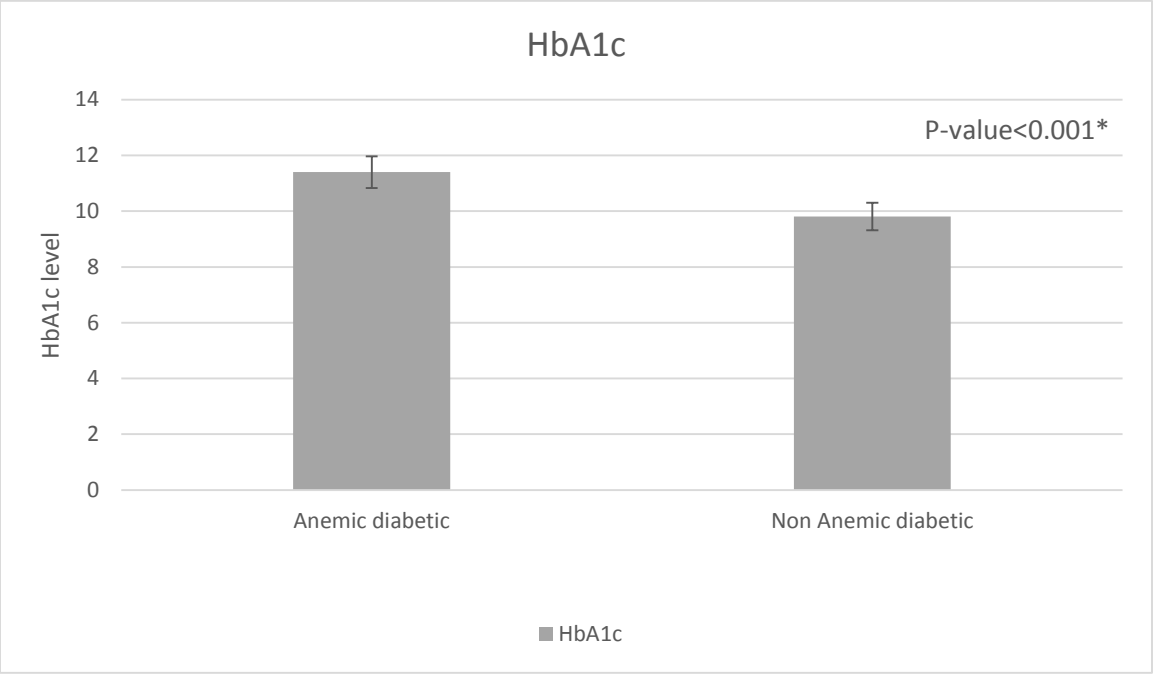



**Figure (2): Difference of HBA1c regarding presence of anaemia in the in Children with Type 1 Diabetes (no=55)**

There was a moderate negative correlation between HB and HBA1c (P-value=0.013, r=-0.333) **(Table 5).**

**Table (5): Correlation between HbA1c and Hb, serum iron and serum ferritin levels in type 1 diabetic children**

| Items | | **HBA1c** |
|---|---|---|
| **HB (g/dL)** | **r** | -0.333 |
| | **p-value** | 0.013* |
| **Serum iron (go/l)** | **r** | -0.086 |
| | **p-value** | 0.531 |
| **Serum ferritin (go/dl)** | **r** | -0.125 |
| | **p-value** | 0.365 |

*P-value is significant at ≤0.05 r: correlation coefficient

## 4. Discussion:

Due to its increasing prevalence, type 1 diabetes (T1D) continues to be difficult for diabetologists, pediatricians, and general practitioners, who increasingly must deal with the issue of providing the best treatment possible for young patients with this condition and its consequences like anemia **[10].**

With time, systemic consequences such as anaemia may develop in children with T1D. Anemia in T1D may have a complex, multifactorial background**.** On the other hand, microcytosis may also be present in anemia associated with inflammation. Normocytic anemia occurs in diabetic nephropathy due to inadequate erythropoietin production **[11].** So, we wanted to report the iron deficiency anaemia in diabetic type I children in a sample of Egyptian children in upper Egypt.

In the current study, Prevalence of anemia among Children with Type 1 Diabetes was 29.1% while among healthy controls was 12.7%. Anemia was significantly more prevalent among type 1 Diabetes than controls (P value = 0.035).

Salah and colleges study was not far from our prevalence, According to his examination of 200 T1D youngsters in Egypt, anemia was identified in 37% of the cases. A parasite infestation was the cause of anemia in 24% of patients, while thalassemia minor was identified in 18.7%, folic acid insufficiency in 18.7%, celiac disease in 4%, and iron deficiency in 54.7 percent of patients **[12].** The small difference between our study and Salah's study that we excluded other causes of anemia to assess the effect of diabetes only not other causes of anemia so, our prevalence was slightly different from his study.

In contrast to our study, based on hemoglobin concentration, Thomas et al. calculated that 14% of persons with T1D who have had the disease for an average of 20 years have anemia. Anemia was seen in 52% of patients with macroalbuminuria, 24% with microalbuminuria, and less than 8% of those with normal albumin production, indicating that impaired renal function and albuminuria were risk factors **[13].** The difference in prevalence of anemia between our results and Thomas study is that he recruited patients with type 1 but in older age as most of his patients were in forties of age and about one third of them were above 50 years old with median disease duration of 20 years.

When comparing between children with type 1 diabetes and controls, we found that mean values of Hb, RBC, MCV, MCHC, MCH, serum iron, serum ferritin were significantly lower in diabetic children than controls (P

value was 0.002, <0.001, 0.014, 0.01, 0.04 and <0.001 respectively). Hence this anaemia was most probably iron deficiency anaemia.

In addition, iron deficiency, which manifests as anemia with microcytosis in the blood count, is one of the most frequent causes of anemia in children with T1D. Compared to those without diabetes, T1D patients have a greater prevalence of it **[14].** Also, Tihić-Kapidžić et al., found a significant lower level haematological indices in cases with type 1 diabetes than healthy controls **[8].**

The pathogenesis of iron deficiency anemia in type I diabetes mellitus is poorly understood, with the main contributing factors being a decrease in the number of interstitial cells that synthesize specific erythropoietin and an impairment of regular processes enabling oxygen sensing through hypoxia-inducible transcription factor-1α (HIF-1α) secondary to interstitial fibrosis or vascular lesions. Additional processes might include glycation of the EPO receptor by or as a result of hyperglycemia, hyporeninemia, cytokine-induced suppression of EPO synthesis, and urine loss of EPO (in individuals with nephritic range proteinuria) **[15].**

Another explanation that the iron deficiency anemia may be caused by autoimmune gastritis. De Block and colleges stated that autoimmune gastritis may be present in 20–30% of individuals with iron deficiency anemia who do not show signs of gastrointestinal blood loss, and it is three–five times more common in people with type 1 diabetes **[16].**

In our study, there was a significant association between lower Hb and MCV mean values among children with type 1 diabetes for disease duration over 6 years than the other diabetic children with shorter disease duration.

In line with our results, Wójciak and colleges reported that the newly diagnosed type 1 diabetes children, significantly lower concentrations of hematocrit (HCT) and hemoglobin (HGB) as well as the amount of RBC and mean corpuscular volume (MCV) have been shown, in comparison with the children who have type 1 diabetes for longer duration **[17].**

Regarding RDW, TIBC, and hepcidin, Rusak and colleges observed statistically significant differences compared to the control participants. The mean values of iron concentration, iron metabolism parameters, and vitamin B12 levels, however, were all determined to be within normal limits. This clarified how maintaining ideal diet, scheduling many follow-up appointments at

the outpatient clinic, and engaging in ongoing education **[11].**

We found a significant difference regarding BMI as lower mean values was among anaemic-diabetic children with serum ferritin ≤20 than non-anaemic children with serum ferritin >20 *(*p value was 0.049). Rahman and colleagues came to the conclusion that moms with low body mass index and stunted children had much higher risks of anemia.**[18].**

We also found in current study that HBA1c mean values was higher among anaemic diabetic children (whose ferritin ≤20) than those non anaemic diabetic children (whose ferritin >20) and there was a significant negative correlation between the glycosylated hemoglobin level and the hemoglobin level.

Previous research supported our negative correlation, Wójciaket et al. discovered a noteworthy negative association between the MCH concentration and children with type 1 diabetes who have had the condition for more than a year.**[17].** Similar results were reported in many studied as reduced iron stores have been related to increased glycation of HbA1c **[14,19].**

The explanation of this finding can be found in a previous study done by Davis et al., in animal models, they discovered that mice lacking iron exhibit symptoms of impaired metabolic balance, such as changes in insulin signalling, as shown by hyperglycemia, hyperinsulinemia, and hyperlipidaemia **[20].**

## 5. Conclusion and recommendations:

In conclusion, our study demonstrated that anemia especially iron deficiency anemia is prevalent among children with type 1 diabetes with association with longer disease duration, poor control of diabetes and lower BMI. So, we recommend routine screening of children with type I DM, strict control of diabetes, and nutritional counseling of those children. The main points of strength of this study are being a case control study and it is one of few works that investigate anemia among children with type 1 diabetes. However small sample size and it was done in one center are the main limitations. Further multicenter studies with large sample size are recommended.

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**Abbreviations**

HbA1c (%), hemoglobin A1c

RBC (1012/L), red blood cells

HGB, HB (g/L), hemoglobin

HCT (%), hematocrit

MCV (fL), mean corpuscular volume

MCH (pg), mean corpuscular hemoglobin

MCHC (g/L), mean corpuscular hemoglobin concentration