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Original Article

Anaemia in patients with type 2 diabetes mellitus without nephropathy is related to iron deficiency

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ABSTRACT

Background and aims: Iron deficiency anaemia, although well reported in diabetic nephropathy, has not been well studied in type 2 diabetes patients in the absence of nephropathy. We studied the prevalence of anaemia and iron deficiency in type 2 diabetes patients without nephropathy.
Material and methods: A total of 89 patients were selected for this study. 24 h urine protein less than 500 mg was used as the criteria to rule out diabetic nephropathy. Complete hemogram, iron profile and high sensitivity C reactive protein (hs CRP) levels were performed in each patient.Functional iron deficiency (FID) was defined as serum ferritin more than 100 μg/l with serum transferrin less than 20% and total iron deficiency state was defined as serum ferritin less than 100 μg/l.
Results: Fifteen patients (16.8%)had anaemia out of which 13 had total iron deficiency and one each had functional iron deficiency and normal iron status respectively. Assessment of the iron status overall showed that 49 patients had TID (55.05%), 16 had FID (17.9%)and 24 (27.05%) had normal iron status. The hs-CRP was significantly higher in those with iron deficiency.
Conclusions: The present study found a high prevalence of iron deficiency anaemia in type 2 diabetic patients even in the absence of nephropathy. Most of the diabetic subjects also displayed an iron deficiency state the cause of which needs further investigation.

Key message: There is high prevalence of iron deficiency anaemia in patients with type 2 diabetes mellitus without nephropathy.

1. Introduction

The prevalence of diabetes mellitus is increasing all over the globe [1] with 8.4% of the global population being affected as of 2017 [2] and predicted to rise to 9.9% by 2045. International Diabetes Federation (IDF) estimates the total number of diabetic subjects in India to be around 72.9 million (as of 2017) which makes India the second most affected country in the world, after China [3,4]. Diabetes mellitus, a major cause of renal disease, retinopathy and cardiovascular complications is often associated with anaemia [5]. Although anaemia in diabetes mellitus is usually attributed to the associated chronic kidney disease, studies have shown that there is increased prevalence of anaemia in patients with diabetes even without renal impairment [6–8]. Anaemia is also a common problem in our country with iron deficiency anaemia contributing to the majority of the cases. While iron deficiency anaemia has been reported earlier in diabetes, few studies have assessed functional iron deficiency (FID) in diabetes. This study was done to ascertain prevalence of anaemia in patients with type 2 diabetes mellitus without nephropathy and relation of iron deficiency with systemic inflammation.

2. Materials and methods

A total sample consisting of 89 patients of type 2 diabetes mellitus with age group of more than 20 years was taken from the diabetes clinic of a tertiary care centre in North India after obtaining approval from the Institutional Ethical Committee. The sample size

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calculation was based on a prevalence rate of 35 ± 10% with 95% confidence interval (as in previous studies) which yielded a sample size of 87 patients. Informed consent was taken from all participants. Pregnant and lactating mothers, patients with acute systemic illness and acute bleeding disorders, previously diagnosed chronic kidney disease/diabetic nephropathy patients were excluded from the study. Stool examination was performed to exclude patients with worm infestations. Diabetes was diagnosed as per WHO criteria [9]. Anaemia was also diagnosed based on WHO criteria haemoglobin level <13 g/dl in adult males &<12 g/dl in adult females [10]. Diabetic retinopathy was diagnosed by an ophthalmologist by fundus examination. A 24 h urine protein test indicative of more than 500 mg/day was used to define diabetic nephropathy. All subjects were called after an over-night fast for a detailed biochemical and haematological investigations. Complete blood count was performed using automated hematology cell counter. Plasma glucose, liver and kidney function tests were done through standard procedure using automated biochemistry analyzer. Measurement of HbA1c was done by high performance liquid chromatography (HPLC) method. Total serum cholesterol, serum triglyceride and HDL-cholesterol were measured using commercially available assay kits. The estimation LDL-cholesterol was done by Friedwald equation. A complete iron profile was done which included estimation of serum iron according to ICSH 1978 [11]. Serum ferritin was done through commercially available kit. Total iron binding capacity (TIBC) values were determined by colorimetric methods (calculation method). Transferring saturation was calculated as (serum iron level x 100%)/TIBC.

Patients were defined as total iron deficiency (TID) if serum ferritin was less than 100 μg/l. Functional iron deficiency (FID) was defined as serum ferritin more than 100 μg/l with serum transferrin less than 20%. Normal iron status was defined as serum ferritin more than 100 μg/l with serum transferrin more than 20%. High sensitivity *C*- reactive protein (hs-CRP) were measured by ELISA method using hs CRP assay kit. Serum vitamin $B_{12,}$ folate and erythropoietin were assayed using commercially available kits.

2.1. Statistical analysis

The patients were divided into Anaemia and non-anaemia groups and various parameters between the two groups were compared. For further analysis, the patients were divided into FID, TID and Normal Iron status groups-clinical and biochemical characteristics were compared between these 3 groups. Data was expressed as mean±SD for continuous variables and percentages for categorical variables. Means were compared using independent samples *t*-test. Comparison between FID, TID and normal iron status was performed using one way ANOVA. Chi square test was used to compare categorical variable between groups. Statistical analysis was carried out on SPSS version 22 (IBM, USA).

3. Results

A total of 89 patients with type 2 diabetes were studied out of which 15 had anaemia (16.8%). Out of this 13 had total iron deficiency and one each had functional iron deficiency and normal iron status respectively. The comparison of biochemical parameters between the anaemia and normal haemoglobin groups is shown in Table 1.

On comparing the biochemical parameters, serum ferritin and serum iron showed obvious differences. Though, other biochemical and inflammatory markers did not show any significant differences.

On assessment of the iron status 49 patients showed TID (55.05%) while 16 showed FID (17.9%)and 24 normal iron status (27.05%). The comparison of clinical and biochemical parameters between the three groups is shown in Table 2 while the serum iron profile and inflammatory markers are compared in Table 3. The mean hs-CRP in the iron deficient group (TID + FID) was greater than the iron sufficient group (1.96 ± 0.91) vs 1.46 ± 0.96, p = 0.04.

4. Discussion

The present study found that a significant proportion of patients with type 2 diabetes mellitus had anaemia even after exclusion of all cases of nephropathy. Most of these patients have iron deficiency anaemia. Iron deficiency was observed in about three-fourths of all patients of type 2 DM irrespective of status of anaemia. Out of these over 75% had total iron deficiency while the remaining had functional iron deficiency.

Our study found a higher prevalence of iron deficiency anaemia i.e. 16.8%than previously reported in several studies. The reported prevalence of anaemia in type 2 diabetes patients ranges from 8% to 23% in studies from other parts of the world [6,12]. Some Indian studies have reported a prevalence of 42% [13] and 92% [14] respectively. However, most of these studies have included patients with diabetic nephropathy and there is scanty data on prevalence of anaemia in type 2 diabetes without nephropathy particularly from India. The reported prevalence of anaemia in these studies isonly around 9–10% [6,12]. The higher prevalence of anaemia in our study may be attributable to higher prevalence of iron deficiency in the Indian population. It could also be secondary to a higher hepcidin response to T2DM related inflammation among Indians.

We found significant iron deficiency in majority of patients with T2DM even though a small proportion of them manifested anaemia. Over half of T2DM patients had TID and nearly one fifth had functional iron deficiency. Functional iron deficiency represents a state in which iron reserves are not available for erythropoiesis as they are locked by chronic inflammation and high hepcidin levels. This phenomenon is known to occur in chronic kidney disease and is well described in diabetic kidney disease. However, functional iron deficiency in diabetic patients without nephropathy has been less well studied. The NHANES study in the United States, reported that prediabetic patients had significantly higher serum ferritin with low transferrin saturation compared to those with NGT. However, anaemic patients were not included in the study [15]. The authors attributed the high serum ferritin to be secondary to associated low grade inflammation in them.

Systemic inflammation has also been suggested as an important factor causing anaemia in type 2 diabetes without renal involvement, since diabetes mellitus is a chronic inflammatory state. Various studies have found that type 2 diabetic patients with anaemia have an increased production of pro-inflammatory cytokines including hs-CRP, IL-6, adiponectin, Intercellular Adhesion Molecule-1 (ICAM-1) And Vascular Cell Adhesion Molecule (VCAM-1) as compared to those without anaemia supporting this hypothesis [16]. Our study also found a significantly higher hs CRP in patients with iron deficiency as compared to those with normal iron status supporting a role for chronic inflammation in its pathogenesis.

Several studies have reported higher serum ferritin levels in type 2 diabetic patients which may represent increased ferritin release from injured cells due to chronic inflammation rather than an alteration in iron metabolism [15]. The normal ferritin levels in the state of functional iron deficiency as opposed to low ferritin levels in iron deficiency anaemia as noted in our study could also represent higher levels of inflammation in this group of patients.

Chronic inflammation and associated markers of inflammation such as IL-6 play a pivotal role in increasing hepcidin levels. Hepcidin inhibits iron transport out of gut enterocytes and macrophages by affecting the iron transporter, ferroportin. It can

Table 1
Biochemical parameters between anaemia and normal haemoglobin groups.

Parameters	Type 2 diabetes mellitus without anaemia (Mean ± SD)	Type 2 diabetes patients with anaemia (Mean ± SD)	P value
Serum ferritin (μg/L)	103.86 ± 20.087	69.93 ± 34.08	**0.001**
Serum iron (μg/L)	101.41 ± 31.00	71.80 ± 34.83	**0.001**
TIBC (μg/dl)	329.54 ± 86.49	333.27 ± 73.70	NS
Transferrin saturation (%)	24.52 ± 24.87	20.97 ± 3.14	NS
Ankle brachial index	1.06 ± 0.13	1.10 ± 0.15	NS
Hs-CRP (ng/ml)	1.84 ± 0.92	1.84 ± 1.07	NS
Erythropoietin (mIU/ml)	27.73 ± 3.66	29.45 ± 4.00	NS
Vit B12 (pg/ml)	223.17 ± 15.55	222.00 ± 19.33	NS
Folic acid (ng/ml)	1.86 ± 0.35	1.82 ± 0.52	NS

Table 2
Comparison of clinical and biochemical parameters among patients with total iron deficiency, functional iron deficiency and normal iron levels.

Parameters	Patients with total iron deficiency (Mean ± SD)	Patients with functional iron deficiency (Mean ± SD	Patients with normal iron levels (Mean ± SD)	P value
Age (years)	49.76 ± 10.38	50.00 ± 10.28	48.67 ± 9.59	NS
BMI (kg/m2)	26.30 ± 4.56	25.38 ± 3.50	26.52 ± 3.95	NS
WHR	0.93 ± 0.10	0.95 ± 0.13	0.92 ± 0.05	NS
Hb (g/dl)	13.02 ± 1.21	13.47 ± 0.91	13.65 ± 0.98	NS
FBS (mg/dl)	185.94 ± 56.75	185.44 ± 43.97	162.04 ± 44.88	NS
PPBS (mg/dl)	262.92 ± 65.65	252.13 ± 39.28	237.04 ± 52.69	NS
HbA1c (%)	10.34 ± 2.30	9.61 ± 1.83	9.58 ± 1.77	NS
T. CHL (mg/dl)	196.33 ± 36.14	265.63 ± 115.05	184.79 ± 42.15	<0.001
HDL (mg/dl)	42.47 ± 11.51	42.06 ± 5.59	44.42 ± 7.16	NS
LDL (mg/dl)	109.24 ± 66.62	124.25 ± 78.66	110.79 ± 50.29	NS
TG (mg/dl)	128.08 ± 40.43	145.94 ± 28.53	130.29 ± 43.89	NS

Table 3
Comparison of serum iron profile and inflammatory markers.

Parameters	Patients with total iron deficiency (Mean ± SD)	Patients with functional iron deficiency (Mean ± SD	Patients with normal iron levels (Mean ± SD)	P value
Sr iron (μg/L)	91.12 ± 33.11	116.00 ± 21.21	94.17 ± 36.76	**0.031**
TIBC (μg/dl)	302.61 ± 79.32	346.94 ± 89.55	375.25 ± 69.00	**0.001**
Transferrin saturation (%)	26.77 ± 30.37	17.56 ± 1.62	22.34 ± 1.49	NS
Ankle brachial index	1.076 ± 0.14	1.06 ± 0.13	1.05 ± 0.13	NS
Hs-CRP (ng/ml)	2.10 ± 0.95	1.42 ± 0.49	1.47 ± 0.99	**0.010**
Erythropoietin (mIU/ml)	28.22 ± 4.25	27.29 ± 2.31	28.08 ± 3.29	NS

therefore be hypothesised that high hepcidin due to inflammation leads to a state of iron insufficiency which manifests initially as FID. At this stage ferritin levels can be expected to be high or normal. As the iron absorption continues to be compromised due to on-going low grade inflammation, the ferritin levels begin to fall and the patients enters a state of TID and subsequently overt iron deficiency anaemia.

There are few limitations in our study. Sample size of the study was low. Findings of our study should be confirmed by larger studies. We used 24 h urine protein to diagnose diabetic nephropathy. Therefore, patients with microalbuminuric range nephropathy may have been included in our study group. Also, we did not perform upper GI endoscopy to definitely rule out H. pylori or small ulcers which can be associated with iron deficiency and anemia. Clinically, the patients did not display any features suggestive of dyspepsia or other abdominal complaints that would have justified an invasive procedure such as upper GI endoscopy. At the same time, in the absence of upper GI endoscopic findings, it may not be possible to firmly conclude that chronic inflammation is the cause of iron deficiency in these patients.

5. Conclusions

In conclusion, the present study found a high prevalence of iron deficiency anaemia in type 2 diabetic patients even in the absence of nephropathy. In addition, most of the diabetic subjects also displayed an iron deficiency state, the cause of which needs further investigation.

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None.

Declaration of competing interest

None.

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