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Original Research Article

Study of anaemia in type 2 diabetes mellitus and its correlation with albuminuria and eGFR

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ABSTRACT

Background: Diabetes mellitus (DM) is a common metabolic disease. Anaemia is a common accompaniment to diabetes, particularly in patients with albuminuria or reduced renal function. Anaemia is more frequent and more severe at any level of glomerular filtration rate (GFR) in diabetics compared to nondiabetic patients.
Methods: One hundred patients with type 2 diabetes mellitus were included in the study. Complete blood count, serum iron profile, ferritin, vitamin B12 and folate, kidney function tests and urine albumin creatinine ratio were assessed for these patients. Estimated glomerular filtration rate (eGFR) was calculated with MDRD-4 variable formula.
Results: Fifty-four patients (54%) were found to be anaemic. Serum ferritin <50 µg/l was taken as cut off for defining iron deficiency anaemia. 21 patients had iron deficiency anaemia (IDA) and 33 had anaemia of chronic disease (ACD). Serum vitamin B_{12} and folate levels were within normal limits in all the patients. There was a rise in the prevalence of anaemia from 25.9% in patients with a normal ACR to 59.2% in those with microalbuminuria and to 75% in macroalbuminuria.
Conclusions: Any degree of renal impairment and albuminuria are the risk factors for anaemia in these patients. Hence screening, characterization and treatment of aneamia in type 2 DM may be helpful in management of these patients.

Keywords: Albuminuria, Anaemia, Diabetes mellitus

INTRODUCTION

Diabetes mellitus is not a single disease entity, but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia. The impacts of Type 2 DM are considerable as a lifelong disease; it increases morbidity and mortality and decreases the quality of life.[1]

It is highly associated with premature mortality, morbidity, and disability, impacting people's overall quality of life. Globally, 537 million people were estimated to have diabetes mellitus (DM) in 2021, projecting to rise to 783 million by 2045.[2]

Anaemia is considered as a key indicator of chronic kidney disease and an important cardiovascular risk factor.[3,4]

The etiology of anaemia in diabetes is multifactorial and includes inflammation, nutritional deficiencies, concomitant autoimmune diseases, drugs and hormonal changes in addition to kidney disease.[5] The reasons for the earlier onset of anaemia in patients with diabetes

include severe symptomatic autonomic neuropathy, causing efferent sympathetic denervation of the kidney and loss of appropriate erythropoietin, damage to the renal interstitium, systemic inflammation and inhibition of erythropoietin release.[6] Previous studies have shown that the incidence of anaemia in diabetic patients is mostly associated with the presence of renal insufficiency. Thus, patients with diabetes have a greater degree of anaemia for their level of renal impairment than non-diabetic patients presenting with other causes of renal failure.

The patho-physiologic basis for elevated urinary albumin excretion entails the binding of glucose to proteins resulting in excessive protein glycosylation with the buildup of advanced glycated end products. This leads to deposition of advanced glycated end products on the glomerulus resulting in renal and glomerular hypertrophy, mesangial matrix accumulation and thickening of glomerular basement membrane. This abnormality permits the leakage of low molecular weight proteins that is albumin. This is the stage of microalbuminuria (incipient nephropathy).[1]

The decreased formation of erythropoietin due to kidney damage is the major cause of anaemia.[7] Both nutritional deficiency and hyporesponsiveness to erythropoietin contribute to anaemia in diabetic patients with chronic kidney disease. The cause of erythropoietin deficiency in these patients is thought to be reduced renal mass with consequent depletion of the hormone. Possible causes of this erythropoietin hyporesponsiveness include systemic inflammation and microvascular damage in the bone marrow.[8,9]

However, anaemia also occurs in diabetes without underlying chronic renal disease.[7] The anaemia may be apparent before demonstrable changes in the glomerular filtration rate (GFR). Anaemia is generally thought to be a complication of overt nephropathy but its relation with pre-clinical chronic kidney disease (microalbuminuria) is not clearly understood.[7] The early detection and correction of anaemia in diabetes is important as the evidence already shows that it occurs earlier and with greater severity in diabetes than in the non-diabetic population.[10]

The early detection and correction of anaemia in diabetes is important as the evidence already shows that it occurs earlier and with greater severity in diabetes than in the non diabetic population.[5,11] The aim of this study was to identify types of anaemia in type 2 diabetes mellitus and to study correlation between anaemia and renal function in type 2 diabetes mellitus.

METHODS

The present study was a cross-sectional conducted from 1st November 2014 to 31st March 2016 at the department of pathology and department of medicine, PGIMER and Dr. R. M. L. Hospital, New Delhi. The subjects for the study included 100 patients with age more than 40 years with type 2 diabetes mellitus attending the OPD. Blood specimens were collected and processed for determination of complete blood count, blood glucose, HbA1C, serum vitamin B_{12}, serum folic acid, serum ferritin, serum iron profile, blood urea, serum creatinine and serum uric acid.

Urine samples were processed for routine and microscopic examination and urine albumin/creatinine ratio was estimated in a spot urine sample. Normal urinary albumin excretion was categorized by a urine ACR of <30 mg/g (gender independent). Macroalbuminuria was defined as ACR>300 mg/g.

eGFR (ml/min/1.73m^2) was calculated using the MDRD 4-variable formula: 175 x (serum creatinine) − 1.154 × (age) − 0.203 (in female × 0.742).[12]

Renal disease was classified into five stages according to eGFR: stage 1 with eGFR≥90 ml/minute, stage 2 with eGFR of 60-89 ml/minute, stage 3 with eGFR of 30-59 ml/minute, stage 4 with eGFR of 15-29 ml/minute and stage 5 with eGFR<15 ml/minute.

The patients with the following diagnosis were excluded from the study: known cases of malignancy, tuberculosis, abnormal bleeding (e.g. piles, menorrhagia, esophageal varices), hypothyroidism, end stage renal disease, acute kidney injury, patients on dialysis, HIV positive, hemoglobinopathies and urinary tract infection.

Anaemia was defined as hemoglobin values <13.0 gm/dl for men and <12.0 gm/dl for women (WHO criteria).

Statistical analysis

Descriptive statistics such as mean, median or proportions was used to describe the study sample. Independent student's t test and ANOVA were applied. P value <0.05 was considered significant. Data was analyzed using SPSS (version 20).

RESULTS

Descriptive statistics

Patient's characteristics

The study included 100 outdoor patients of type 2 diabetes mellitus. There were 53 males and 47 females. The age of the study population ranged from 40 to 82 years, with a mean of 51.80 years. The duration of disease ranged from 4 months to 10 years, with a mean of 3.99 years. The mean hemoglobin in the study population was 11.55 gm/dl and mean HbA1c was 7.06%. Anaemia was present in 54 patients and the mean hemoglobin in anaemic patients was 10 gm/dl. The mean hemoglobin in

anaemic males was 9.86 gm/dl and in anaemic females was 10.12 gm/dl.

Table 1: Demographic and clinical features of the patients.

Variables	Age±SD (years)	Median (years)
Age	51.80±8.78	50
Duration of disease	3.99 ±2.45	3

Using the definition for anaemia as Hb<13 gm/dl in men and <12 gm/dl in women, 54 (54%) patients out of 100 were found to be anaemic. Twenty-four (24) were men and 30 were women. Total leucocyte count was normal in 98% of the patients. Two of the patients had mild leucocytosis. Platelet count was normal in all the patients (Table 2).

Serum ferritin, serum vitamin B_{12}, serum folate and iron profile were done in all the patients. All the patients had normal serum vitamin B_{12} and folate levels. For characterization of anaemia, Serum ferritin <50 µg/l was taken as cut off for defining IDA and serum ferritin >100 µg/l was taken to rule out IDA.[13] Twenty one anaemic patients (21%) had serum ferritin <50 µg/l, 3 anaemic patients (3%) and 1 non-anaemic patient (1%) had serum ferritin levels in between 50-100 µg/l while 30 anaemic (30%) and 45 non-anaemic patients (45%) had serum ferritin >100 µg/l.

Table 2: Hematological parameters in anaemic and non-anaemic patients.

Parameters	Anaemic (n=54) (mean±SD)	Non-anaemic (n=46) (mean±SD)
Hemoglobin (gm/dl)	10.00±1.71	13.37±0.94
Total leucocyte count (cells/mm^3)	7707.41±2087.32	7406.52±1807.32
Platelet count (platelets/mm^3)	2.32±0.68	2.23±0.53

Iron profile was also done in all these patients to evaluate iron status. Iron profile in all the 21 patients with serum ferritin <50 µg/l reflected iron deficiency.

Four patients had serum ferritin levels between 50-100 µg/l. Out of these 3 patients were anaemic and 1 was non-anaemic. Iron profile in 3 anaemic patients with serum ferritin 50-100 µg/l reflected anaemia of chronic deficiency.

Out of 75 patients with serum ferritin >100 µg/l, 30 were anaemic and 45 were non -anaemic. The iron profile of the anaemic patients reflected anaemia of chronic disease.

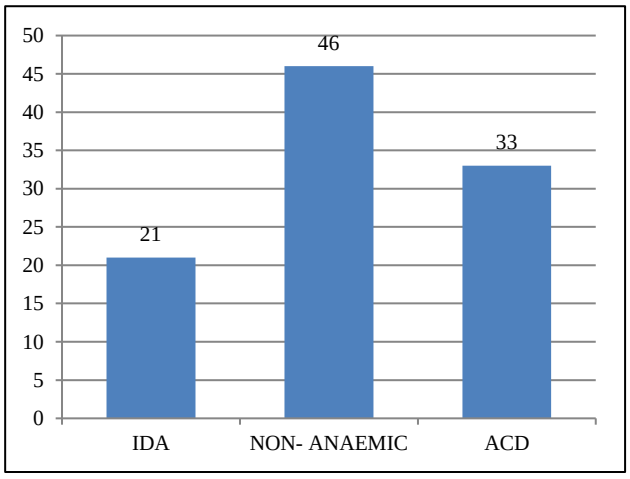

Figure 1: Prevalence and type of anaemia.

Thus, on the basis of serum ferritin and Iron profile, anaemic group was divided into iron deficiency anaemia (IDA) and anaemia of chronic disease (ACD). Out of 54 anaemic patients 21 (21%) had IDA and 33 (33%) had ACD (Figure 1).

Liver function tests were within normal limits in all the patients.

Renal function tests

The mean blood urea levels were 31.79 mg/dl, mean serum creatinine 0.87 mg/dl and mean uric acid 4.73 mg/dl. The mean urine ACR level was 239.80 mg/g. Twenty seven (27) patients were normoalbuminuric, 49 patients were microalbuminuric and 24 patients had macroalbuminuria. The mean estimated eGFR was 101.49 ml/minute/1.73 m^2.

Anaemia and renal function

Urine ACR was higher in anaemic patients (Mean±SD = 328.89±396.92) as compared to non-anaemic patients (Mean±SD =135.22±141.34). This difference was statistically significant (p value =0.002). The eGFR levels were lower in anaemic patients (Mean±SD =89.06±34.65) as compared to non-anaemic patients (Mean±SD =116.09±44.37). This difference was statistically significant (p value =0.001) (Table 3).

Table 2: Comparison of urine ACR and eGFR in anaemic and non-anaemic diabetes mellitus patients.

Parameter	Group	Number of patients	Mean	Standard deviation	P value
Urine ACR (mg/g)	Anaemic	54	328.89	396.92	0.002
	Non-anaemic	46	135.22	141.34	
eGFR (ml/min/1.73m^2)	Anaemic	54	89.06	34.65	0.001
	Non-anaemic	46	116.09	44.37	

Further, the comparison of urine ACR and eGFR was done between the patients with iron deficiency anaemia and anaemia of chronic disease. The urine ACR in patients with iron deficiency anaemia was 294.29±286.45 mg/g while the patients with anaemia of chronic disease had urine ACR of 350.91±456.47 mg/gm. This difference was not statistically significant (p value =0.61). The eGFR in patients with iron deficiency anaemia was 89.87±35.90 ml/minute/1.73 m^2 while the patients with anaemia of chronic disease had eGFR of 88.54±34.38 ml/minute/1.73 m^2. This difference was not statistically significant (p value =0.89).

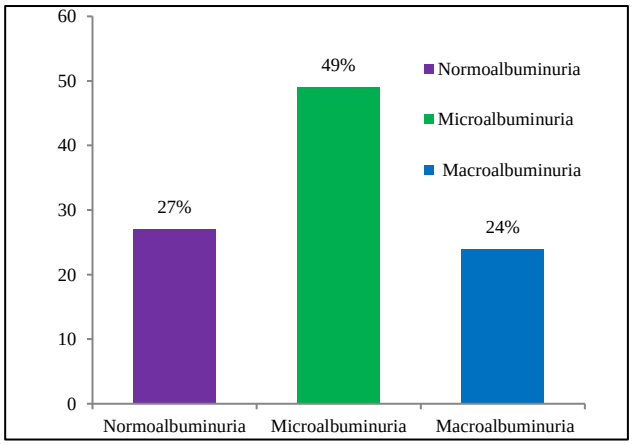

Figure 2: Distribution of the patients according to albuminuria.

According to urine ACR, the patients were further divided into three groups namely: normoalbuminuria, microalbuminuria and macroalbuminuria. The prevalence of anaemia in each group was assessed. Twenty seven patients (27%) had normoalbuminuria, 49 (49%) had microalbuminuria and 24 patients (24%) had macroalbuminuria (Figure 2).

The mean urine ACR levels in the three groups were 15.56 mg/gm, 182.65 mg/gm and 608.75 mg/gm respectively.

Seven patients out of 27 patients with normoalbuminuria had anaemia (25.9%), 29 patients out of 49 patients with microalbuminuria had anaemia (53.7%) and 18 patients out of 24 patients with macroalbuminuria had anaemia (75%). This shows that there is sharp and steady increase in the prevalence of anaemia with increasing urinary albumin excretion (Figure 3).

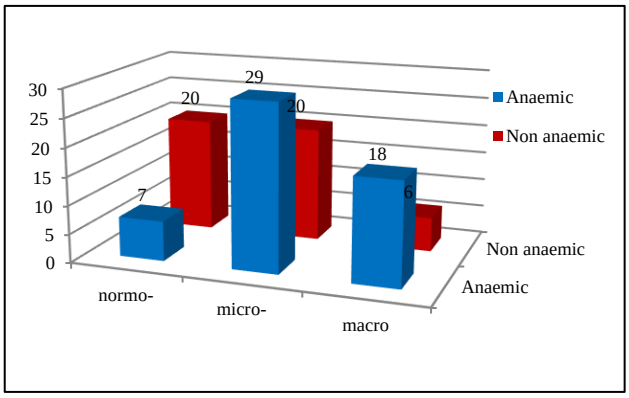

Figure 3: Prevalence of anaemia among groups based on levels of albuminuria.

Table 3: Distribution of patients based on eGFR.

Stage	Anaemic (n=54)	Non-anaemic (n=46)	Total (n=100)
Stage 1	24 (41.4%)	34 (58.6%)	58
Stage 2	20 (64.5%)	11 (35.5%)	31
Stage 3	8 (100%)	0 (0%)	8
Stage 4	2 (66.67%)	1 (33.33%)	3

The prevalence of anaemia increased from 41.4% in stage 1, 64.5% in stage 2, 100% in stage 3 and 66.6% in stage 4.

Correlation of hemoglobin with urine ACR and eGFR

There was significant negative correlation between hemoglobin and ACR (p=0.005; r=-0.280) (Figure 4).

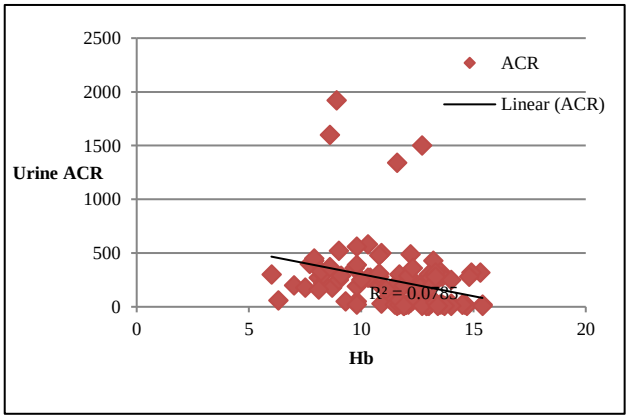

Figure 4: Correlation of hemoglobin with urine ACR.

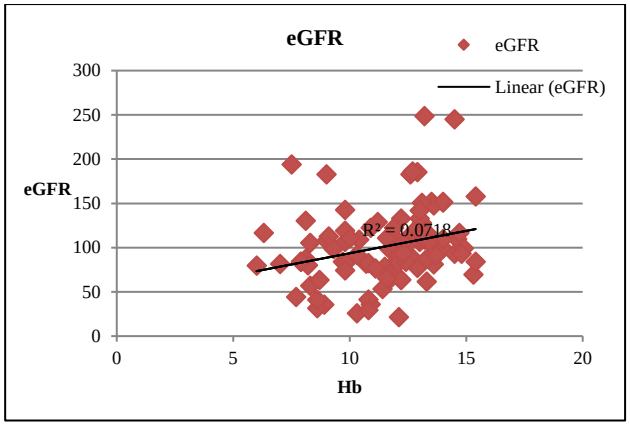

Figure 5: Correlation of hemoglobin with eGFR.

A significant positive correlation was seen between hemoglobin and eGFR (p=0.007; r=0.268) (Figure 5).

DISCUSSION

In this study prevalence and type of anaemia in patients with type 2 diabetes mellitus and its correlation with renal function was assessed. One hundred patients (47 females and 53 males) were included in the study.

Laboratory abnormalities

In this study, anaemia was defined as a hemoglobin level <13 gm/dl in males and <12 gm/dl in females (according to WHO criteria). Fifty four patients (54%) were found to be anaemic. This was higher than other reported studies (13-40%).[3,7,8,12-18] This is due to the fact that in this study WHO criteria was used to define anaemia while only some of the other studies had not used WHO criteria to define anaemia.

A study by Jones et al characterized the nature of previously unrecognized anaemia in 7331 diabetic patients. They found anaemia in 15% of the patients and concluded that 34% of these patients had erythropoietin deficiency, 40% had anaemia due to nutritional deficiencies (vitamin B_{12}, folate or ferritin) and 26% had an unexplained anaemia.[19]

Serum ferritin was done in all the patients. A value of <50 µg/l of serum ferritin was seen in 21 patients with mean of 15.28 µg/l, all of these had low serum iron levels (mean±SD =44.48±5.52), increased total iron binding capacity (mean±SD =328.52±54.27), increased unbound iron binding capacity (mean±SD =274.52±59.38) and decreased transferrin saturation (mean±SD =13.73±2.96). These patients were classified as patients with iron deficiency anaemia (IDA).

Four patients had serum ferritin levels between 50-100 µg/l. Among these three were anaemic and one was non-anaemic. Anaemic patients had mean serum ferritin levels of 76.53±11.93µg/l with low to normal serum iron levels (mean±SD =57.33±5.13), low total iron binding capacity (mean±SD =223.67±26.69), low unbound iron binding capacity (mean±SD =166.33±21.59) and normal transferrin saturation (mean±SD =25.66±0.83). Thus, these patients were classified as patients with anaemia of chronic disease (ACD). The non-anaemic patient had normal iron profile.

Seventy five patients (75) had serum ferritin levels >100 µg/l. Out of which, 30 were anaemic and 45 were non-anaemic. The mean serum ferritin in these anaemic patients was 156.21±30.35µg/l with low to normal serum iron levels (mean±SD =52.63±19.97), low total iron binding capacity (mean±SD =193.13±61.07), low unbound iron binding capacity (mean±S.D =140.67±43.35) and normal transferrin saturation (mean±SD =26.68±3.68).These patients were classified as patients with anaemia of chronic disease (ACD). Iron profile of 45 non-anaemic patients was within normal limits.

Thus, on the basis of serum ferritin and iron profile, the anaemic patients were divided into ACD and IDA. Thirty three patients (33%) had ACD and 21 patients (21%) had IDA.

ACD was the commonest type of anaemia in patients with DM in this study. The prevalence of IDA in diabetic patients was higher from other reported studies. This may be explained by dietary deficiency in the study population.[20]

Only few studies have evaluated the cause of anaemia in diabetic patients.

Hosseini et al used RBC indices for typing of anaemia. They found anaemia in 93 patients out of 305 diabetic patients (30.4%). Out of which, normocytic normochromic was seen in 46 patients (15.1%), microcytic hypochromic in 44 patients (14.4%) and macrocytic hyperchromic anaemia in 3 patients (1%).[21]

Pranay et al assessed the type of anaemia in diabetic patients by peripheral blood smear examination. They found anaemia in 80 patients out of 200 diabetic patients (40%). Fifty six anaemic patients (70%) showed normochromic normocytic anaemia, 20 anaemic patients (25%) showed microcytic hypochromic anaemia and 4 anaemic patients (5%) showed macrocytic hypochromic anaemia.[22]

Salma et al found the prevalence of anaemia is significantly higher in the poorly controlled diabetics. Also, the average age of the patients with anaemia is significantly higher than average age of patients without anaemia.[23]

However, in our study serum vitamin B12 and folate levels were within normal limits in the anaemic patients. The mean levels of vitamin B12 and folate in the anaemic

population were 471.34±95.15 and 9.15±2.68 respectively.

Anaemia and renal function

In this study, renal function of the patients was assessed with urine ACR and eGFR.

The prevalence of anaemia was highest in patients with macroalbuminuria (75%) followed by 59.2% in patients with microalbuminuria and 25.9% in patients with normoalbuminuria.

Renal disease was classified by eGFR calculated by MDRD 4-variable formula. The prevalence of anaemia was highest in stage 3 patients (100%) followed by 66.6% in stage 4, 64% in stage 2 and 43% in Stage 1. Thus, there was increase in the prevalence of anaemia with an increase in the stage of renal disease.

The end stage renal disease patients (stage 5) were not included in this study.

These results were in concordance with the previously reported studies.[3,8,15,16]

Adetunji et al found elevated ACR in 136 patients out of 502 diabetic patients. The prevalence of anaemia was 19%, 29% and 41% in the patients with normoalbuminuria, microalbuminuria and macroalbuminuria respectively. On stratification of patients according to eGFR, they found increase in prevalence of anaemia from 8% in stage 1 CKD to 100% in end stage renal disease (stage 5).[8]

Thomas et al found anaemia in 190 patients out of 820 diabetic patients (according to WHO criteria). They further stratified patients according to GFR and albumin excretion rate. Sixty one percent (61%) patients had normoalbuminuria, 27% had microalbuminuria and 12% had macroalbuminuria. On comparing normoalbuminuric patients with a GFR>80 ml/minute/1.73 m^2 and patients with persistent microalbuminuria, the latter had 2 times the risk of anaemia (OR 2.3, p<0.02). On comparing normoalbuminuric patients with a GFR>80 ml/minute/1.73 m^2 and patients with persistent macroalbuminuria, the latter had >10 times the risk of anaemia (OR 10.1, p<0.0001). They also found that patients with normoalbuminuria but impaired renal function (<60 ml/minute/1.73 m^2) had 6 times the risk of anaemia (OR 5.9, p<0.0001) compared with patients with normoalbuminuria and normal renal function (GFR >80 ml/minute/1.73 m^2).[3]

Jones et al found increase in the prevalence of anaemia from 9% in patients with eGFR≥60 ml/minute/1.73 m^2 to 36% in those with eGFR<60 ml/minute/1.73m^2.[15]

In a study by Mama et al, it showed 20.1% of the participants were anaemic. Being age >60 years, poor glycemic control, eGFR 60-89.9 ml/minute/1.73 m^2, DM duration >10 year and having diabetic complications were significantly associated with anemia.[24]

Limitations of the study were: present study was a cross sectional study so long term follow up are needed for conclusive results, sample population was from already high prevalent anaemic geographical areas. So, other causes need to be ruled out. Control groups were not used (observational study).

CONCLUSION

This study showed that there is significant association between anaemia and renal function. The prevalence of anaemia increases as kidney function declines. Anaemia was also found in the patients with normoalbuminuria and normal eGFR. Hence anaemia can occur in type 2 DM without overt renal impairment. Thus screening, characterization and treatment of anaemia in type 2 DM may be helpful in management of these patients.

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