



Morphological Classification, Associated Factors and Prevalence of Anaemia in Type 2 Diabetes Mellitus Patients at Fort Portal Regional Referral Hospital, Western Uganda

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Abstract

Purpose: To determine the morphological classification, associated factors and prevalence of anaemia among T2DM patients at Fort Portal Regional Referral Hospital, Southwestern Uganda. **Methods:** This cross-sectional study enrolled 230 T2DM patients, 60 (26.1%) males and 170 (73.9%) females. The socio-demographic characteristics were collected using structured questionnaires, 4ml of venous blood was collected from each study participant into an EDTA vacutainer. The random blood sugar level of each participant was measured using a OneTouch glucometer and recorded. The blood samples were then transported to the central laboratory for Complete Blood Counts using a SYSMEX XN 550 haematology analyser. Thin smears were made for participants who had low Hb (<12.0 g/dl for females and <13.0 g/dl for males) according to the WHO definition of anaemia. The thin films were fixed with absolute methanol and stained using May–Grünwald Giemsa stain then dried and examined for the morphological types of anaemia using ×1000 magnification. **Results:** The overall prevalence of anaemia in T2DM in this study was 8.3%. Anaemia was equally distributed among males and females (8.3% and 8.2% respectively). As far as morphological types of anaemia are concerned, normocytic normochromic anaemia was more prevalent, 12 (63.2%) followed by normocytic hypochromic, 3 (15.8%), microcytic normochromic, 2 (10.5%) and microcytic hypochromic, 2 (10.5%). The factors which were associated with anaemia were level of education (AOR 4.2, p-value 0.174) and age 50 years and above (AOR: 1.2, p-value 0.712). However, they were not statistically significant; p-value > 0.05. **Conclusion:** The prevalence of anaemia in T2DM in this study was low compared to other studies done within the country. Normocytic normochromic was

the commonest type of anaemia and low level of education and age 50 years and above were associated with anaemia.

Subject Areas

Diabetes Mellitus

Keywords

Type 2 Diabetes Mellitus, Anaemia, Prevalence, Morphological Classification, Associated Factors

1. Introduction

Anaemia is a common comorbidity among patients with Type 2 Diabetes mellitus (T2DM). It has been shown to accelerate the progression and development of other T2DM-associated complications such as nephropathy, retinopathy, neuropathy, impaired wound healing, and macro-vascular disease [1]-[3]. The manifestations of anaemia are more severe in diabetic patients, who are also twice more likely to develop anaemia than non-diabetic patients [2] [4]-[6]. T2DM accounts for approximately 90% of the cases of diabetes worldwide [7] [8]. The number of individuals living with DM is rising at an exponential rate and is predicted to affect 693 million people aged 18 - 99 years by 2045, globally, which is approximately a 65% increase from the 451 million affected in 2017 [9] [10].

The most prominent cause of anaemia in DM is Chronic Kidney Disease (CKD), often resulting from Diabetic Kidney Disease (DKD) [11] [12]. Development of anaemia is often common during the later stages of chronic kidney disease [13]. Its pathogenesis is mainly attributed to a fall in erythropoietin levels [1] [3] [14], absolute or functional iron deficiency and shortened red cell survival [4]. A key factor in CKD-related anaemia is the reduction in erythropoietin (EPO) production [15] [16]. In CKD, reduced blood flow and oxygen delivery to the kidneys impair the activation of Hypoxia Inducible Factor (HIF), preventing the EPO gene from being triggered, consequently lowering EPO levels [15]. Anaemia in DM is triggered by several other mechanisms as well. DM induced hyperglycaemia, hypertension, and dyslipidaemia, stimulate pro-inflammatory cytokines (e.g., IL-1, TNF α) and adhesion molecules (ICAM-1, VCAM-1) that suppress EPO production under hypoxic conditions [17]. Chronic hyperglycaemia also affects RBC structure and lifespan, further contributing to anaemia. Elevated glycated haemoglobin (HbA1c) levels increase the β -sheet formation in haemoglobin molecules [18]. Additionally, hyperglycaemia alters RBC membranes, exposing phosphatidylserine on their surface, which signals macrophages to remove them prematurely, shortening their lifespan (Gabreanu and Angelescu, 2016; Tujuba *et al.*, 2021). These processes, along with changes in RBC membrane proteins, lead to greater RBC destruction and consequently lower RBC counts in diabetic patients

[19]. Kidney damage from microvascular injury during DKD causes severe proteinuria, leading to a significant drop in plasma EPO levels through ycurine loss [20].

Anaemia in diabetes mellitus has been associated with nutritional deficiencies, inflammation, concomitant autoimmune diseases, advanced age, lower Body Mass Index (BMI), longer duration of diabetes, peripheral vascular disease, specific medications and hormonal changes [21].

The prevalence of anaemia among DM patients varies in different areas; 41.7% - 63% in Pakistan [22], 29.7% in Kuwait [23], 59% in UK [24], 11.5% - 17.8% in Australia (Gauci, *et al.*, 2017) and 67% in India [25]. In Africa, there were observed variations in the prevalence of anaemia in T2DM with 41.4% in Cameroon [26], and 29.8% in Ethiopia [27].

In Uganda, there is an increasing burden of people living with DM [28] [29]. However, there is limited published data on the prevalence of anaemia in T2DM. This study, aimed at investigating the morphological classification, associated factors, and prevalence of anaemia among T2DM patients at Fort Portal Regional Referral Hospital (FRRH) in Western Uganda.

2. Methods

2.1. Study Site

Fort Portal Regional Referral Hospital also known as Fort Portal Hospital or Buhinga Hospital is located in Fort Portal City, Kabarole district, Western Uganda.

The hospital has a bed capacity of 333 and serves the districts of Kabarole, Bundibugyo in the West, Kamwenge in the South-east, Kasese in the South-west, Ntoroko in the North and Kyenjojo in the East.

Fort Portal Regional Referral Hospital has a diabetic clinic with approximately 650 enrolled Diabetes Mellitus patients and receives an average of 80 T2DM patients per clinic day.

2.2. Target Population

This study was conducted among Type 2 Diabetes Mellitus patients attending the diabetic clinic at Fort Portal Regional Referral Hospital.

2.3. Study Design

This study followed a cross-sectional design. Simple random sampling was used for participant recruitment. Data collection included blood samples, which were analysed using the SYSMEX XN 550 analyser and structured questionnaires. The data were further processed in STATA 15.0 (See **Figure 1**).

2.4. Sample Size

The sample size was calculated using the 1965 Kish and Leslie formula based on a prevalence study from Mulago National Referral Hospital with an 18.2% prevalence of anaemia in DM patients [30]. The resulting sample size was 230 participants.

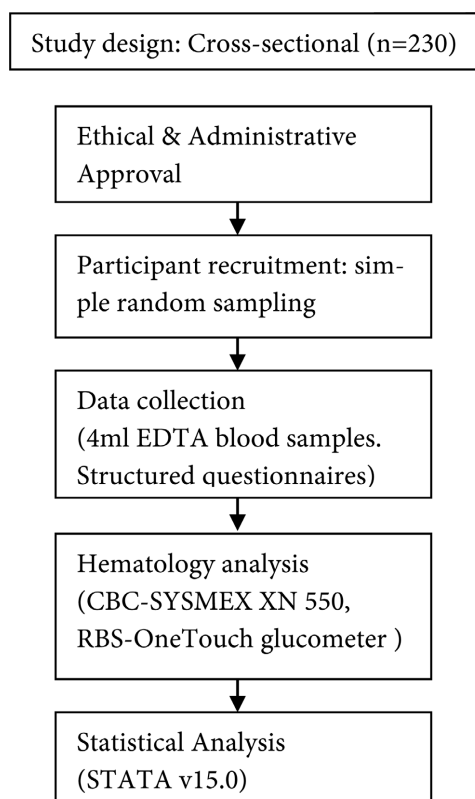


Figure 1. Research process.

2.5. Sampling Technique

Simple random sampling technique was employed to enrol T2DM patients who fitted the study inclusion criteria attending the diabetic clinic at Fort Portal Regional Referral Hospital during a weekly diabetes clinic day.

2.6. Inclusion Criteria

All adult patients with T2DM attending the diabetic clinic at Fort Portal Regional Referral Hospital.

2.7. Exclusion Criteria

T2DM patients were excluded if they were actively bleeding, had received a blood transfusion within two weeks prior to the study, had known hematological diseases, were critically ill, or were taking iron supplements during or within two weeks before the study.

2.8. Data Collection

Demographic data and factors associated with anemia were collected using structured questionnaires. Four milliliters of venous blood were drawn from each participant into EDTA vacutainer tubes. Random blood sugar levels were measured with a OneTouch glucometer and recorded. Blood samples were transported to the central laboratory for Complete Blood Count analysis using the SYSMEX XN

550 hematology analyzer. For participants with low hemoglobin levels (<12.0 g/dL for females and <13.0 g/dL for males) based on WHO criteria, thin smears were prepared, fixed with methanol, stained with May-Grünwald Giemsa stain, and examined at $\times 1000$ magnification to determine the morphological types of anemia.

2.9. Quality Control

The hematology analyzer was calibrated, and control samples were run to ensure accuracy and reproducibility. Data were double entered into an Excel spreadsheet, checked for completeness, cleaned, and analyzed.

2.10. Data Analysis

The dataset was imported into STATA[®] 15.0 software (College Station, Texas, USA) for analysis. Participants' characteristics were described using means or medians for continuous variables and proportions for categorical variables and presented in a table.

The proportion of participants with anaemia was calculated as a fraction of participants with anaemia out of all those enrolled in the study and expressed as a percentage with its corresponding 95% confidence interval (CI). Using Chi-square test, the proportion of participants with anaemia was established and compared between categories of age and gender. A significance level of 5% was used.

The proportion of participants with each morphological type of anaemia was calculated as a fraction of participants with anaemia and expressed as a percentage and presented on a bar-graph.

All participants' factors (socio-demographic, medical, behavioural) were used as independent variables in bivariate analysis, based on both Chi-square test and Logistic regression where repeated analysis comparing each independent variable with anaemia was done. Unadjusted odds ratios with their corresponding 95% CI were reported. A variable was considered significant in this analysis if it had a $p < 0.05$.

All statistically significant factors ($p < 0.05$), those with p -value < 0.2 and those which are biologically plausible with anaemia in T2DM were considered in the multivariate analysis which was performed to control confounding.

The factors in the final multivariate model were then reported together with their adjusted odds ratios and 95% confidence intervals. A variable was considered statistically significant in this analysis if it had a $p < 0.05$.

2.11. Ethical Considerations

Approval was sought from the Department of Medical Laboratory Science and Faculty Research Committee of the Faculty of Medicine (Ref: MUST/MLS/030). Ethical clearance was obtained from the Mbarara University of Science and Technology Research and Ethics Committee (MUST-REC). Administrative clearance to conduct the study was obtained from the Director, of Fort Portal Regional Referral Hospital.

The data obtained from this study was and will be strictly used for study

purposes and was only accessible to the individuals carrying out the research. Participants' privacy was ensured. Patient samples and data were collected in accordance with the principles of the Declaration of Helsinki.

3. Results

Socio-demographic characteristics

A total of 230 participants were enrolled in the study, the mean age of the study participants was 53.9 years (SD: 14.9 years) and the majority aged 50 years and above, 150 (65.2%). The majority of the study participants were females, 170 (73.9%), with no or with primary education (86.5%), residing in rural settings (92.2%), with an estimated monthly income of less than 200,000 Ugandan shillings (86.1%) and were peasant farmers, 134 (58.3%) (See **Table 1**).

Table 1. Participants' socio-demographic characteristics, n = 230.

Characteristic	Frequency, n (%)
Mean age of in years (SD)	53.9 (14.9)
Age categories	
18 - 29	14 (6.1)
30 - 49	66 (28.7)
50 and above	150 (65.2)
Sex	
Male	60 (26.1)
Female	170 (73.9)
Occupation	
None	47 (20.4)
Peasant farmer	134 (58.3)
Business	43 (18.7)
Formal employment	6 (2.6)
Level of education	
None	115 (50.0)
Nursery/primary	84 (36.5)
Secondary	25 (10.9)
Tertiary	6 (2.6)
Marital status	
Single/unmarried	110 (47.8)
Married	120 (52.2)
Residency type	
Rural	212 (92.2)

Continued

Urban	18 (7.8)
Monthly income	
0-50,000	137 (59.6)
500,001-200,000	61 (26.5)
Above 200,000	32 (13.9)

Medical Characteristics

The majority of the study participants had been diagnosed with Type 2 Diabetes Mellitus in 5 or less than 5 years (75.2%), overweight or obese (67%), hypertensive (83%) and on Metformin for treatment of T2DM (88.7%) (See **Table 2**).

Table 2. Participants' medical characteristics, n = 230.

Characteristic	Frequency, n (%)
Duration since diagnosis of T2DM in years	
≤5	173 (75.2)
6 - 10	40 (17.4)
>10	17 (7.4)
Weight status	
Underweight	7 (3.0)
Healthy weight	69 (30.0)
Overweight	60 (26.1)
Obese	94 (40.9)
Hypertension	
No	39 (17.0)
Yes	191 (83.0)
Peptic\duodenal ulcers	
No	94 (40.9)
Yes	136 (59.1)
Diabetic foot	
No	149 (64.8)
Yes	81 (35.2)
Micro-vascular disorders	
No	228 (99.1)
Yes	2 (0.9)
Other disorders	
HIV	1 (0.4)

Continued

Insomnia		1 (0.4)
No		228 (99.1)
Metformin		
No		48 (20.9)
Yes		182 (79.1)
Insulin		
No		204 (88.7)
Yes		26 (11.3)

Proportion of Type 2 Diabetic Patients with Anaemia Attending Fort Portal Regional Referral Hospital, Western Uganda

The overall prevalence of anaemia was 8.3% (95% Confidence interval: 5.3-12.6%). There were no gender or age disparities in the prevalence of anaemia, $p > 0.05$ (See [Table 3](#)).

Table 3. Anaemia prevalence.

Anaemia prevalence type	N	Frequency, n	% (95% CI)	p value*
Overall	230	19	8.3 (5.3 - 12.6)	NA
Age—specific				0.466
18 - 29	14	0	0.0 (NA)	
30 - 49	66	5	7.6 (3.1 - 17.3)	
50 and above	150	14	9.3 (5.6 - 15.2)	
Gender—specific				0.981
Male	60	5	8.3 (3.4 - 18.9)	
Female	170	14	8.2 (4.9 - 13.5)	

*p values based on Pearson chi-square test.

Morphological Types of Anaemia in Type 2 Diabetic Patients Attending Fort Portal Regional Referral Hospital, Western Uganda

The most prevalent morphological type of anaemia was Normocytic normochromic, 12 (63.2%) followed by Normocytic hypochromic, 3 (15.8%) and both microcytic normochromic and microcytic hypochromic had an equal prevalence of, 2 (10.5%) (See [Figure 2](#)).

Factors Associated with Anaemia in Type 2 Diabetic Patients Attending Fort Portal Regional Referral Hospital, Western Uganda

Several factors thought to be associated with anaemia in Type 2 Diabetic patients were analysed. These included: Age, Sex, Occupation, Level of education, Marital status, Residency type, monthly income, Duration since diagnosis of T2DM, Weight status of participant, Hypertension, Peptic\duodenal ulcers,

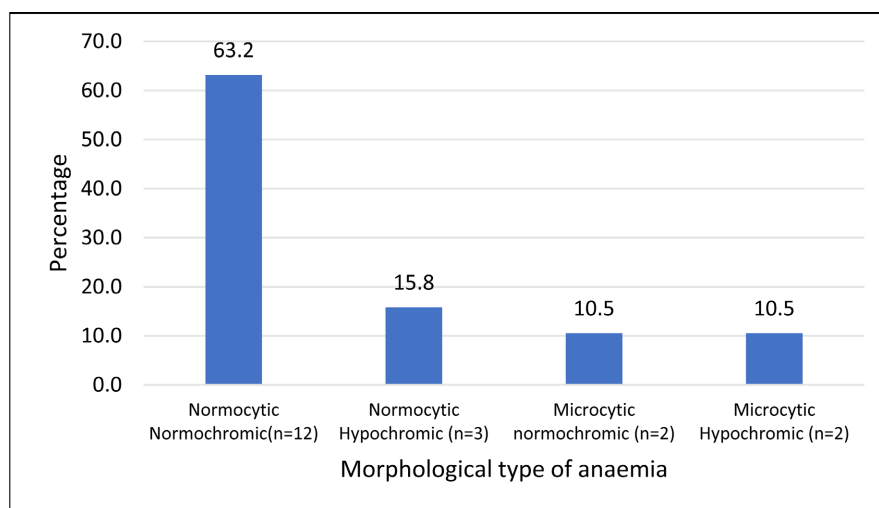


Figure 2. Proportions of the different morphological types of anaemia based on the peripheral blood smears, n = 19.

Diabetic foot, Metformin usage, Insulin usage, Dietary Composition, alcohol consumption and Physical exercise. In bivariate analysis, only the level of education showed a significant association with anaemia in T2DM patients (p value < 0.031), as indicated in **Table 4**.

Table 4. Results of bivariate analysis for factors associated with anaemia in Type 2 diabetic patients.

Variable	No anaemia n (%)	Anaemia n (%)	Unadjusted OR (95% CI)	p value
Age categories				0.419
18 - 49	75 (93.75)	5 (6.25)	1.0	
50 and above	136 (90.7)	14 (9.3)	1.5 (0.54 - 4.45)	
Sex				0.981
Male	55 (91.7)	5 (8.3)	1.0	
Female	156 (91.8)	14 (8.2)	1.0 (0.34 - 2.87)	
Occupation				0.729
None	43 (91.5)	4 (8.5)	1.0	
Peasant farmer	124 (92.5)	10 (7.5)	0.9 (0.26 - 2.91)	
Business	38 (88.4)	5 (11.6)	1.4 (0.35 - 5.65)	
Formal employment	6 (100.0)	0 (0.0)	1.0 (NA)	
Level of education				0.031
None	100 (87.0)	15 (13.0)	4.5 (0.57 - 35.48)	
Nursery/primary	81 (96.4)	3 (3.6)	1.1 (0.11 - 11.10)	
Secondary/tertiary	30 (96.8)	1 (3.2)	1.0	

Continued

Marital status				0.317
Single/unmarried	103 (93.6)	7 (6.4)	1.0	
Married	108 (90.0)	12 (10.0)	1.63 (0.62 - 4.31)	
Residency type				0.664
Rural	194 (91.5)	18 (8.5)	1.0	
Urban	17 (94.4)	1 (5.6)	0.6 (0.08 - 5.04)	
Monthly income				0.254
0 - 50,000	123 (89.8)	14 (10.2)	1.0	
500,001 - 200,000	59 (96.7)	2 (3.3)	0.3 (0.07 - 1.35)	
Above 200,000	29 (90.6)	3 (9.4)	0.9 (0.24 - 3.37)	
Duration since diagnosis of T2DM				0.281
≤5 years	161 (93.1)	12 (6.9)	1.0	
6 - 10 years	36 (90.0)	4 (10.0)	1.5 (0.45 - 4.89)	
>10 years	14 (82.35)	3 (17.65)	2.9 (0.72 - 11.40)	
Weight status of participant				0.459
Underweight/Healthy weight	70 (92.1)	6 (7.9)	1.0	
Overweight	57 (95.0)	3 (5.0)	0.6 (0.15 - 2.56)	
Obese	84 (89.4)	10 (10.6)	1.4 (0.48 - 4.01)	
Hypertension				0.435
No	37 (94.9)	2 (5.1)	1.0	
Yes	174 (91.1)	17 (8.9)	1.8 (0.40 - 8.16)	
Peptic\duodenal ulcers				0.909
No	86 (91.5)	8 (8.5)	1.0	
Yes	125 (91.9)	11 (8.1)	0.9 (0.37 - 2.45)	
Diabetic foot				0.729
No	136 (91.3)	13 (8.7)	1.0	
Yes	75 (92.6)	6 (7.4)	0.8 (0.31 - 2.29)	
Metformin				0.542
No	43 (89.6)	5 (10.4)	1.0	
Yes	168 (92.3)	14 (7.7)	0.7 (0.24 - 2.10)	
Insulin				0.385
No	186 (91.2)	18 (8.8)	1.0	
Yes	25 (96.15)	1 (3.85)	0.4 (0.05 - 3.23)	

Continued

Dietary composition				
Vegetables				0.38
No	20 (87.0)	3 (13.0)	1.0	
Yes	191 (92.3)	16 (7.7)	0.6 (0.15 - 2.08)	
Red meats				0.909
No	125 (91.9)	11 (8.1)	1.0	
Yes	86 (91.5)	8 (8.5)	1.1 (0.41 - 2.74)	
Fast foods				0.838
No	202 (91.8)	18 (8.2)	1.0	
Yes	9 (90.0)	1 (10.0)	1.2 (0.15 - 10.40)	
Alcohol consumption				0.762
No	196 (91.6)	18 (8.4)	1.0	
Yes	15 (93.75)	1 (6.25)	0.7 (0.09 - 5.82)	
Physical exercise				0.206
Never	82 (87.2)	12 (12.8)	1.0	
Daily	56 (94.9)	3 (5.1)	0.4 (0.10 - 1.36)	
1 - 3 days a week	65 (94.2)	4 (5.8)	0.4 (0.13 - 1.37)	
4 - 6 days a week	8 (100.0)	0 (0.0)	1.0 (N/A)	

On performance of multivariate analysis, none of the factors showed statistically significant association with anaemia in T2DM patients. However, participants with no education had 4.2 times higher odds of having anaemia as compared to those with secondary/tertiary education (OR = 4.2, 95% CI: 0.53 - 33.71, p-value: 0.174). Participants who were 50 years and above had 1.2 times higher odds of having anaemia compared to participants below 50 years (AOR = 1.2, 95% CI: 0.41 - 3.64, p-value: 0.712) (See **Table 5**).

Table 5. Results of multivariate analysis for factors associated with anaemia in Type 2 diabetic patients.

Variable	Adjusted OR (95% CI)	p value
Education level		
None	4.2 (0.53 - 33.71)	0.174
Nursery/primary	1.1 (0.11 - 10.85)	0.946
Secondary/tertiary	1.0	
Age		
<49	1.0	
50 and above	1.2 (0.41 - 3.64)	0.712

Continued

Metformin

No	1.0	
Yes	0.78 (0.26 - 2.32)	0.652

4. Discussion**Prevalence of Anaemia in Type 2 Diabetes Mellitus**

From this study, the prevalence of anaemia in T2DM was 8.3% which is lower than the 18.2% reported from Mulago National Referral Hospital [30]. The difference in the findings could be attributed to the variation in the residence of the populations assessed; a large majority of our participants were rural inhabitants, whereas those from the study at Mulago were largely urban dwellers. These varying environments could influence factors associated with the development of anaemia in T2DM, such as diet and exercise [21] [31]. Other participant characteristics that could be attributed to a low prevalence of anaemia in our study population are shorter duration of diabetes, with 75.2% of participants having a diagnosis for DM less than 5 years from the study period. Several studies have demonstrated a relationship between longer duration of diabetes and anaemia [32]-[35].

The prevalence of anaemia in T2DM patients from this study is comparable to the 8.06% (6) and 7% [34] reported from Ethiopia and Sudan respectively.

Morphological Classification of Anaemia among Participants

The most prevalent type of anaemia was normocytic normochromic, followed by normocytic hypochromic, and the least prevalent was microcytic hypochromic anaemia. This is consistent with the findings of other studies that revealed normocytic normochromic anaemia to be more prevalent in T2DM patients [3] [5] [36]. These findings support observations that chronic illness is more likely to develop normocytic normochromic anaemia as a result of impaired RBC production [37].

On the other hand, other studies have indicated a higher prevalence of microcytic hypochromic anaemia in T2DM patients; 57.4% [38] from India, 55.4% [39] from Egypt, 57.14% (34) from Sudan. This could be attributed to iron deficiency status [40]. Iron deficiency anaemia is the most common type of anaemia in low and middle-income countries [41].

Factors Associated with Anaemia among Type 2 Diabetes Mellitus Patients

None of the factors assessed showed a statistically significant association with anaemia in T2DM patients. However, participants with no education had 4.2 times higher odds of having anaemia as compared to those with secondary/tertiary education. This is similar to findings from Ethiopia [42] where uneducated DM participants were more likely to develop anaemia compared to participants with college and above education. This can be attributed to a lower level of health awareness among non-educated patients. Participants who were 50 years and above were 1.2 times more likely to be anaemic than those below 49 years of age which is concurrent with findings from similar studies [1] [43] where older age

was associated with presence of anaemia in T2DM.

5. Conclusions

In this study, the overall prevalence of anaemia among individuals with Type 2 Diabetes Mellitus (T2DM) was 8.3%. Normocytic normochromic anaemia was the most prevalent morphological type and microcytic hypochromic anaemia being the least prevalent.

Although factors such as level of education and age 50 years and above were associated with anaemia, these associations were not statistically significant. Therefore, no strong conclusions can be made regarding their influence on anaemia in T2DM patients. Further research is needed to identify significant factors for anaemia in this population.

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