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# Anaemia in Type 2 Diabetes Mellitus (T2DM) Patients in Hospital Putrajaya

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## ABSTRACT

Patients with diabetes have an earlier onset and increased severity of anaemia compared to those with similar degree of renal impairment from other causes. Anaemia is associated with an increased risk of vascular complications. In this study, we determined the prevalence of anaemia in T2DM patients and its association with sociodemographic, clinical and laboratory parameters in an endocrine tertiary hospital in Malaysia. This was a cross-sectional study using retrospective electronic data from January 2011 to December 2013 of 165 T2DM patients in Hospital Putrajaya. Data was analysed using IBM SPSS Statistics version 21.0 for Windows. The prevalence of anaemia was 39.4% and majority had normocytic normochromic (80%), mild (58.5%) anaemia. Majority were Malays (73.9%), aged below 60 with comparable gender percentage and long-standing, poorly-controlled DM [median fasting blood sugar (FBS) 8mmol/L; glycated haemoglobin (HbA1c) 7.9%]. Using the KDIGO chronic kidney disease (CKD) staging system, 86% of these patients were in stages 3-5. Anaemic patients had a significantly higher serum urea, creatinine and a lower FBS, estimated glomerular filtration rate (eGFR) compared to non-anaemic patients. Anaemic patients with diabetic nephropathy had a significantly lower haemoglobin (Hb) compared to those without this complication (p=0.022). The sensitivity and specificity at a cut-off eGFR value of 38.3 ml/min/1.73 $m^2$ (maximum Youden index = 0.462) was 66.7% and 79.5%, respectively to discriminate mild from moderate anaemia. This study shows that anaemia is already present in T2DM patients in Hospital Putrajaya at initial presentation to the specialist outpatient clinic and is significantly associated with CKD. Hence, it emphasises the obligatory need for routine and follow-up full blood count monitoring in T2DM patients in primary care as well as tertiary settings in Malaysia to enable early detection and aggressive correction of anaemia in preventing further complications.

**Keywords: Anaemia, Type 2 diabetes mellitus (T2DM), Chronic kidney disease (CKD), Diabetic nephropathy, Estimated glomerular filtration rate (eGFR).**

## INTRODUCTION

The Third National Health and Nutrition Examination Survey (NHANES-III) reported that patients with diabetes were twice as likely to have anaemia compared to those with similar degree of renal impairment from other causes.[1] Nevertheless, there is an increasing number of diabetic patients without renal impairment who are anaemic. The pathogenesis of anaemia in these patients is unclear. However, various hypotheses have been proposed including tubulointerstitial disease, chronic renal hypoxia, hyperglycaemia, systemic inflammation, symptomatic autonomic neuropathy causing efferent denervation of the kidney and loss of appropriate erythropoietin (Epo) production, altered iron metabolism, inhibition of Epo release and drugs.[2, 3]

Anaemia is associated with an increased risk of the vascular complications of diabetes including nephropathy, retinopathy, neuropathy, impaired wound healing and macrovascular disease.[4.] In the current hospital setting in Malaysia, laboratory parameters to determine anaemia are only measured at acute clinical presentations and not routinely at follow-up consultations.

To date, there is limited data to determine the occurrence of anaemia in diabetics, particularly in a South-East Asian population. Thus, this research aimed to determine the prevalence of anaemia in type 2 diabetes mellitus (T2DM) and its association with sociodemographic, clinical and selected laboratory parameters in a multiethnic Malaysian population in Hospital Putrajaya, a tertiary endocrine centre.

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## MATERIALS AND METHODS

### Study design

This was a retrospective cross-sectional study using electronic data of 165 T2DM patients ≥18 years of age, who visited the endocrine clinic of Hospital Putrajaya from January 2011 to December 2013. Sample size calculation for hypothesis testing purpose was done using the prevalence of diabetic complications in T2DM patients and the largest sample size was used. The prevalence of diabetic retinopathy ($P_1$=0.368)[5] and diabetic nephropathy ($P_2$=0.54)[6] in DM patients were used as the calculation gave the largest sample size of 140 patients after multiplying by two.

Pregnant women were excluded from the study. Only electronic records (clinical and laboratory) of initial visit to the clinic were extracted for the purpose of this study. Laboratory data included fasting blood sugar (FBS), glycated haemogobin (HbA1c), serum sodium (Na), potassium (K), urea and creatinine levels, estimated glomerular filtration rate (eGFR), haemoglobin (Hb) and haematocrit. Other information obtained electronically were sociodemographic factors (gender, age, ethnicity, smoking status and duration of T2DM) and clinical findings on first visit [blood pressure (BP), medications, eGFR and diabetic complications].

### Definition of terms

Anaemia was classified according to clinical grading and red blood cell (RBC) morphology as follows:

- Clinical grading based on Hb level:[7]
    - Mild (female: 11 – 11.9 g/dL; male: 11-12.9 g/dL)
    - Moderate (8-10.9 g/dL )
    - Severe (< 8 g/dL )
- Morphology based on RBC indices [mean cell volume (MCV) and mean cell haemoglobin (MCH)]:[8]
    - Microcytic, hypochromic (MCV < 80fL, MCH < 27 pg)
    - Normocytic, normochromic (MCV 80-95fL, MCH ≥ 27 pg)
    - Macrocytic (MCV > 95fL )

For the purpose of classification of anaemia and non-anaemia patients, based on the upper limit of mild anaemia, the haemoglobin value was rounded up to Hb < 13g/dL for anaemic male patients and Hb < 12 g/dL for anaemic female patients. Based on the CKD staging from The National Kidney Foundation Kidney Disease Improving Global Outcomes (KDIGO) 2012 guidelines, eGFR values were categorised using the following cut-off points:[9]

- Stage 1: ≥ 90 mL/min/1.73m$^2$
- Stage 2: 60 – 89 mL/min/1.73m$^2$
- Stage 3a: 45 – 59 mL/min/1.73m$^2$
- Stage 3b: 30- 44 mL/min/1.73m$^2$
- Stage 4: 15 – 29 mL/min/1.73m$^2$
- Stage 5: < 15 mL/min/1.73m$^2$

### Laboratory measurements

Plasma glucose was measured by UV hexokinase method on automated biochemistry analyser, UNICEL® DXC 800 (Beckman Coulter, Massachusetts, USA). Creatinine was analysed by enzymatic method, urea was determined by kinetic test using urease and glutamate dehydrogenase and sodium and potassium were done using ion-selective electrode on the same platform. Plasma glycated haemoglobin (HbA1c) utilised the principle of ion-exchange high performance liquid chromatography (HPLC) on the D10 BIORAD system (Biorad Laboratories, Hercules, California, USA). Hb, red blood cell counts and red cell indices were measured on an automated blood counter (Coulter STKS, Coulter Corporation, 11800 SW, 147, Miami, Florida 33196-2500, United States of America) using volume, conductivity and light scatter (VCS) technology.

## Statistical analysis

Statistical calculations were performed using the standard statistical software package, IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. Non-parametric tests (Mann Whitney, Kruskal Wallis and Chi Square) were used for analysis of variables, which were not normally distributed, and median values with 25 to 75 percentiles were used. Receiver operating curve (ROC) analysis was done to determine the optimal eGFR in discriminating mild from moderate anaemia in T2DM patients. In all statistical analyses, a 'p' value of $< 0.05$ (95% confidence interval) was considered to be statistically significant.

## Ethics

Approval to conduct the study was obtained from the Medical Research and Ethic Committee, Ministry of Health Malaysia ((NMRR-14-429-20752) and the Director of Hospital Putrajaya (ref. no: (24) dlm. HPJ/180/6 Jld. 7 dated 26/06/2014). Ethical approval was also obtained from The Ethics Committee for Research involving Human Subjects of Universiti Putra Malaysia (JKEUPM) [FPSK(EXP14-medic)U052].

# RESULTS

A total of 165 T2DM patients' data were obtained for this study. Table 1 shows the distribution of demographic and clinical characteristics of the patients. There were 51.5% male patients. More than half of the patients were from the age group of < 60 years old (53.3%). Median age was 58.8 years old (IQR= 63 years old). Majority were Malays (73.9%), followed by Indians (13.3%) and Chinese (12.7%). Patients with T2DM ≥ 5 years were 54.5% of the study population. The results also showed that most patients did not smoke (80.4%). Coronary heart disease (23.6%) was the most common complication followed by diabetic neuropathy (11.5%), cerebrovascular disease (11.5%), diabetic nephropathy (7.9) and diabetic retinopathy (3.0%). Majority were on metformin (72.1%) whilst 24.2% were on an angiotensin converting enzyme inhibitor (ACEI). The systolic BP was raised in 51.5% of the patients whereas 78.2% of patients had normal diastolic BP. The study population was further classified according to the KDIGO CKD classification using eGFR: stage 1 (6.1%), stage 2 (7.9%), stage 3 (27.2%), stage 4 (28.5%) and stage 5 (30.3%). Based on this staging, 86% of patients had eGFR < 60ml/min/1.73m$^2$. The prevalence of anaemia in this study population was 39.4%. The median FBS and HbA1c was 8 mmol/L and 7.9%, respectively [Table 2]. In those with anaemia, 80% had normocytic normochromic and of mild grade (58.5%) [Table 3].

**Table 1.** Demographics and clinical characteristics of study population

| Variable | n (%) |
|---|---|
| | **(N=165)** |
| **Gender** | |
| Male | 85 (51.5) |
| Female | 80 (48.5) |
| **Age (years)** | |
| < 60 | 88 (53.3) |
| ≥ 60 | 77 (46.7) |
| **Ethnicity** | |
| Malay | 122(73.9) |
| Chinese | 21 (12.7) |
| Indian | 22 (13.3) |
| **Smoking** | |
| Yes | 32 (19.4) |
| No | 133(80.6) |
| **Duration of T2DM (years)** | |
| < 5 | 75 (45.5) |
| ≥ 5 | 90 (54.5) |
| **Diabetic Complications** | |
| Nephropathy | 13 (7.90) |
| Neuropathy | 19 (11.5) |
| Retinopathy | 5 (3.0) |
| CHD | 39 (23.6) |
| CVA | 19 (11.5) |
| **Medication** | |
| Metformin | 119 (72.1) |
| ACE inhibitor | 40 (24.2) |
| Neither on metformin nor ACE inhibitor | 6 ( 3.7) |
| **Blood Pressure (mmHg)** | |
| Systolic Blood Pressure | |
| ≥ 140 | 85 (51.5) |
| < 140 | 80 (48.5) |
| Diastolic Blood Pressure | |
| ≥ 90 | 36 (21.8) |
| < 90 | 129 (78.2) |
| **CKD Staging (ml/min/1.73m$^2$)** | |
| Stage 1 (≥ 90) | 10 (6.1) |
| Stage 2 (60-89) | 13 (7.9) |
| Stage 3a (45-59) | 21 (12.7) |
| Stage 3b (30-44) | 24 (14.5) |
| Stage 4 (29-15) | 47 (28.5) |
| Stage 5 (< 15) | 50 (30.3) |
| **Anaemia Status** | |
| Present (male: Hb < 13g/dL; female Hb < 12g/dL) | 65 (39.4) |
| Absent | 100 (60.6) |

**Table 2.** Laboratory parameters of study population

| Parameter | Median (IQR) | Min – Max | Reference range |
|---|---|---|---|
| FBS (mmol/L) | 8.00 (4.57) | 4.08 – 23.18 | 3.5 – 6.0 * |
| HbA1c (%) | 7.90 (3.65) | 4.50 – 18.4 | < 6.5 * |
| Na (mmol/L) | 139.9 (5.95) | 117.7 – 148.0 | 135 – 145 * |
| K (mmol/L) | 4.15 (2.84) | 2.44 – 8.25 | 3.5 – 5.0 * |
| Urea (mmol/L) | 4.80 (3.80) | 1.3 – 149.0 | 1.7 – 8.3 * |
| Creatinine (µmol/L) | 87.00 (62.0) | 30 – 1223 | 44 – 80 * |
| eGFR (mL/min/1.73m$^2$) | 68.9 (204.0) | 3.7 – 207.7 | ~130 (Male)*<br>~120 (Female)* |
| Hb (g/dL) | 12.9 (3.05) | 5.2 – 18.10 | > 13 (Male)**<br>> 12 (Female)** |
| Haematocrit (%) | 38.3 (122.5) | 14.9 – 137.4 | 40 – 52 (Male)***<br>36 – 48 (Female)*** |

*based on Hospital Putrajaya Laboratory Information System (LIS) 2013
** based on reference (5)
***based on reference (6)

**Table 3.** Classification of anaemia based on morphology and clinical grading

| Features | n (%) |
|---|---|
| | **(N=65)** |
| **Morphology*** | |
| Normocytic Normochromic (MCV 80-95 fL, MCH ≥ 27 pg) | 52 (80.0) |
| Microcytic Hypochromic (MCV < 80 fL, MCH < 27 pg) | 11 (16.9) |
| Macrocytic Normochromic (MCV > 100 fL) | 2 ( 3.1) |
| **Grading**** | |
| Mild (female: 11 – 11.9 g/dL; male: 11-12.9 g/dL) | 38 (58.5) |
| Moderate (8-10.9 g/dL ) | 21 (32.3) |
| Severe (< 8 g/dL ) | 6 ( 9.2) |

* based on reference (6)
**based on reference (5)

Table 4 compares T2DM patients with and without anaemia. There is significant difference between all stages of CKD and anaemic status ($p < 0.001$). All other variables showed no significant difference.

**Table 4.** Sociodemographic factors and clinical findings between T2DM patients with and without anaemia

| Variable | Anaemia (Male Hb < 13g/dL) (Female Hb < 12g/dL) N (%) | Non-Anaemia (Male Hb ≥ 13g/dL) (Female Hb ≥ 12g/dL) N (%) | $X^2$ | p-value |
|---|---|---|---|---|
| **Gender** | | | | |
| Male | 32 (37.6) | 53 (62.4) | 0.224 | 0.636 |
| Female | 33 (41.3) | 47 (58.8) | | |
| **Age (years)** | | | | |
| < 60 | 36 (40.9) | 52 (59.1) | 0.181 | 0.670 |
| ≥ 60 | 29 (37.7) | 48 (62.3) | | |
| **Ethnicity** | | | | |
| Malay | 50 (41.0) | 72 (59.0) | 1.588 | 0.452 |
| Chinese | 9 (42.9) | 12 (57.1) | | |
| Indian | 6 (27.3) | 16 (72.7) | | |
| **Smoking** | | | | |
| Yes | 12 (37.5) | 20 (62.5) | 0.060 | 0.807 |
| No | 53 (39.8) | 80 (60.2) | | |
| **Duration T2DM (years)** | | | | |
| <5 | 25 (33.3) | 50 (66.7) | 2.115 | 0.146 |
| ≥5 | 40 (44.4) | 50 (55.6) | | |
| **Systolic BP (mmHg)** | | | | |
| <140 | 31 (38.8) | 49 (61.3) | 0.027 | 0.870 |
| ≥140 | 34 (40.0) | 51 (60.0) | | |
| **Diastolic BP (mmHg)** | | | | |
| <90 | 54 (41.9) | 75 (58.1) | 1.507 | 0.220 |
| ≥90 | 11 (30.6) | 25 (69.4) | | |
| **Metformin** | | | | |
| Yes | 44 (37.0) | 75 (63.0) | 1.046 | 0.306 |
| No | 21 (45.7) | 25 (54.3) | | |
| **ACE Inhibitor** | | | | |
| Yes | 14 (35.0) | 26 (65.0) | 0.427 | 0.513 |
| No | 51 (40.8) | 74 (59.2) | | |
| **CKD Staging (ml/min/1.73m²)** | | | | |
| Stage 1 (≥ 90) | 9 (13.8) | 1 (1.00) | 25.091 | **<0.001*** |
| Stage 2 (60-89) | 10 (15.4) | 3 (3.00) | | |
| Stage 3a (45-59) | 10 (15.4) | 11 (11.0) | | |
| Stage 3b (30-44) | 9 (13.8) | 15 (15.0) | | |
| Stage 4 (29-15) | 15 (23.1) | 32 (32.0) | | |
| Stage 5 (< 15) | 12 (18.5) | 38 (38.0) | | |

*Statistical significance at p<0.05.

Table 5 shows the comparison in laboratory parameters between anaemic and non-anaemic groups. All laboratory parameters showed significant difference except HbA1c, sodium and potassium. Anaemic patients had a significantly higher urea, creatinine, and a lower FBS and eGFR compared to non-anaemic patients.

**Table 5.** Laboratory parameters between anaemic and non-anaemic patients

| Variable | Anaemia (Male Hb < 13g/dL) (Female Hb < 12g/dL) | Non-Anaemia (Male Hb ≥ 13g/dL) (Female Hb ≥ 12g/dL) | z | p-value |
|---|---|---|---|---|
| | Median (IQR) | Median (IQR) | | |
| FBS (mmol/L) | 7.2 (4.150) | 8.3 (4.500) | - 2.681 | **0.026*** |
| HbA1c (%) | 7.8 (4.100) | 7.9 (3.475) | - 0.265 | 0.791 |
| Na (mmol/L) | 136 (8.000) | 137.0 (5.175) | - 1.718 | 0.086 |
| K (mmol/L) | 4.22 (0.645) | 4.08 (0.970) | - 1.394 | 0.163 |
| Urea (mmol/L) | 6.2 (9.850) | 4.30 (2.875) | - 3.619 | **<0.001*** |
| Creatinine (µmol/L) | 115 (155.5) | 79 (45.75) | 4.002 | **<0.001*** |
| eGFR (mL/min/1.73m$^2$) | 53.3 (56.90) | 80.4 (49.63) | - 4.307 | **<0.001*** |
| Hb level (g/dL) | 10.9 (2.460) | 14 (2.175) | - 10.440 | **<0.001*** |

*Statistical significance at p<0.05.

Table 6 shows the association between sociodemographic factors (gender, age, ethnicity, smoking status and duration of T2DM) and clinical findings (BP, medications, eGFR and diabetic complications) with anaemia in T2DM patients. None of the factors were significantly associated with anaemia apart from diabetic nephropathy. Anaemic patients with diabetic nephropathy had a significantly lower Hb compared to those without this complication (p=0.022).

**Table 6.** Association between sociodemographic factors and clinical findings with Hb level

| Variable (n=65) | Anaemia [Hb( g/dL)] (Male Hb < 13g/dL) (Female Hb < 12g/dL) | z / $X^2$ | p-value |
|---|---|---|---|
| | Median (IQR) | | |
| **Gender** | | | |
| Male | 11.3 (7.70) | -0.472[a] | 0.637 |
| Female | 10.1 (6.50) | | |
| **Age (years)** | | | |
| < 60 | 10.7 (7.70) | -0.425[a] | 0.671 |
| ≥ 60 | 10.9 (6.50) | | |
| **Ethnicity** | | | |
| Malay | 10.95 (7.70) | 0.322[b] | 0.579 |
| Chinese | 9.00 (6.20) | | |
| Indian | 11.00 (2.80) | | |
| **Smoking** | | | |
| Yes | 11.20 (4.06) | -0.243[a] | 0.808 |
| No | 10.40 (7.70) | | |
| **Duration T2DM (years)** | | | |
| <5 | 11.20 (7.70) | -1.450[a] | 0.147 |
| ≥5 | 10.30 (6.50) | | |

| | | | | |
|---|---|---|---|---|
| **Smoking** | | | | |
| Yes | 11.20 | (4.06) | -0.243[a] | 0.808 |
| No | 10.40 | (7.70) | | |
| **Duration T2DM (years)** | | | | |
| <5 | 11.20 | (7.70) | -1.450[a] | 0.147 |
| ≥5 | 10.30 | (6.50) | | |
| **Blood Pressure (mmHg)** | | | | |
| **Systolic BP** | | | | |
| <140 | 10.40 | (7.70) | -0.164[a] | 0.870 |
| ≥140 | 11.50 | (6.50) | | |
| **Diastolic BP** | | | | |
| <90 | 10.40 | (7.70) | -1.224[a] | 0.221 |
| ≥90 | 11.00 | (2.62) | | |
| **Metformin** | | | | |
| Yes | 11.00 | (7.70) | -1.020[a] | 0.308 |
| No | 10.00 | (5.40) | | |
| **ACE Inhibitor** | | | | |
| Yes | 11.20 | (3.26) | -0.651[a] | 0.515 |
| No | 10.30 | (7.70) | | |
| **CKD Staging (ml/min/1.73m$^2$)** | | | | |
| Stage 1 (≥ 90) | 9.00 | (3.15) | 5.621[b] | 0.345 |
| Stage 2 (60-89) | 10.00 | (1.67) | | |
| Stage 3a (45-59) | 10.25 | (1.70) | | |
| Stage 3b (30-44) | 11.40 | (1.95) | | |
| Stage 4 (29-15) | 11.20 | (2.30) | | |
| Stage 5 (< 15) | 11.20 | (3.18) | | |
| **Diabetic Complications** | | | | |
| Nephropathy | 10.00 | (5.20) | -2.287a | **0.022*** |
| Present | 10.95 | (7.70) | | |
| Absent | | | | |
| Neuropathy | 10.25 | (7.70) | -1.252a | 0.211 |
| Present | 10.90 | (7.50) | | |
| Absent | | | | |
| Retinopathy | 10.00 | (0.00) | -0.899a | 0.369 |
| Present | 10.90 | (7.70) | | |
| Absent | | | | |
| CHD | 10.00 | (5.90) | -1.360a | 0.174 |
| Present | 10.95 | (7.70) | | |
| Absent | | | | |
| CVA | | | | |
| Present | 11.90 | (4.80) | -0.754[a] | 0.451 |
| Absent | 10.35 | (7.70) | | |

*****Statistical significance at $p<0.05$

[a] Mann Whitney statistical test (z)

[b] Kruskal Wallis statistical test ($\chi^2$)

Receiver operating curve (ROC) analysis done to determine the optimal eGFR in discriminating mild from moderate anaemia in T2DM patients showed that the area under the curve (AUC) of the ROC was 0.717 with a 95% confidence interval (CI) of 0.575 to 0.858 (Figure 1). The optimal cut-off point for eGFR was determined using the Youden index. The sensitivity and specificity at a cut-off eGFR value of 38.3 ml/min/1.73m$^2$ (maximum Youden index = 0.462) was 66.7% and 79.5%, respectively to discriminate mild from moderate anaemia in T2DM patients.

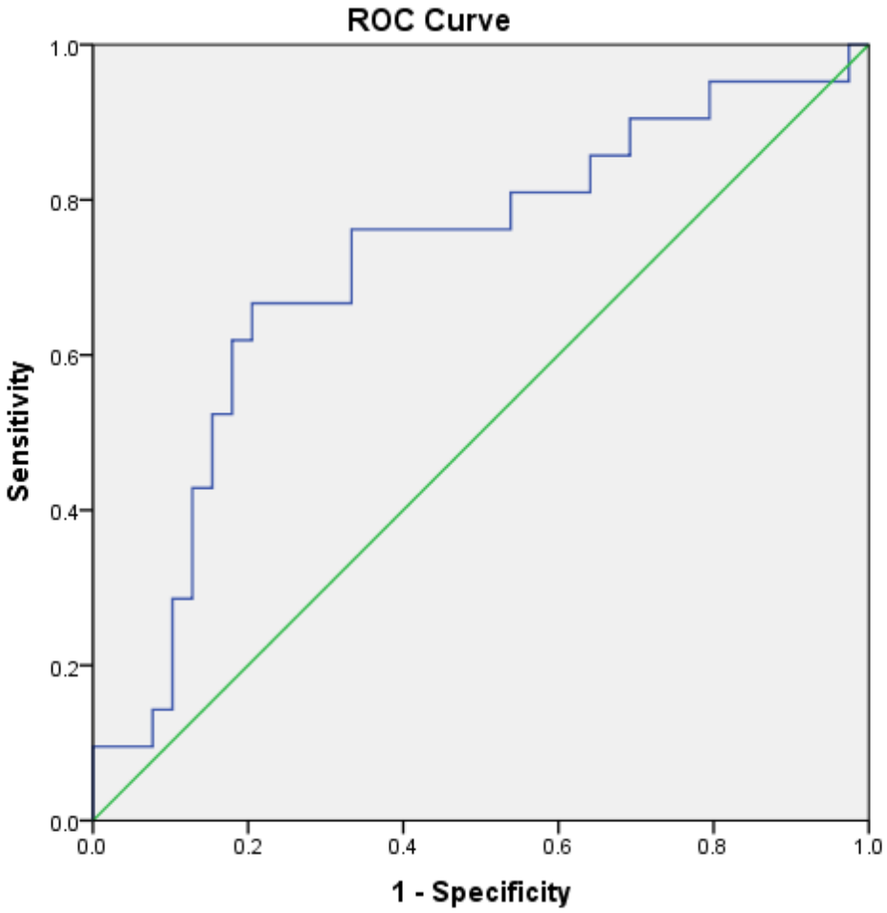


**Figure 1.** ROC analysis of eGFR (ml/min/1.73m$^2$) with grading of anaemia (mild versus moderate) with AUC = 0.717; 95% CI: 0.575-0.858.

## DISCUSSION

In this study, the population consists of mainly Malays (73.9%), aged below 60 (53.3%) with comparable gender percentage. High number of Malays with T2DM reflects the ethnic majority in Malaysia, whereby Malays constitute 63.1% of the population in Peninsular Malaysia.[10] Most of these patients were diagnosed with T2DM more than 5 years ago (54.5%) and the glucose control was generally poor with a median FBS of 8 mmol/L and HbA1c of 7.9%. Although majority did not smoke, most patients had raised systolic BP and the main complication was coronary heart disease. Hypertension is known to be prevalent in T2DM patients.[11] Using the KDIGO CKD staging system, 86% of these patients were in stages 3 to 5 (eGFR < 60ml/min/1.73m$^2$). Patients with end-stage renal disease (ESRD) with concomitant DM have a significantly greater risk of CVD mortality than patients without DM as anaemia may further mediate some of the effects of renal impairment.[12] Thus, due to these multiple risk factors, patients may have an increased risk of cardiovascular disease early in life.[13]

The prevalence of anaemia in T2DM in Hospital Putrajaya between the years 2011 to 2013 was 39.4%. This is relatively high compared to other populations whereby the prevalence ranged between 11 to 23%.[3, 14, 15, 16] This higher incidence could be attributed to the smaller study population with long-standing, poorly controlled DM

with possible increased susceptibility to impaired Epo production and release as a result of diabetic autonomic neuropathy.[17] Erythropoietin production and release is regulated in part by autonomic nervous system, suggesting that erythropoietin production could be prematurely impaired in patients with poor glycaemic control with diabetic autonomic neuropathy.[18] In addition, diabetic patients with stable metabolic control and milder complications are more likely to be managed in the primary care and therefore have a lower prevalence of anaemia compared to those managed in this tertiary setting.

The majority of the study population had normochromic normocytic, mild anaemia, similar to a recent study in Hong Kong,[19] while fewer had microcytic (16.1%) and macrocytic (3.1%) anaemia. Previous studies on diabetic patients have shown that longstanding poorly controlled diabetes is associated with normocytic normochromic anaemia and precedes clinical evidence of renal impairment.[3,18] Normocytic mild anaemia is a characteristic presentation of anaemia in chronic diseases and it evolves into microcytic as the severity of the anaemia increases.[20]

Microcytic anaemia, which made up 16.1% of the study population may be primarily due to iron deficiency, which is prevalent in patients with DM and CKD. Absolute iron deficiency anaemia defined as depletion of iron stores (serum ferritin < 100ng/ml) may be found in these patients as a result of dietary deficiency, impaired intestinal absorption and increased risk of bleeding from uraemic-associated platelet dysfunction. More common in CKD though, is functional iron deficiency anaemia (adequate tissue iron with serum ferritin ≥ 100 ng/ml), which is strongly linked to the upregulation of inflammatory cytokines and defective tissue responsiveness to Epo inhibiting iron transport from tissue stores to erythroblasts.[21] Unfortunately, serum ferritin was not available in the database to distinguish between the two types of iron deficiency in this study. More importantly, thalassaemia, a common public health problem in Malaysia whereby 4.5% to 6% of the Malays and Chinese are carriers[22] was not excluded by Hb analysis in this study and may have contributed to the microcytic anaemia in these patients.

Metformin, being the drug of choice in the treatment of T2DM[23] was used in 72.1% of our study population. This medication is known to decrease the absorption of vitamin B12 leading to vitamin B12 deficiency, which causes macrocytic anaemia, estimated to occur in 10-30% of patients using metformin.[24] This percentage is considerably much higher than that found in our study population (3.1%). This lower percentage in our population may be due to the fact that most diabetics in Malaysian tertiary centres are supplemented with vitamin B12. Vitamin B12 serum levels and history of supplementation, however, were not available from the electronic data to determine its association with metformin use in this study.

It has been proposed that the rampant use of angiotensin converting enzyme inhibitor (ACEI) may contribute to anaemia in DM by directly inhibiting the proerythropoeitic effects of angiotensin II on erythrocyte precursors, degradation of physiological inhibitors of haematopoiesis and suppression of IGF-1.[25,26] However, recent evidence has found no association between ACEI use and Hb level,[14] concurring with our study. Nevertheless, it has to be noted that only a small number of patients (24.2%) were prescribed ACEI in our study.

Most studies showed a greater prevalence of anaemia in patients > 60 years, reflecting the higher rate of CKD in the older age group and lower eGFRs with aging.[2] Conversely, this study, although insignificant revealed a higher percentage of patients who were < 60 years old within the anaemic group (Table 4). The higher number may suggest a different mechanism of anaemia apart from CKD, given the lower prevalence and less severe CKD in younger patients. The insignificant lower prevalence of anaemia in smokers is consistent with previous data.[27] The increased Hb levels in smokers is thought to be caused by secondary erythrocytosis, causing an upward shift of the Hb distribution curve.[27]

There was a significant difference between all stages of CKD (Stages 1 to 5) and anaemia status, corresponding to the significant lower eGFR in the anaemic group as compared to the non-anaemic group. This significant difference, especially in Stages 1 and 2 CKD where the eGFR is normal may be explained by the fact that early in the course of DM, tubulointerstitial damage may occur even before a fall in GFR is observed.[14] Craig et al (2005) demonstrated a state of relative erythropoietin (Epo) resistance in a cohort of diabetic patients in the absence of renal disease and rationalised that this suboptimal response to Epo may be caused by chronic inflammation associated with increased production of cytokines, such as tumour necrosis factor-α, interleukin-1, or interferon-g, which might suppress erythrocyte stem cell proliferation. Therefore, it is hypothesised that overt inflammation associated with diabetes may contribute to Epo unresponsiveness before the onset of nephropathy.[3] Thomas et al (2003) had similar findings whereby the prevalence of anaemia was higher in diabetic patients despite having preserved renal function and found that the severity of this early injury correlated better with albumin excretion rate (AER) than with GFR.[2] Unfortunately, Epo levels were not measured and results for AER and albuminuria were not consistently recorded in patients' notes, thus limiting the analysis.

The risk of anaemia increases significantly with deterioration of renal function[2] is supported by the higher urea and creatinine in the anaemic group compared to the non-anaemic group. Within the anaemic group, significantly lower Hb levels were found in patients with diabetic nephropathy (DN) than those without DN. Anaemia is usually more severe and occurs at an earlier stage in patients with DN than in patients with CKD of other causes.[12] These strong associations between kidney failure and anaemia in diabetes most likely reflect the unique vulnerability of the renal microcirculation to injury in DM.[4]

ROC analysis of eGFR with grading of anaemia (AUC = 0717; 95% CI =0.575-0.858) revealed that a cut-off eGFR value of 38.3 ml/min/1.73m$^2$, with a sensitivity = 66.7% and specificity = 79.5% was the optimal discriminator for moderate anaemia (Hb between 8-10.9 g/dL). The eGFR of 38.3 ml/min/1.73m$^2$ is at Stage 3b of CKD, which is moderately to severely decreased kidney function. The KDIGO Clinical Practice Guidelines for Anaemia in Chronic Kidney Disease[28] states that:

> 'For CKD patients without anaemia, measure Hb concentration when clinically indicated and at least annually in patients with CKD Stage 3a to 3b'.[28]

However,

> 'For CKD patients with anaemia not being treated with erythropoietin-stimulating agent, measure Hb concentration when clinically indicated and at least every three (3) months in non-dialysis patients with CKD Stage 3 to 5'.[28]

This guideline emphasises the need to be more vigilant in monitoring Hb in CKD patients in our population considering that in Stage 3b the anaemia is at moderate level and from the data, most patients are not treated with erythropoietin-stimulating agents.

In patients with CKD and anaemia (regardless of age and CKD stage), it is further recommended to include the following tests in the initial evaluation of the anaemia: FBC, absolute reticulocyte count, serum ferritin level, serum transferrin saturation, serum vitamin B12 and serum folate.[28] Apart from FBC, the other tests were not routinely done in all T2DM patients with anaemia in Hospital Putrajaya. Incorporating these tests in routine management of CKD patients will aid in the specific treatment of anaemia, thus reducing associated morbidity and mortality.

Surprisingly, anaemic patients had a significantly lower FBS compared to non-anaemic patients, although both were in the uncontrolled range. In terms of pre-analytical factors, there is no significant correlation between haematocrit and FBS. It is an established fact that haematocrit affects glucose measurement determined using test strip technology whereby increases in haematocrit are known to decrease glucose measurement and vice versa.[29] However, in this case, the sample was analysed using an automated analyser in the core laboratory and there is no known association with haematocrit when FBS is analysed in this way. Considering FBS is not an indicator of long-term glycaemic control and remains in the uncontrolled range in both groups, the slightly lower value in the anaemic group compared to the non-anaemic group, although significant may be irrelevant with respect to anaemic status.

The lower HbA1c in individuals with anaemia compared to the non-anaemic group, however, although insignificant may be a spurious result indicating reduced glycation of Hb due to reduced Hb concentration or increased red cell turnover.[30] Thus, the glycaemic control in the anaemic group could probably be much worse, consistent with previous research.[18] Apart from that, as mentioned before, haemoglobinopathies were not excluded in this study and could contribute to falsely low HbA1c. To ensure that the HbA1c is a true reflection of the patient's diabetic control, the laboratory could calculate and report estimated average glucose (eAG) whenever HbA1c is reported. The report should state that the eAG is solely for comparison with the average of actual glucose measurements, preferably from the patient's own home blood glucose monitoring.[31]

The mechanisms behind the higher risk of anaemia in uncontrolled diabetes as compared to controlled diabetes are not clearly understood. Nevertheless, it has been established that diabetic autonomic neuropathy is a complication of poor glycaemic control, and thought to give rise to a defect in the 'anaemia sensing' mechanism, contributing to Epo deficiency.[17] Other factors which have been reported to increase the risk of anaemia in DM include; systemic inflammation;[32] injury to renal architecture due to chronic hyperglycemia and the resulting formation of advanced glycation end products; and lowered androgen levels. It is speculated that poorly controlled DM may further aggravate these conditions.[18]

This study has several limitations. Firstly, other aetiologies of anaemia such as iron and vitamin B12 deficiency or haemoglobinopathies were not ruled out. Also, albuminuria and AER, markers of glomerular damage for CKD staging 1 and 2, eAG and Epo levels were not ascertained due to lack of recorded data. Secondly, for all variables, only the results of the initial referral was taken for analysis so a one-off result compared to an average of multiple values may not reveal the true details of the clinical condition. Furthermore, the variability of finding may confound the effects of covariates on outcomes. Lastly, since this study was a cross-sectional epidemiologic investigation, potential unmeasured confounders were not adjusted for. Thus, temporal relationship could not be established.

Further studies should be carried out on anaemic patients with T2DM in Malaysian multiethnic population using iron studies, folate, vitamin B12 and Epo levels, Hb analysis, eAG, albuminuria, AER and albumin-creatinine ratio, specifically taking into account the different stages of CKD. These parameters would help shed some light on the mechanisms behind anaemia in T2DM. Studies of interventions and therapeutic strategies for anaemic T2DM patients should also be considered to prevent associated complications.

## CONCLUSION

This study shows that anaemia is already present in T2DM patients in Hospital Putrajaya at initial presentation to the specialist outpatient clinic and is significantly associated with CKD. As such, regular, early monitoring of Hb level of T2DM should begin at the primary care setting. Management of DM at tertiary level should include mandatory routine haematological tests at follow-up visits enabling aggressive correction of anaemia to prevent other diabetic complications. This will probably lead to institution of early reno-protective measures, providing timely intervention in the high-risk group. It is known that early identification and correction of anaemia will benefit these patients. However, to what extent and which treatment is the most ideal in terms of balancing the potential benefits against the adverse risks of treatment is not known.

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