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Development and performance evaluation of a moderate positive reference material containing genapol x-080 for hemolysis testing

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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).

1. 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.

2. MATERIALS AND METHODS

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 =0.368) [5] and diabetic nephropathy (P =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].

3. 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/1.73m2. 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].

4. 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- 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]. 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] 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.

5. 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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