ORIGINAL ARTICLE

Prevalence of Anaemia and Abnormal RBC Indices among T2DM Patients during HbA1c Measurement in Hospital Kuala Lumpur

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ABSTRACT

Introduction: Anaemia in subjects with T2DM is not an infrequent finding. HbA1c, a biomarker of choice for glycaemic control is altered by anaemia and abnormal red blood cell (RBC) indices. Hence, this study's main objective is to determine the prevalence of anaemia and abnormal RBC indices among type 2 diabetes mellitus (T2DM) subjects who had HbA1c measurement performed in Hospital Kuala Lumpur (HKL). **Method:** A retrospective study of 305 subjects with concurrent HbA1c and FBC performed in Pathology Laboratory, HKL from January 2017 to December 2017. The demographic and laboratory parameters of the subjects (total Hb, MCV, MCH, MCHC, HbA1c, iron, ferritin, folate, B12) were attained from the laboratory information system (LIS). **Results:** A total of 113 (37%) subjects had anaemia, whilst 131 (43%) had abnormal RBC indices. The anaemia was mostly mild (n=70, 61.9%) and normocytic normochromic (n=63, 55.8%). The median HbA1c for the anaemia group was lower (HbA1c=7.52%) compared to the non-anaemia group (HbA1c=8.3%) (p=0.03). The median HbA1c was significantly higher in those with microcytic hypochromic anaemia (HbA1c=7.9%) compared to normocytic normochromic anaemia (HbA1c=7.2%) (p=0.025). **Conclusion:** The prevalence of anaemia among T2DM subjects was 37% with the majority being mild and normocytic normochromic. HbA1c measurement is affected by anaemia and hence, whenever possible, HbA1c results should be reviewed together with the FBC results.

Keywords: Anaemia, Glycated haemoglobin, Diabetes mellitus, Red blood cell indices, Prevalence

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INTRODUCTION

Haemoglobin A1c (HbA1c) is a biomarker widely used for diagnosis of type 2 diabetes mellitus (T2DM) and monitoring of diabetes control (1). However, HbA1c is affected by numerous factors, including anaemia (2). Anaemia can cause falsely low or high HbA1c levels, depending on the aetiology of anaemia (2,3). In iron deficiency anaemia (IDA), HbA1c is falsely increased, with the effects more notably seen in cases of moderate to severe anaemia (2, 4, 5). Folate and vitamin B12 deficiencies have also been associated with higher HbA1c; nevertheless, the studies on this are limited (2,3). In contrast, conditions associated with a shorter RBC lifespan, such as haemolytic anaemia are associated with spuriously decreased HbA1c levels (2, 3). Some studies have shown an association between HbA1c and RBC indices [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), total haemoglobin] in both diabetic and non-diabetic subjects (2, 5). A low MCV and low MCH are related to higher HbA1c levels in subjects with iron deficiency, even in the absence of apparent anaemia (2).

DM is a common non-communicable disease in Malaysia, with a reported prevalence of 18.3%, while the prevalence of anaemia is 21.3% (6). Among Malaysians aged 35 to 70 years, anaemia prevalence was 13.8%, with microcytic hypochromic being the most common (59.7%) (7). The prevalence of anaemia specifically among T2DM with chronic kidney disease (CKD) is between 31.7% and 39.4% in Malaysia (8,9). The most common type in T2DM is normocytic normochromic followed by microcytic hypochromic (8). Interestingly, most subjects were unaware of being anaemic (9).

Considering the significance of HbA1c in diabetes management and the possibility of false interpretation of HbA1c in the presence of anaemia, the study aimed to determine anaemia (including morphological types and severity) and abnormal RBC indices prevalence in

T2DM patients who had HbA1c samples measured in Hospital Kuala Lumpur (HKL). The study also aimed to determine if further investigations were done to exclude nutritional anaemia and, to evaluate the association between HbA1c and Hb as well as RBC indices.

MATERIALS AND METHODS

Study design

This was a retrospective cross-sectional study of subjects with HbA1c samples analysed in the Pathology Laboratory of HKL from January 2017 - December 2017. The inclusion criteria were T2DM patients aged >18 years old who had concurrent HbA1c, and full blood count (FBC) sent on the same day. Pregnant subjects and subjects with end-stage renal disease (ESRD) and haemoglobinopathy were excluded from this study. Each subject had only one HbA1c result included. Demographic (age, gender, race) and laboratory data (total Hb, MCV, MCH, MCHC, HbA1c, ferritin, iron, folate, B12) of these patients were obtained from the laboratory information system (LIS).

Calculation of sample size was based on N = 4p(1-p)/ e2 (10), in which the prevalence (p) was based on the study by Thambiah et al. 2015, with the final calculated sample size of 305 subjects.

Definition

Anaemia in adults was defined by the World Health Organization (WHO) diagnostic criteria as <13.0 g/ dL for males and <12.0 g/dL for females (11). Mild, moderate, severe anaemia was defined as haemoglobin of 11.0–12.9 g/dL for men and 11.0–11.9 g/dL for women, 8.0–10.9 g/dL for both genders, and Hb ≤7.9 g/dL for both genders, respectively (11). Based on MCV and MCH values, subjects with anaemia were also categorised into hypochromic microcytic (MCH <27pg and MCV <80 fL), normochromic normocytic (MCV 80–100 fL and MCH ≥27 pg) and macrocytic anaemia (MCV >100 fL).

Statistical analysis

Descriptive statistics for categorical variables were shown as frequency and percentage [n(%)]. Normally distributed continuous variables were presented as mean and standard deviation (SD) whilst median and interquartile range (IQR) for skewed data. Chi-squared and Mann-Whitney tests were used for analysis of categorical and continuous variables, respectively. A p-value <0.05 was considered as significant.

RESULTS

A total of 305 subjects with a mean age of 59.6±12.5 years were included. The majority were Malays (50.5%) and females (51.5%) as shown in Table I. The majority (73.5%) had an estimated glomerular filtration rate (eGFR) ≥60 ml/min/1.73m2. A median HbA1c of 7.9% (IQR=3.2) was obtained. Out of 305 patients, 113 (37%) had anaemia and 131 (43%) had abnormal RBC indices. In those with anaemia, the majority (n=60, 53.1%) were females whilst 40 (35.4%) subjects had eGFR less than <60 ml/min/1.73m2. In those with abnormal RBC indices, 64 (48.9%) were anaemic, whilst 67 (51.1%) were not (Table II). The majority had normocytic (70.5%), normochromic (73.8%) and normal MCHC (78.4%). Table III shows the proportion of subjects with anaemia according to the morphology type and severity of anaemia. The majority had mild anaemia (61.9%), whilst morphologically, the majority (55.8%) had normochromic normocytic anaemia, 44.2% had microcytic hypochromic anaemia and none with macrocytic anaemia. Most anaemic subjects (n=91, 80.5%) had no further laboratory investigations for nutritional anaemia performed, whilst the remainder had investigations for ferritin, iron, folate and B12.

Table I: Demographics and clinical characteristics of study participants (N=305)

Characteristics	Frequency	Percentage (%)
Age (years)		
<60	148	48.5
≥60	157	51.5
Gender		
Male	148	48.5
Female	157	51.5
Race		
Malay	154	50.5
Chinese	51	16.7
Indian	97	31.8
Others	3	1
HbA1c (%)		
≤6.5	154	50.5
>6.5	150	49.5
Anaemia		
Yes	113	37.0
No	192	63.0
RBC indices		
Normal	174	57.0
Abnormal	131	43.0
eGFR (ml/min/1.73m^2)		
≥90	117	38.4
60-89	107	35.1
45-59	42	13.8
30-44	35	11.5
29-15	4	1.3

No significant difference in proportion was demonstrated with regards to age, gender, and ethnicity between those with anaemia and without anaemia (Table IV). The median HbA1c was, however, significantly lower in anaemia (7.5%) compared to the non-anaemia group (8.3%), (p=0.03). A significant difference in the median HbA1c level was also obtained between the microcytic

Table II: RBC indices pattern among study participants (N=305)

RBC indices	Frequency (n)	Percentage (%)
MCV (fl)		
Microcytic (<80)	89	29.2
Normocytic (80-100)	215	70.5
Macrocytic (>100)	1	0.3
MCH (pg)		
Hypochromic (<27)	78	25.6
Normochromic (27-32)	225	73.8
Hyperchromic (>32)	2	0.6
MCHC (g/dL)		
Low (<31.5)	40	13.1
Normal (31.5-34.5)	239	78.4
High (>34.5)	26	8.5
Abnormal RBC indices	131	43.0
with anaemia	64	48.9
without anaemia	67	51.1

Table III: Morphology type and severity of anaemia (n=113)

Anaemia	Frequency (n)	Percentage (%)
Morphology		
Microcytic hypochromic	50	44.2
Normocytic normochromic	63	55.8
Severity of anaemia		
Mild	70	61.9
Moderate	40	35.4
Severe	3	2.7

Table IV Demographic characteristics and HbA1c levels between anaemia and non-anaemia

	Non –anaemia (N=192) n (%)	Anaemia (N=113) n (%)	χ^{2a}	p-value*
Age (years)				
<60	100 (52.1)	48 (42.5)	2.628	0.105
≥60	92 (47.9)	65 (57.5)		
Gender				
Male	95 (49.5)	53 (46.9)	0.189	0.722
Female	97 (50.5)	60 (53.1)		
Ethnicity				
Malay	102 (53.1)	52 (46.0)	1.437	0.231
Non-Malay	90 (46.9)	61 (54.0)		
	Non-Anaemia Median (IQR)	**Anaemia Median (IQR)**	**Z[b]**	**p-value***
HbA1c (%)	8.3 (3.25)	7.5 (2.35)	-2.953	0.03

*statistical significance at p<0.05; [a]Chi-Square test; [b]Mann-Whitney test

hypochromic anaemia group (7.9%) and the normocytic normochromic anaemia group (7.2%); (p=0.025) (Table V). In those with anaemia, an inverse correlation was seen with Hb, MCH and MCV (Table VI).

Table VI: Correlation between RBC indices and total Hb with HbA1c in subjects with anaemia

Variables	HbA1c (%)	
	r(s)	p-value*
Hb (g/dL)	-0.0278	<0.003
MCV (fL)	-0.331	<0.0001
MCH (pg)	-0.237	<0.011
MCHC (g/dL)	0.104	0.272

Spearman correlation (r_s). statistical significance at p<0.005

Table V: Comparison in HbA1c level between the types of anaemia (n=113)

	Microcytic hypochromic anaemia (n=50)	Normocytic normochromic anaemia (n=63)	Z[c]	p-value*
Median (IQR) HbA1c (%)	7.9 (2.40)	7.2 (2.00)	-2.238	0.025

*statistical significance at p<0.05; [c]Mann-Whitney test

DISCUSSION

The anaemia prevalence was 37%, consistent with the prevalence previously reported among T2DM subjects in the Endocrine Clinic of Hospital Putrajaya (39.4%), another tertiary centre in Malaysia (8). A lower prevalence of anaemia (31.7%) was, however, reported among T2DM subjects in primary care clinics, suggesting that study site contributes to the disparity possibly due to the diabetes complexity managed between the centres (9). In other parts of the world, the prevalence of anaemia reported over the last ten years in subjects with T2DM was between 20.1% - 41.4% (12-17). Often the factors that contribute to anaemia are multifactorial and poorly understood. Causes include diabetic nephropathy, inhibition of erythropoietin release and production because of autonomic neuropathy, altered iron metabolism because of systemic inflammation, and nutritional deficiency (18). Other causes include the use of certain drugs, including oral antidiabetic drugs and ACE inhibitors (18). It was also reported that those with poor glycaemic control had a higher prevalence of anaemia compared to those with good diabetes control (14).

Consistent with other studies, the anaemia was primarily mild (61.9%) and normochromic normocytic (55.8%) (8,9,13,15,16). A higher percentage of normochromic normocytic anaemia was reported (85%) in Ireland (19) whilst in India, most had hypochromic microcytic anaemia (57.14%) (20). Iron deficiency (often associated with microcytic hypochromic anaemia) is the commonest cause of nutritional deficiency worldwide and has a higher prevalence in low and middle-income nations, with women, adolescents and children most at risk (21). Thus, the prevalence and the type of anaemia in diabetes are highly dependent on the population being studied, with factors such as dietary intake, age of study participants, duration of diabetes, geographical location, and economic status contributes to the difference (15).

Anaemia causes spuriously decreased or increased HbA1c depending on anaemia types (2, 3). In this study, the hypochromic microcytic anaemia group had a significantly higher HbA1c than those with

normochromic normocytic anaemia. We could not fully ascertain the cause of hypochromic microcytic anaemia as the majority was without further investigations for nutritional deficiency such as iron and ferritin. Nevertheless, IDA and thalassaemia trait have been reported as the most common causes for microcytic anaemia (22). The mechanisms for the increased HbA1c in IDA remain uncertain (3). One of the postulated mechanisms is alteration in the haemoglobin's quaternary structure which allows the glycation process of β-globin chain to occur more easily (21). Another possible mechanism is that the longer RBC life span allows for a higher glycation time (3,5).

Clinicians and laboratorians must be aware of factors that affect HbA1c levels including anaemia, more so if HbA1c is used for diagnostic purposes. It is recommended to be cautious when interpreting HbA1c results of anaemia patients with HbA1c of 5.7% (threshold for prediabetes) and 6.5%, which are diagnostic cut-off values for diabetes (6.3% for Malaysia) (3). Madhu et al 2017 showed that iron supplementation results in a significant decrease in HbA1c (4). Seventy percent (14/20) of subjects categorised as pre-diabetic prior to treatment were recategorised to normal glucose tolerance (NGT) following iron supplementation (4). Five out of six subjects who had pre-treatment HbA1c levels in the diabetic range reverted to pre-diabetes and one to NGT levels following supplementation (4).

The study is not without limitation. Being a retrospective study, the cause of anaemia in this study cannot fully be ascertained as there was a lack of data on further investigations performed particularly for nutritional deficiency. In addition, other investigations for haematological disorders such as thalassaemia were also not assessed in this study.

CONCLUSION

The prevalence of anaemia among T2DM patients in HKL was 37%. The presence of anaemia and the type of anaemia affect HbA1c measurement and should be considered during interpretation of HbA1c as it may impact negatively on the management of T2DM patients. Further investigations for anaemia should also be performed.

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