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RESEARCH ARTICLE

ASSESSMENT OF ERYTHROPOIETIN AND RENAL BIOMARKERS OF DIABETICS WITH COMPLICATIONS IN BENIN CITY, EDO STATE

UI Abdulkadir[1], RO Omoregie[2], BIG Adejumo[2], FO Oyakhire[3], EO Akhaumere[4], WP Udoh[5], AJP Kamdem[6], IMM Otutu[2], GE Obasuyi[2], AO Ogbebor[3], FO Aiyesoro[7] E Samson[8], EOA Egwuakhide[9]

[1]*Department of Medical Laboratory Science, Federal University, Lafia, Nasarawa state*
[2]*Department of Medical Laboratory Science, University of Benin, Benin City, Edo State.*
[3]*Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Benson Idahosa University, Benin City, Edo state.*
[4]*Department of Chemical Pathology, National Hospital, Abuja.*
[5]*Federal College of Medical Laboratory Science and Technology, University Teaching Hospital, Jos, Plateau State.*
[6]*Department of Hematology, College of Medical Sciences, University of Benin, Edo State.*
7 Department of Medical Laboratory Science, College of Health Sciences, Akure, Ondo State.
[8]*Department of Natural Science, Faculty of Science and Technology, Middlesex University, London, United Kingdom.*
[9] *Department of Pediatric, Noel Specialist Hospital, Benin City.*

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ABSTRACT: Chronic diabetes mellitus is a metabolic disorder characterized by persistent hyperglycemia and associated complications. Assessing erythropoietin (EPO) levels and glycated hemoglobin (HbA1c) levels in diabetic patients can provide valuable insights into the management and progression of the disease. This study aimed to assess the levels of erythropoietin and renal parameters in patients with complications of diabetes mellitus and evaluate the clinical implications of these assessments. A total of eighty-one consenting participants including 21 patients presenting complications of diabetes mellitus, 30 diabetic patients without any complications, and 30 heathy control persons without history of diabetes mellitus. Erythropoietin level was measured using enzyme-linked immunosorbent assay (ELISA), and HbA1c levels were also determined through modified enzymatic reaction, packed cell volume, haemoglobin, urea and creatinine values were determined using standard methods. Demographic and clinical data, including age, gender, diabetes duration, and medication history, were collected via questionnaires. Statistical analysis was performed to determine the correlation between EPO levels and HbA1c levels. Values obtained in this study was presented as mean ± standard deviation (levels of significance were accepted at $p<0.001$). In this study, high HbA1c levels among diabetic patients with complications were associated with a diet rich in protein and carbohydrates. Additionally, the increased erythropoietin levels were attributed to the absence of nephropathy, a condition typically linked to lower erythropoietin levels, in these patients. Further research is warranted to explore the clinical implications of these assessments in predicting diabetes complications and guiding therapeutic interventions.

Keywords: DWOC, diabetics without complications; DWC, diabetics with complications

Corresponding Author:
Fidelis Ohiremen Oyakhire,
Department of Medical Laboratory Science,
Faculty of Allied Health Sciences, Benson Idahosa University, Benin City, Edo State, Nigeria.

INTRODUCTION:

Diabetes mellitus (DM) is a carbohydrate metabolism disorder which is caused due to impairment in insulin secretion and/or the activity of insulin, leading to chronic hyperglycemia with defective carbohydrate, fat and protein metabolism [1]. DM could also be referred to as a diverse group of metabolic disorders with associated high disease burden in developing countries, for example Nigeria [2]. It is known as a leading global health problem, and risk factor for blindness, vascular brain diseases, renal failure, and limb amputations, among others [3]. In Nigeria, the current prevalence of DM among adults aged 20-69 years is 1.7% [4].

In patients with diabetes mellitus, years of poorly treated diabetes mellitus are associated with a myriad of microvascular (affects small vessels) and macrovascular (affects large vessels) complications [5]. The most commonly documented microvascular complications of diabetes mellitus are neuropathy, nephropathy, and retinopathy while macrovascular complications include myocardial infarction, peripheral arterial disease, and stroke [5].

Diabetic retinopathy is a common cause of adult blindness and is characterized initially by retinal capillary microaneurysms (background retinopathy) and later by neovascularization (proliferative retinopathy) and macular edema [5]. There are no early symptoms or signs, but focal blurring, vitreous or retinal detachment, and partial or total vision loss eventually develop; rate of progression is highly variable [5]. Diabetic nephropathy is a leading cause of chronic kidney disease and is characterized by thickening of the glomerular basement membrane, mesangial expansion, and glomerular sclerosis. These changes cause glomerular hypertension and progressive decline in glomerular filtration rate [5]. Systemic hypertension may accelerate progression. The disease is usually asymptomatic until nephrotic syndrome or renal failure develops [5]. Diabetic neuropathy is the result of nerve ischemia due to microvascular disease, direct effects of hyperglycemia on neurons, and intracellular metabolic changes that impair nerve function [5].

Anemia is the most common blood disorder and a common finding in patients with diabetes [6]. The development and progression of microvascular and macrovascular complications in diabetes is associated with anemia [7]. Anemia mostly occurs in diabetes mellitus patients who also have renal impairment and can be considered as a marker of kidney damage, and anemia in diabetics has increased risk of mortality [7]. Evidence indicates that the existence of anemia among type 2 diabetes mellitus is typically associated with the failure of the kidney to produce appropriate erythropoietin [6a][8][6b]. Diabetic neuropathy, chronic inflammatory activity, increased levels of advanced glycation end products (AGEs), erythropoietin hypo-responsiveness, effects of oxidative stress, and anti-diabetic medications are other possible cause of anemia in DM patients [9][10][11][12]. Growing evidence indicates the anemia in type 2 diabetes mellitus patients is a strong and independent indicator of increased risk for diabetes-related macrovascular and microvascular complications [13][6][14][15][16]. It causes early occurrence and rapid progression of complications like diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, end-stage renal diseases, ischemic heart disease, and non-healing diabetic foot ulcers [16].

Factors suggested as the reason for the earlier onset of anemia in patients, 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 [9]. Damage to the peritubular fibroblasts can occur and lead to erythropoietin deficiency and anemia before the loss of filtration [9]. Correction of the anemia not only leads to less fatigue, greater exercise tolerance, and improved quality of life but also to a reduction in mortality and hospital admissions for congestive heart failure (CHF) [9]. Promptly diagnosing and treating anemia in patients

with diabetes may result in an improved quality of life and decreased morbidity and mortality [9].

Many literatures have reported studies on complications of diabetes mellitus in Edo state, but to the best of our knowledge, none has dwelled extensively on the distribution pattern of this disease. Hence, the justification of this study. The aim of this study therefore is to assess the levels of erythropoietin and renal parameters in patients with complications of diabetes mellitus and evaluate the clinical implications of these assessments in Edo State.

MATERIALS AND METHODS:

Study Design

This is a case control study of patients with complicated and non-complicated diabetes mellitus. The study was carried out at University of Benin Teaching Hospital. A total of thirty (21) diabetics with complications, thirty (30) without complications and thirty (30) healthy controls participated in this study. The diabetics were the patients who were attending diabetes clinics at the University of Benin Teaching Hospital, Benin City. The controls were the healthy individuals of same age range with no history of diabetes or any underlying ailments. They were recruited within Benin City. A properly structured questionnaire was administered to every participant to obtain basic demographic details as well as anthropometric characteristics after seeking their consent.

Study participants

1. Diabetes with Complications - All adult males and females with complicated diabetic mellitus who gave consent to participate in the study. The complications include: retinopathy, neuropathy and nephropathy respectively.
2. Diabetes without Complications - Adult males and females with diabetes mellitus (without complication) who gave consent to participate in the study.
3. Controls - Apparently healthy males and females with no history of diabetes mellitus or underlying illness who gave consent to participate in the study.

Sample Size

The sample size for this study was determined based on three factors:

1. The estimated prevalence of variable interest from literature review
2. Confidence interval of 95%
3. The acceptable margin of error

The sample size was calculated according to the following formula;

$$N = \frac{z^2 * p(1-p)}{d^2}$$

Where;

N= required sample size

z = confidence level interval at 95% (standard value of 1.96)

p = estimated prevalence of diabetes mellitus of variable interest from literature review (2.1%) [17].

d = margin of error at 5% (standard value of 0.05)

$$N = \frac{1.92^2 \times 0.021 (1- 0.021)}{0.05^2} = 30$$

Ethical Approval

Ethical approval with Reference number ADM/E22/A/VOL.VII/148301129 was obtained from the Health Research Ethics Committee, University of Benin Teaching Hospital, Benin City, Edo State to carry out this research. In addition, the recruited participants gave their consents after a thorough explanation of the rationale for the study and information on the nature of the research, pains and gains, as well as confidentiality by making use of a questionnaire.

Questionnaire

The participants were administered with a structured open-ended questionnaire, made up of simple questions to elicit details about their personal data

including age, sex, state of residence, level of education, state of origin, occupation, alcohol consumption, smoking history, marital status, level of involvement in exercise, consumption of high calorie carbohydrate meal, family history of diabetes mellitus, current medications, as well as any underlying disease. Other questions on the questionnaire included recent loss of blood and the type of diabetic complications.

Sample Collection

Under aseptic conditions, about 5 millimeters of venous blood samples was obtained from the ante-cubital vein of each subject using a sterile needle and syringe. Three millilitres of the blood was dispensed into an ethylenediamineetraacetic acid (EDTA) container, and other part (2millilitres), into a clean dry plain container. The sample in the EDTA container was used to examine the HbA1c, hemoglobin, and PCV levels of the subject. The sample in the plain container was left undisturbed for a few minutes to clot and centrifuged at 4000 revolutions per minute for about 5 minutes to separate serum from the clot. The serum was then dispensed into another clean and dry plain container, then stored at -20^{0}C prior to analysis of erythropoietin urea and creatinine levels respectively.

Analysis of Parameters

Determination of Fasting Blood Glucose (FBG) Level

Glucose levels of the participant was determined using glucose oxidase method, using a Randox glucose reagent with LOT number GAB2002R, and the test was done following the manufacturer's instructions (Kumar, 2018) [18].

Glycosylated Haemoglobin (HbA1c)

The estimation of HbA1c was done by the modified enzymatic method from the EDTA sample within 12 hours by following the manufacturer's protocols. The kit was commercially purchased from Fortress with LOT number 220525. The diagnosis of diabetes was established considering the American Diabetes Association diagnostic criterion of HbA1c level ≥ 6.5% [19].

Determination of Packed Cell Volume

The Packed Cell Volume was determined using microhematocrit method, according to the manufacturer's instruction [20].

Determination of Hemoglobin Level

Hemoglobin levels of the participants were determined using cyanmethemoglobin method, according to the manufacturer's instruction [21].

Determination of Urea Level

The urea levels of the participants were determined using colorimetric method, according to the manufacturer's instructions. The reagent was commercially purchased from Randox company, United Kingdom with LOT number 637861 [22].

Determination of Creatinine Level

Creatinine level was determined by Jaffe's method, according to the manufacturer's instruction [23]. Creatinine reagent was gotten from Randox company with LOT number 630901.

Determination of Erythropoietin Level

Erythropoietin level was determined using Elabscience ELISA kit with LOT number ER164VFB1442, according to the manufacturer's instructions [24].

Statistical Analysis

The questionnaires were screened for completeness, coded and data will be entered into the independent student t- Test version 25.0 software. Values obtained in this study will be presented as mean ± standard deviation (levels of significance were accepted at $p<0.05$).

RESULTS:

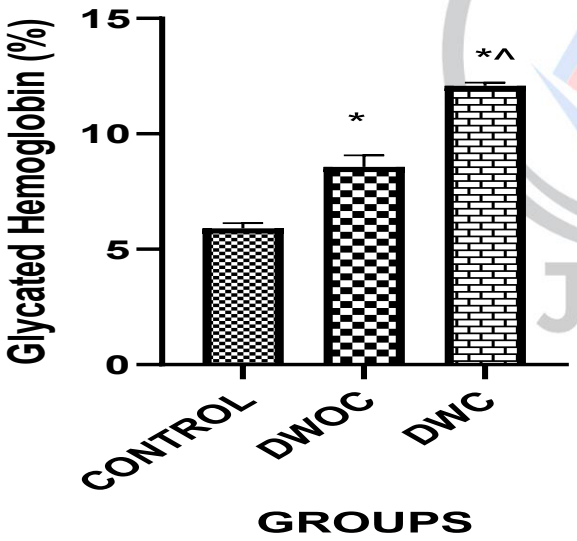
Table 1 shows the socio-demographic characteristics of the study population. The study population comprises 30 diabetes patients without complications, 21 diabetes patients with complications and 30 healthy non-diabetic controls. A greater percentage of the non-diabetic control were young adults (96.7%), females (96.7%), had no family history of diabetes (86.7%), were single (93.3%), unemployed (86.7%), had obtained tertiary education (100%) and of the Benin ethnic group (33.3%). A greater proportion of the diabetic patients without complications had suffered from the disease for 5 – 10 years (50%); were of the middle-age class (56.7%); were females (66.7%); had family history of diabetes mellitus (90%); were married (96.7%), retired (63.3%), had obtained tertiary education (80%) and from Benin city (70%). Majority of the diabetic patients with complications had suffered from diabetes for 5 – 10 years (66.7%); were of the elderly adult class (57.1%); were males (85.7%); had family history of diabetes mellitus (81%); were married (100%), retired (85.7%), had obtained tertiary education (85.7%) and from Benin city (52.4%).

Table 1. The Socio - Demographic Characteristics of the Study Population

Characteristics		Study Groups			Total
		Non-Diabetic	Diabetic Without Complication	Diabetic With Complication	
Duration of Diabetes Mellitus	>10yrs	0 (0)	8 (26.7)	7 (33.3)	15(18.5)
	0-5yrs	0 (0)	7 (23.3)	0 (0)	7 (8.6)
	5-10yrs	0 (0)	15 (50.0)	14 (66.7)	29(35.8)
	Nil	30 (100)	0 (0)	0 (0)	30(37.0)
Age Groups	Young	29 (96.7)	1 (3.3)	0 (0)	30(37.0)
	Middle-Age	1 (3.3)	17 (56.7)	9 (42.9)	27(33.3)
	Elderly	0 (0)	12 (40.0)	12 (57.1)	24(29.6)
Sex	Females	29 (96.7)	20 (66.7)	3 (14.3)	52(64.2)
	Males	1 (3.3)	10 (33.3)	18 (85.7)	29(35.8)
History of Diabetes Mellitus	No	26 (86.7)	3 (10.0)	4 (19.0)	33(40.7)
	Yes	4 (13.3)	27 (90.0)	17 (81.0)	48(59.3)
Marital Status	Married	2 (6.7)	29 (96.7)	21 (100.0)	52(64.2)
	Single	28 (93.3)	1 (3.3)	0 (0)	29(35.8)
Occupation	Employed	4 (13.3)	8 (26.7)	0 (0)	12(14.8)
	Retired	0 (0)	19 (63.3)	18 (85.7)	37(45.7)
	Unemployed	26 (86.7)	3 (10.0)	3 (14.3)	32(39.5)
Educational Status	Primary	0 (0)	5 (16.7)	3 (14.3)	8 (9.9)
	Secondary	0 (0)	1 (3.3)	0 (0)	1 (1.2)
	Tertiary	30(100.0)	24 (80.0)	18 (85.7)	72(88.9)
Ethnicity	Agbor	0 (0)	1 (3.3)	0 (0)	1 (1.2)
	Auchi	0 (0)	1 (3.3)	0 (0)	1 (1.2)
	Benin	10 (33.3)	21 (70.0)	11 (52.4)	42(51.9)
	Eshan	5 (16.7)	2 (6.7)	3 (14.3)	10(12.3)
	Etsako	1 (3.3)	0 (0)	0 (0)	1 (1.2)
	Ghana	1 (3.3)	0 (0)	0 (0)	1 (1.2)
	Igbo	6 (20.0)	1 (3.3)	0 (0)	7 (8.6)
	Ika	1 (3.3)	0 (0)	0 (0)	1 (1.2)
	Isoko	1 (3.3)	0 (0)	0 (0)	1 (1.2)
	Unknown	1 (3.3)	0 (0)	0 (0)	1 (1.2)
	Urhobo	2 (6.7)	3 (10.0)	0 (0)	5 (6.2)
	Yoruba	2 (6.7)	1 (3.3)	7 (33.3)	10(12.3)

Table 2 shows selected life-style and clinical characteristics of the study population. Majority of the control subjects did not take nutritional supplements (96.7%); received daily sunlight (100%); performed daily exercise (96.7%); do not take alcohol (80%); do not smoke (100%); consume high carbohydrate, high

protein diets (90%); indicated no blood loss (83.3%); and did not show any diabetes complications (100%). A greater percentage of the diabetes patients without complications had type 2 diabetes (80%); were taking diabetes medications (100%); were not taking nutritional supplements (86.7%); received daily sunlight (100%); performed daily exercise (100%); do not take alcohol (100%); do not smoke (100%); consume high carbohydrate, high protein diets (70%); indicated no blood loss (86.7%); and did not show any diabetes complications (100%). Most of the diabetes patients with complications had type 2 diabetes (51.9%); were taking diabetes medications (63%); were not taking nutritional supplements (93.8%); received daily sunlight (100%); performed daily exercise (98.8%); do not take alcohol (90.1%); do not smoke (100%); consume high carbohydrate, high protein diets (80.2%); indicated no blood loss (88.9%); and did not show any diabetes complications (74.1%).



Key – DWOC – Diabetes without Complication
DWC – Diabetes with Complication

Figure 1. The Mean Glycated Hemoglobin Levels of the Study Population.

The mean glycated hemoglobin levels of the study population are shown in **Figure 1**. Analysis of variance and a post-hoc analysis indicated that non-diabetic control had a significantly lower ($p < 0.001$) mean HBA1c (5.90 ± 1.28%) compared with diabetes patients without complications (8.57 ± 2.71%) and those with complications (12.08 ± 0.73%). Diabetic patients with complications also indicated significantly higher ($p < 0.001$) mean HBA1c level compared with patients without complications.

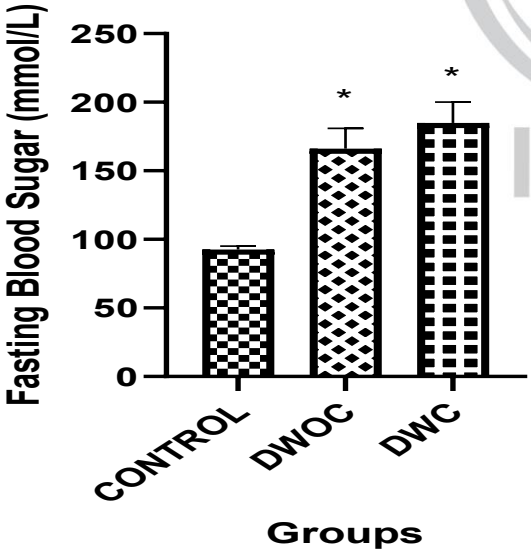
Table 2. Selected Life - Style and Clinical Characteristics of the Study Population

		GROUP			Total
		Control	DWOC	DWC	
Type of Diabetes Mellitus	Nil	30 (100)	0 (0)	0 (0)	30(37.)
	Type 1	0 (0)	6 (20.0)	3 (14.3)	9 (11.1)
	Type 2	0 (0)	24(80.0)	18(85.7)	42(51.9)
Diabetes Medication	Yes	0 (0)	30(100)	21(100)	51(63.0)
	None	30 (100)	0 (0)	0 (0)	30(37.0)
Nutritional Supplement	No	29(96.7)	26(86.7)	21 (100)	76(93.8)
	Yes	1 (3.3)	4 (13.3)	0 (0)	5 (6.2)
Daily Sunlight	No	0 (0)	(0)	(0)	(0)
	Yes	30 (100)	30 (100)	21 (100)	81 (100)
Daily Exercise	Yes	29(96.7)	30 (100)	21 (100)	80(98.8)
	No	1 (3.3)	0 (0)	0 (0)	1 (1.2)
Alcohol	No	24(80.0)	28(93.3)	21 (100)	73(90.1)
	Yes	6 (20.0)	2 (6.7)	0 (0)	8 (9.9)
Smoking	No	30 (100)	30 (100)	21 (100)	81 (100)
	Yes	(0)	(0)	(0)	(0)
Dietary Intake	High Carbohydrate, High Protein	27(90.0)	21(70.0)	17(81.0)	65 (80.2
	High Carbohydrate, Low Protein	1 (3.3)	0 (0)	0 (0)	1 (1.2)
	Low Carbohydrate, High Protein	2 (6.7)	9 (30.0)	4 (19.0)	15(18.5)
Blood Loss	Injury	0 (0)	4 (13.3)	0 (0)	4 (4.9)

	Menstruation	5 (16.7)	0 (0)	0 (0)	5 (6.2)
	None	25(83.3)	26(86.7)	21 (100)	72(88.9)
Complications	Foot Ulcer	0 (0)	0 (0)	4 (19.0)	4 (4.9)
	Retinopathy	0 (0)	0 (0)	13(61.9)	13(16.0)
	Neuropathy	0 (0)	0 (0)	4 (19.0)	4 (4.9)
	None	30 (100)	30 (100)	0 (0)	60(74.1)

Abbreviations: DWOC, diabetics without complications; DWC, diabetics with complications.

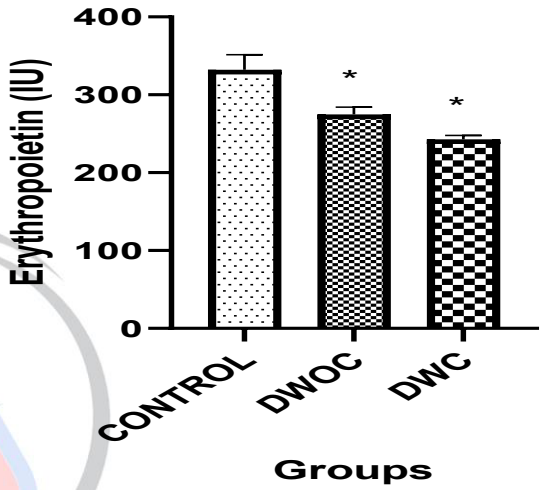
Figure 2 shows the mean fasting glucose level of the study population. Data indicate significantly lower ($p < 0.001$) mean fasting blood sugar in non-diabetic control (92.60 ± 12.21 mmol/L) compared with diabetic patients without complications (166.16 ± 80.8 mmol/L) and those with complications (184.85 ± 83.43 mmol/L). There was no significant difference in mean FBS between diabetic patients with complications compared with patients without complications ($p = 0.319$).



Key – DWOC – Diabetes without Complication
DWC – Diabetes with Complication

Figure 2. The Mean Fasting Glucose Level of the Study Population

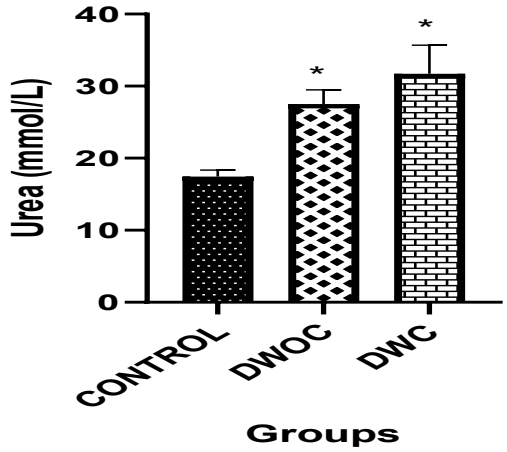
The mean erythropoietin level of the study population is shown in **Figure 3**. Data indicates a significantly higher mean erythropoietin level in the control group (332.27 ± 106.04 IU) compared with patients without complications (274.73 ± 52.19 IU) and those with complications (242.86 ± 23.44 IU). However, no significant differences were observed between diabetic patients with complications and those without complications ($p = 0.129$).



Key – DWOC – Diabetes without Complication
DWC – Diabetes with Complication

Figure 3. The Mean Erythropoietin Level of the Study Population

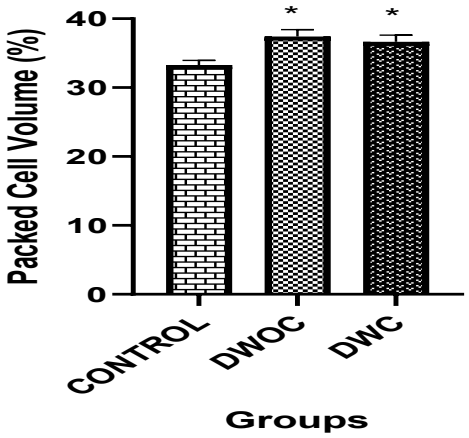
The mean urea level of the study population is presented in **Figure 4**. Data shows that the control group (17.46 ± 4.82 mmol/L) indicated significantly lower ($p < 0.001$) mean urea level compared with patients without complications (27.50 ± 10.94 mmol/L) and those with complications (31.71 ± 18.16 mmol/L). No significant differences were observed between diabetic patients with complications and those without complications ($p = 0.211$).



Key – DWOC – Diabetes without Complication
DWC – Diabetes with Complication

Figure 4. Mean Urea Level of the Study Population

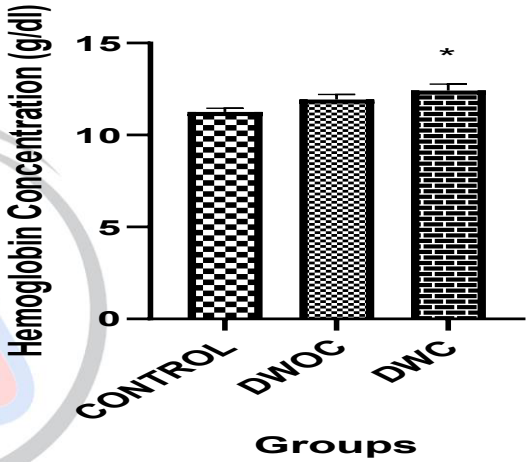
The mean packed cell volume of the study groups is presented and compared among them in **Figure 5**. Analysis of variance shows that the control group (33.30 ± 3.55%) indicated significantly lower mean urea level compared with patients without complications (37.40 ± 5.49%) and patients with complications (36.66 ± 4.37%). No significant differences were observed between diabetic patients with complications and those without complications (p = 0.514).



Key – DWOC – Diabetes without Complication
DWC – Diabetes with Complication

Figure 5. The Mean Packed Cell Volume of the Study Population

The mean hemoglobin concentration of the study population is shown in **Figure 6**. There was no significant difference (p = 0.067) in hemoglobin concentration between the control group (11.26 ± 1.08 g/dl) and diabetic patients without complications (11.93 ± 1.50 g/dl). Similarly, no significant difference (p = 0.214) was observed in Hb concentration between diabetic patients with complications and those without complications (12.42 ± 1.58 g/dl). In contrast, the diabetic patients with complications indicated significantly greater (p = 0.004) level of hemoglobin compared with the non-diabetic controls.

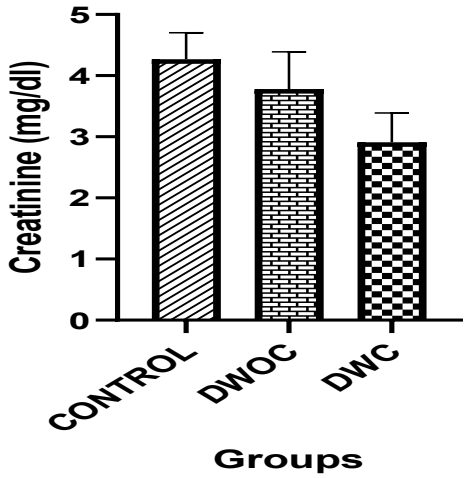


Key – DWOC – Diabetes without Complication

DWC – Diabetes with Complication

Figure 6. The Mean Hemoglobin Concentration of the Study Population

Figure 7 shows the mean creatinine levels of the study population. Data shows that there were no significant differences in mean creatinine level between the control (4.27 ± 2.36 mg/dl) and patients without complications (3.78 ± 3.34 mg/dl; p = 0.489) and patients with complications (2.91 ± 2.19 mg/dl; p = 0.084). Similarly, no significant difference was found in creatinine level between the patients with complications and those without complications (p = 0.267).



Key – DWOC – Diabetes without Complication

DWC – Diabetes with Complication

Figure 7. The Mean Creatinine Levels of the Study Population

DISCUSSION :

Diabetes mellitus (DM) is a metabolic disorder, characterized by either absolute or relative deficiency of insulin, resulting in hyperglycaemia, which if not managed properly can lead to acute or chronic complications such as diabetic retinopathy, nephropathy and neuropathy [25]. It is associated with reduced life expectancy, significant morbidity due to specific diabetes related-microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease) and diminished quality of life [26].

In this study, the demographics, lifestyle and clinical characteristics showed that a greater proportion of patients with complications of diabetes mellitus were of the elderly age range (Table 1). This is in accordance with Bai, *et al*. (2021) [27], who found the prevalence of diabetes to increase with advanced age with the highest prevalence for ages ≥65 years.

In this study, majority of the diabetics with complications were males 18(85.7%), while females were 3 (14.3%) **(Table 1)**. This finding agreed with previous studies done by Adogu *et al.*, 2015; Aguocha *et al*., 2013; Chijioke *et al.*, 2010; Ojobi *et al.,* 2014; Unadike *et al*., 2013[28] [29] [30] [31] [32], but disagreed with Agofure *et al.*, 2020 who recorded more female participants in their study. However, Khan, *et al*. (2020) [33], reported that the rates of occurrence of diabetes mellitus are similar among men and women. Of the 21(100%) participants with diabetes complications, 17(81%) had family history of diabetes mellitus compared to 4(19%) who did not have. According to literatures, it has been reported in many populations that genetic vulnerability and familial aggregation are responsible for diabetes mellitus [34] [35]. It is estimated that having one or both parents with type 1 diabetes mellitus increase the chances of the transmission by two to four folds [36]. Whereas, 25% and 33% percent of those with type 2 diabetes also have family relatives who also have the disease [37].

All the participants living with diabetes complications were married, of which 18(85.7%) were retired and 3(14.3%) were unemployed. This attest to the fact that diabetes mellitus is a disease of adult and increases with age [37] **(Table 1)**. All the participants were educated, primary school 3(14.3%) and tertiary level 18(85.7%). All the participants with diabetes complications were on medication and nutritional supplements. They were all exposed to daily sunlight, engaged in daily exercise, they neither smoked nor consume alcohol. According to their response when asked about diet, 17(81%) of the total participants who were diagnosed with diabetes complications said they do take high carbohydrate and protein foods. None of the participants lost blood through injury or other means prior to the study. This agree with the fact that being educated cannot stop the development of diabetes mellitus, rather, a well-coordinated lifestyle, exercise, routine medical checks and strict adherence to medications will prevent the onset of the disease and subsequent complications.

In this study, Bini 11(52.4%), Yoruba 7(33.3%) and Esan 3(14.3%) ethnic groups dominated the race of the patients with diabetes complications

respectively **(Table 1)**. According to Uloko *et al*., 2018, [38] the pooled prevalence of DM in the six geopolitical zones of Nigeria were 3.0% (95% CI 1.7–4.3) in the North-West, 5.9% (95% CI 2.4–9.4) in the North-East, 3.8% (95% CI 2.9–4.7) in the North-Central zone, 5.5% (95% CI 4.0–7.1) in the South-West, 4.6% (95% CI 3.4–5.9) in the South-East, and 9.8% (95% CI 7.2–12.4) in the South-South zone respectively. Though, their study was not majorly on complications of diabetes mellitus, rather, it was done on prevalence and on larger section of the country. Also, according to Parm *et al.* [39] the prevalence of type 2 diabetes was higher in the Asian and Black ethnic groups, compared with the White group. It is believed that majority of them, if live long enough will eventually develop diabetes complications.

Eighteen 18(85.7%) of the participants with complications had type 2 diabetes, while, 3(14.3%) were diagnosed with type 1 diabetes **(Table 2)**. This supports the fact that the prevalence of type 2 is higher than type 1. According to Brutsaert, 2023[40], people with either type 1 or type 2 diabetes are likely to have complications as a result of the elevated glucose level. However, because type 2 diabetes may be present for some time before it is diagnosed, complications in type 2 diabetes may be more serious or more advanced when they are discovered.

The pattern of diabetes complication in this study showed that retinopathy had the highest number of occurrence 13(61.9%), neuropathy and foot ulcer recorded 4(19%) each. Surprisingly, no case of diabetes nephropathy was recorded **(Table 2)**. This is in contrast with previous study by Agofure *et al.,*2020 [41] In their study, they reported kidney failure 27.87%, heart attack 27.27%, erectile dysfunction 21%, as well as diabetes foot ulcer and blindness. Adogun *et al*., 2018 [28] recorded lower prevalence of complications and pattern of presentation in Imo state Teaching Hospital, Nigeria. Tracey *et al*. (2015) [42]; reported that the prevalence of diabetes complications ranged widely depending on study population and methodology used (6.5-25.2% retinopathy; 3.2-32.0% neuropathy; 2.5-5.2% nephropathy).

In this study, patients with diabetes complications had the highest mean level of glycosylated hemoglobin (Figure 1), it indicated a significant difference in mean glycated haemoglobin levels between non-diabetic control compared with the diabetic patients without complications and those with complications. Similarly, diabetic patients with complications also indicated significantly higher mean HBA1c level compared with patients without complications. This is in agreement with Sherwani *et al*. (2016) [43], which noted that higher levels of glycated haemoglobin have been linked to cardiovascular disease, nephropathy, neuropathy, and retinopathy, and indicate poorer control of blood glucose levels.

In this study, Figure 2 showed that the mean fasting blood sugar in non-diabetic control compared with diabetic patients without complications and those with complications is statistically significant. However, there was no significant difference in mean FBS between diabetic patients with complications compared with patients without complications which implies that the fasting blood sugar level in both diabetic patients with complications and those without complications is not dependent on the patient having complications.

In this study, the mean erythropoietin level of the study population indicated a significantly higher mean erythropoietin level in the control group compared with patients without complications and those with complications **(Figure 3)**. However, no significant differences were observed between diabetic patients with complications and those without complications. This finding is in disagreement with the study carried out by Gluba-Brzózka *et al*. (2020) [44], which reported that erythropoietin level decreases as a result of complication (especially nephropathy) in diabetic patients. McGill and Bell in 2006 [8], also opined that anemia that is associated with erythropoietin deficiency may have prognostic significance for persons with nephropathy or heart failure. In early diabetic nephropathy, damage to the peritubular

fibroblasts can occur and lead to erythropoietin deficiency and anemia prior to the loss of filtration. It has also been documented that low erythropoietin (EPO) levels in diabetics with complications other than nephropathy can be attributed to several factors. One potential cause is chronic inflammation, which is common in diabetes. Inflammation can suppress EPO production by affecting the kidneys and other organs involved in erythropoiesis. Additionally, poor glycemic control in diabetic individuals can lead to oxidative stress, which may impair EPO production in the kidneys or other tissues. Another factor is microvascular damage, a hallmark of diabetes, which can impact various organs beyond the kidneys, including the bone marrow. This damage can affect the marrow's ability to respond to EPO signals effectively. Diabetic individuals are also at increased risk for cardiovascular complications, such as heart disease, which can reduce oxygen delivery to tissues and trigger lower EPO production as the body attempts to balance oxygen levels. Lastly, certain medications used to manage diabetes, such as ACE inhibitors or specific antidiabetic drugs, might influence EPO levels. Overall, a combination of metabolic dysregulation, organ damage, and medication effects can contribute to lower EPO levels in diabetics with complications other than nephropathy. The reason for these results is not known, but may be due to the fact that none of the patients with complications in this study was diagnosed with nephropathy, which would have affected the production of erythropoietin that play a vital role in erythropoiesis.

Diabetes patients without complications recorded higher packed cell volume (PCV) in this study compared to those with complications **(Figure 5)**. Surprisingly, those participants with complications recorded highest mean concentration of haemoglobin. This may be due to the fact that the targeted organs in these patients were not kidneys, as none was reported to have diagnosed with nephropathy. The erythropoiesis is not affected hence, the normal packed cell volume and haemoglobin concentration levels. Although, all the participants with diabetes complications admitted to be taking nutritional supplements which may have compensated them with loss minerals and vitamins, they were also exposed to daily early morning sunlight which is a good source of vitamin D.

The participants with diabetes complications had the highest concentrations of urea, but lowest level of creatinine among the study group **(Figure 4)**. This may be due to the fact that urea is an unreliable biomarker for renal function compared to creatinine, because many factors affect its blood concentration. Also, none of the participants was reported to have diagnosed with renal complication within the period of this study. In the study of Harita *et al*., 2009[45] titled ‘’Lower serum creatinine is a new risk factor of type 2 diabetes,’’ they found that during the 4-year follow-up period, 877 men developed type 2 diabetes. This may suggest that there is ongoing cell destruction within the kidney which may eventually result to nephropathy.

Limitations of the study

The study was limited to the small available sample size as at the time of this study. Also, many participants opted out due to personal, religious and cultural reasons The study area was also restricted to Edo state which prevented us from assessing other part of the country.

CONCLUSION:

This study showed that more males had diabetes complications compared to females. The pattern of diabetes complications showed that retinopathy had the highest number of occurrences, while, neuropathy and foot ulcer followed. Federal Government should encourage the Ministries, departments and agencies responsible for creating awareness on the danger of diabetes. Citizens should be able to embrace medical checks and to live healthy lifestyles.

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Author Contribution:
BIGA, UIA and FOO were responsible for the conceptualization of the study. The methodology was developed by ROO, EOA, WPU and GEO. Samples and Data analysis was performed by FOO and AOO. The literature review was prepared by BIGA, IMMO, ES, FOO and EOAE. All authors contributed to the review and editing of the manuscript. BIGA provided supervision throughout the study. All authors contributed to funding of this research. All authors read and approved the final version of the manuscript.

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