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Prevalence of Ketosis-Prone Diabetes Mellitus in Specialist Hospital Sokoto, Nigeria

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Abstract

Ketosis-prone diabetes mellitus is a diabetes subtype that occurs in adults with hyperglycemia and ketosis without evidence of autoimmunity. Ketosis-prone diabetes is one of the diabetes subtypes that are on the rise in our black community. This study was designed to estimate the prevalence of ketosis-prone diabetes mellitus at a specialist hospital, in Sokoto, Nigeria. Seventy patients were recruited, and demographic data of the patients were obtained using a prepared questionnaire. Biochemical parameters such Fasting plasma glucose, Plasma ketones, Glycated Hemoglobin, insulin, and Glutamic Acid Decarboxylase determined using a standard method. Insulin resistance and beta-cell function were determined using the homeostasis model and Aβ classification was adopted in the selection of KPDM among participants. A prevalence of 17.14% was recorded with a 1:1 ratio between males and females. prevalence of 50% (6:12), 25% (3:12), 8.3% (1:12), 8.3% (1:12), and 8.3% (1:12) were also recorded among Hausa, Yoruba, Fulani, Igbo and others respectively. A significant difference was observed in FPG, HOMA-IR and HOMA-B between male and female subjects with the female having a greater value at $p<0.05$. Also, a significant difference was observed among Hausa, Fulani, Yoruba, Igbo and others in PK, FPG, HOMA-IR and HOMA-B. Knowing the prevalence of ketosis-prone diabetes mellitus was important for proper management to reduce costs and avoid mortality.

Nomenclature and units

1.0 Introduction

Diabetes is a metabolic disease that occurs when the pancreas is unable to produce insulin or when the body cannot make good use of the insulin it produced (Banu, Sur, & Targets, 2023). Its burden is increasingly high in many developed countries and the prevalence is higher in Africa, Asia and some part of American countries (Gninkoun, Alassani, Sagna, Adjagba, & Djrolo, 2016; Hu et al., 2023). It was estimated that 10.5% of adults between 20–79 years of age (meaning 536.6 million) people are living with diabetes in the world (Sun et al., 2022). The global prevalence of diabetes region-wise in 2021 according to the International Diabetes Federation was 7.1% in North America and the Caribbean while in Europe it is 7.8%. Similarly, the (IDF, (2009) estimated the prevalence of diabetes in South America and Central America to be 10.4% and it is slightly higher than that of Africa which is 14.2%. Lastly, in Western Pacific, it was estimated to be 14.7% and in South-East Asia as well as the Middle East and North Africa it was 20.8% and 27.6% respectively (Hu et al., 2023)

The highest number of undiagnosed diabetes was found in Africa (53.6%), Western Pacific (52.8) and Southeast Asia (51.3) regions (Ogurtsova et al., 2022). In Nigeria, diabetes has a varied range of prevalence across the regions of the country. An estimate shows less than 2% and greater than 10% of people with diabetes lived in rural and urban cities respectively (A. Sabir et al., 2013). (A. A. Sabir et al., 2017) reported a prevalence of 4.3% of diabetes in the sub-Saharan population of northwest, Nigeria. The difference resulted from the lifestyle and demographic transition of different regions. The rise in migration of people from rural to urban areas and the development and expansion of cities to sub-urban areas raised a fear that the diabetes epidemic would rise more than expected (Fasanmade & Dagogo-Jack, 2015).

Diabetes Mellitus is classified according to the American Diabetes Association classification as Type 1 diabetes is autoimmune β-cell destruction leading to absolute insulin deficiency. Type 2 diabetes is a progressive loss of β-cell insulin secretion on the background of insulin resistance. Gestational diabetes mellitus (GDM) is diabetes diagnosed in the second or third trimester of pregnancy (Nguyen et al., 2020). Specific types of diabetes due to other causes include monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation). Other emerging diabetes includes ketosis-prone diabetes which shares certain similarities with type 1 and type 2 diabetes (Redondo et al., 2020).

Ketosis-prone diabetes mellitus (KPDM) is a rare form of diabetes mellitus affecting middle-aged (mean age at presentation 39.1 years), mildly obese or overweight (mean BMI 28.5 kg/m^2) black African, African-American (H. E. Lebovitz & M. A. Banerji, 2018), African-Caribbean Asian and Hispanic people with a family history of diabetes (Smiley, Chandra, & Umpierrez, 2011) and duration of symptom (5-44 days) (Mauvais-Jarvis, Smith, et al., 2004). It was reported in the literature as Flatbush diabetes (Banerji et al., 1994), atypical diabetes (Sobngwi & Gautier, 2002), idiopathic type 1 (Smiley et al., 2011) and Ketosis-prone diabetes (H. E. Lebovitz & M. A. Banerji, 2018). Some symptoms of type 1 diabetes are observed in KPDM, although there is no evidence of autoimmune markers. However, diabetes ketoacidosis (DKA) is present with impaired insulin resistance and insulin action (Imran & Ur, 2008). However, the syndrome was reported in previous studies as a sub-class of type 2 diabetes (Volk & Wellmann, 1985). Other symptoms of diabetes are notable in KPDM such as polyurea, polyphagia, and polydipsia though it appears to be in a short time usually less than 4 weeks and weight loss is usually between 4-12 Kg (Mauvais-Jarvis, Sobngwi, et al., 2004).

Diabetic ketoacidosis has been reported in 75% of the cases of KPDM in early diagnosis with similarities in biochemical and acid-base features in diabetes type I. It had been poorly diagnosed principally due to its complex nature at diagnosis and lack of clinical experiences (Smiley et al., 2011).

Identification of people with a potential diagnosis of KPDM is vital to optimizing treatment. For example, performing pathology tests for the presence or absence of islet cell or GAD autoantibodies, glycated haemoglobin estimation (HbA1c), with assessing beta-cell functional reserve will enable researchers to identify whether people can be treated with insulin for a short period or if they are likely to need life-long insulin replacement (Colloby, 2014).

The prevalence of KPDM is on the rise. Insulinopaenia at presentation requires intensive insulin treatment at diagnosis. However, these individuals present a challenge to healthcare professionals concerning how their diabetes should be managed over the longer term. With the opportunity to discontinue insulin therapy and with the introduction of oral hypoglycaemic agents, confidence in managing this group of people is growing. This research was conducted to determine the prevalence of KPDM in a specialist hospital in Sokoto, Nigeria and among some specific ethnicities comprises of Hausa, Fulani, Yoruba, Igbos and others.

2.0 Materials and Methods

This section presents the materials and methods used in this research work.

2.1 Ethical Approval

Ethical approval was sought at Sokoto State Specialist Hospital, Sokoto, using a letter of introduction from the Department of Biochemistry Usmanu Danfodiyo University, Sokoto.

2.2 Study Area

This study was carried out at Sokoto State Specialist Hospital, Sokoto, Nigeria. Sokoto State has twenty-three Local Government Areas (LGA) and a land mass of 31,041 km2 with an estimated population of 6,391,000 projected for 2022 (Ukpong & Adetunji, 2020). The State has a predominance of Hausa and Fulani ethnic groups, while the non-natives belong to Igbo, Yoruba and other minority ethnic groups. Occupants are Farmers, civil servants, traders, artisans and people of other occupations like tanning and dyeing. Specialist Hospital, Sokoto, is among two tertiary health institutions in the Sokoto metropolis. It has a bed capacity of 570 and provides medical care/services to the residents in the metropolis as well as those referred from the other Local Government Areas in the state and some patients from the neighbouring states like Kebbi, Zamfara and sometimes from some parts of Niger republic.

2.3 Inclusion and Exclusion Criteria

The participants included in the study were adult diabetic patients with Type 2 diabetes, patients with non-autoimmune diabetes and Ketoacidosis and patients with a hyperglycemic crisis. And the participants excluded from the study were patients with autoimmune diabetes and are below 18 years of age (< 18 years), pregnant women, patients in trauma or with known infection and patients aged above 70 years.

2.4 Participants and Sample Size

The participants were adult patients coming to the diabetology unit of Sokoto Specialist Hospital, Sokoto with symptoms of diabetes and obesity within the study period. Fisher statistical formula for sample size was used to calculate sample size, i.e., $N = Z^2pq/D^2$, where N = Minimum sample size, Z = Standard deviation set at 1.96 which corresponds to a 95% confidence interval, P = Prevalence of diabetes (4.3%) among residents of Sokoto, $q = 1 - P$, and D = Margin of unacceptable error or measure of precision (0.05).

$N= (1.96)^2*0.046*0.954/(0.05)^2 =67.43$

The minimum sample size was considered to be 68 people.

2.5 Study Protocol

Informed consent was obtained from all the participants. Demographic, clinical and laboratory data of interest were collected during the study.

2.6 Demographic Profile

The demographic profiles of the participants were recorded from the consent form filled out by the researcher which include gender, ethnicity, age, smoking habit and family history of diabetes.

2.7 Anthropometric Profile

Height

Measurement of height using a stadiometer was done by standing upright against a ruler and sliding a horizontal headpiece adjustable on the head of the patient and values were recorded in meters.

Body Mass

Measurement of body mass was taken using a weighing balance (Seca). The patients were free from additional objects like shoes, phones, keys etc measured and recorded in Kilograms.

Body Mass Index

Body mass index (BMI) was calculated using the method of (Mei et al., 2002).

BMI (Kg/M^2) = Body Mass / $Height^2$ the BMI was categorized based on WHO 2002 guidelines.

2.8 Blood Sample Collection

The blood sample was drowned after 8-10 hours of fasting using the aseptic technique by the Venipuncture method. Briefly, 5 ml of blood was taken in fluoride oxalate, EDTA and a plain container for fasting blood glucose, HbA1c and ELISA test respectively in a ratio (2:2:1) centrifuged and kept frozen at -20 ^{0}C before use.

2.9 Estimation of Plasma Ketones

Plasma Ketones were estimated using the electrical impedance method by On Call GK dual meter for estimation of Beta-ketone (Ketan, 2016).

Principle

The thin strip with a chemical reagent system measures the blood β-ketone (β-hydroxybutyrate) concentration in whole blood. Blood was applied to the end tip of the test strip and automatically absorbed into the reaction cell where the reaction

takes place. A transient electrical current was formed in the reaction and blood β-ketone concentration is calculated based on the electrical current detected by the meter then the result is shown on the meter display. The meters are calibrated to display plasma equivalent results.

Procedure

The test strip was used for in vitro diagnosis only. Sterile finger sticks or venous blood obtained using an aseptic technique are placed on the tip of the strip. The meter absorbed and analyzed the blood to display the result on the meter monitor in mmol/l within 10 seconds.

Reference range

Individuals with Ketones levels less than 0.6 mmol/L are considered normal.

Individuals with ketone levels of 0.6-1.5 mmol/L are considered to have a risk of developing DKA.

Individuals with Ketones levels greater than 1.6 mmol/L are at high risk of developing DKA.

2.10 Estimation of Plasma Glucose

Plasma glucose was estimated via the glucose oxidase method using the Randox kit according to Lott, (1975).

Principle

Glucose was determined after enzymatic oxidations in the presence of glucose oxidase. The hydrogen peroxide formed reacted under the catalysis of peroxidase with 4-aminophenazone and phenol and produced a red-violet-coloured quinonimine complex which was measured using a spectrophotometer at 500nm. The intensity of the colour is proportional to the glucose concentration in the sample. The equation for the reaction is as follows:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\textit{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4\text{-aminophenazone} + \text{phenol} \xrightarrow{\textit{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Procedure

Test tubes were set up in triplicates and labelled as blank, test and standard as shown below:

	Blank	Test	Standard
Serum /plasma (µl)	-	10	-
Standard (µl)	-	-	10
Distilled water (µl)	10	-	-
Glucose oxidase Reagent (µl)	1000	1000	1000

The contents were mixed and incubated at 37°C for 5 minutes and the absorbance of the standard and test were read against the blank.

Calculation

Glucose concentration was determined using the formula.

$$\text{Serum glucose}\left(\frac{\text{mmol}}{\text{l}}\right) = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Conc. of standar}$$

Reference Value: 3.9-5.8mmol/L

2.11 Estimation of Glycated Haemoglobin

Glycated Hemoglobin was determined using the Ion exchange resin method of Trivelli*et al.*(1971).

Principle

Whole blood was mixed with lysing reagent and hemolysate was prepared. It was then mixed with a weakly binding cation exchange resin. The non-Glycosylated Hemoglobin bound to the resin leaving Bound-Glycated Hemoglobin (GHb) free in the supernatant. The GHb percentage was determined by measurement of the absorbance of the GHb fraction and of the total Hb.

Procedure

Assay Temperature: 30°C ±1°C

Wave Length: 415 nm

Step I: Hemolysate preparation: 0.25 ml of lysing reagent was pipetted into a test tube. Added to it 0.05 ml of well-mixed whole Blood/control. Solution mixed well and allowed to stand at room temperature for 5 minutes.

Step II: Bound Glycated Hemoglobin GHb separation and assay: Resin Tubes were brought to assay temperature by incubation of the tubes in a water bath with 0.1 ml of hemolysate (from step 1) and then added to a test tube. The resin separator was positioned in the tube so that the rubber sleeve was approximately 3 cm

above the resin level. Contents were mixed in a vortex mixer continuously for 5 minutes. The resin was allowed to settle at assay temperature for 5 minutes, the resin separator was pushed down the tube until the Resin was firmly packed. The supernatant was poured directly into a cuvette and absorbance was measured against deionized water.

Step III: Total bound Hemoglobin (THb) assay: 5.0 ml of deionized water Pipetted into a test tube. Added to it 0.02 ml of hemolysate (from step 1). Mixed and read absorbance against deionized water.

Calculation

$$\text{Ghb \%} = \frac{\text{Absorbance of GHb}}{\text{Absorbance of Thb}} \times 7.2 \text{ x temperature Factor}$$

Assay Temperature: 30±1°C, Wave Length: 415 nm
Values greater than or equal to 8% are considered diabetic Subjects.

2.10 Estimation of Insulin

Insulin was estimated by Insulin Accubind ELISA Method, using the method of Boehm and Lebovitz, (1979).

Principle

The principle was based on the interaction of streptavidin coated on the surface of a microplate well and exogenously added biotinylated monoclonal insulin antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labelled antibody and a serum containing the native antigen reaction results between the native antigen and the antibodies, without competition or steric hindrance formed a soluble sandwich complex. The reaction was as follows:

$EnzAb_{(M)}$ + Ag(Ins)+ $BtnAb_{(M)} \leftrightarrow$Enz $Ab_{(M)}$- Ag(Ins) – $BtnAb_{(M)}$

Simultaneously, the complex was deposited to the well through the high-affinity reaction of streptavidin and biotinylated antibody.

$EnzAb_{(M)}$- Ag(Ins) – $BtnAb_{(M)}$ + Streptavidin → Immobilized Complex

After equilibrium was attained, the antibody-bound fraction was separated from the unbound by decantation or aspiration. The enzyme activity in the antibody-bound fraction was directly proportional to the native antigen concentration by utilizing several different serum references of known antigen values. A dose-response curve was generated from which the antigen concentration of unknown was extrapolated.

Procedure

In calibrators, 50 controls and samples were pipetted into assigned duplicate wells. 100µl of the insulin enzyme reagent was added to each well. The microplate was swirled gently for 20-30 seconds to mix and was incubated for 120 minutes at room temperature (20-27°C). The content of the microplate was discarded by decantation or aspiration. 350µl of wash buffer was added and decanted, and that was repeated twice to make 3 washes. 100µl of working substrate solution was added to all the wells and then incubated for 15 minutes. After that 50µl of the stock solution was added to each well and mixed gently for 15-20 minutes. The absorbance in each well was read at 450nm in a microplate reader. A dose-response curve was used to ascertain the concentration of insulin in the unknown specimen.

2.12 Glutamic acid decarboxylase 65 (GAD65) Assay

Human glutamic acid decarboxylase 65 (GAD65) antibodies (IgG) were assayed using the ELISA method (Grubin *et al*., 1994)

Principle

This assay employed the qualitative enzyme immunoassay technique. The microtiter plate provided in this kit was pre-coated with antigen. Samples were pipetted into the wells with anti-human Ig G conjugated Horseradish Peroxidase (HRP). Antibodies specific to the antigen present were bound to the pre-coated antigen. Washed to remove any unbound reagent, a substrate solution was then added to the wells and the colour developed is proportional to the amount of human GAD65 antibody (Ig G) bound in the initial step. The colour development stopped and the intensity of the colour was measured.

Procedure

All reagents and samples were at room temperature before use. The sample was Centrifuged after thawing before the assay. All samples were assayed in duplicate.

Three negative control wells were set, two positive control wells and one blank wall. 100µl sample diluents per well added, but not to blank well. Then 10µl of negative control, positive control and diluted sample per well was also added and then covered with the adhesive strip provided, and incubated for 45 minutes at 37°C. Each well was aspirated and washed, repeating the process four times for a total of five washes. Washed by filling each well with wash buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser or auto-washer and allowed to stand for 30 seconds, the liquid was completely removed at each step which aided good performance. After the last wash, the wash buffer remaining was removed by decantation. The plate was inverted and blotted against clean paper towels.

100µl of Horseradish peroxidase conjugate (HRP-conjugate) were added to each well (not to Blank!). Micro titer plate covered with the adhesive strip, Incubated for 30 minutes at 37°C. The aspiration process was repeated five times. 50µl each of substrate A and B was added to each well and incubated for 10 minutes at 37°C protected from light. 50µl of Stop Solution was added to each well and gently taped to the plate to make thorough mixing. Blank well taken as zero, determined the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

Calculation

For calculating the value of the human GAD65 antibody (Ig G), compare the sample well with the control.

(a) Negative control Optical Density (OD) values must not be more than 0.1. If one of the negative controls OD values is higher than 0.1, discard it. If two or more two negative control ODs values are higher than 0.1, repeat the test. If the average value of OD is negative **<** 0.05, calculate it as 0.05.

(b) Positive Control OD Values must not be less than 0.6. If one of the positive controls OD values is less than 0.6, discard it. If two positive control OD values are less than 0.6, repeat the test.

(c) A cutoff value was defined as the average Negative Control value plus 0.25. When OD of (sample) **<** Cutoff Value: "Negative" While OD (sample) ≥ Cutoff Value: "Positive".

2.13 Homeostasis Model Assessment

Homeostasis model assessment (HOMA) is derived from a mathematical assessment of the balance between hepatic glucose output and insulin secretion from fasting levels of glucose and insulin (Matthews et al., 1985). The HOMA model requires only a single measurement of insulin and glucose in the basal state.

HOMA of Insulin Resistance (HOMA-IR)

The HOMA of insulin resistance (HOMA-IR) index was regarded as a simple, inexpensive and reliable surrogate measure of insulin resistance (Matthews et al., 1985). Calculated as:

$$HOMA - IR = \frac{Fasting\ glucose\,(mmol/l) \times Fastinginsulin\,(\mu U/}{22.5}$$

The normal HOMA-IR values of healthy humans range from 0.5-1.4 less than 1.0 indicating insulin sensitivities and are optimal and above 1.9 indicate early insulin resistance. Above 2.9 indicates significant insulin resistance.

β-cell function (HOMA-B)

The β-cell function index was calculated as proposed by (Yamada et al., 2006). The product of 20 and fasting insulin levels divided by the value of fasting glucose concentrations minus 3.5 have been proposed to be a good measure of β-cell function.

$$\beta - \text{cell function (HOMA} - \text{B)} = \frac{20 \text{ x fasting insulin } (\mu U/ml)}{\text{fasting Glucose } (mmol/l) - 3.5}$$

2.14 Statistical Analysis

All data were reported as means ± Standard Deviation (SD). The values were analyzed using Statistical Package for Social Sciences (SPSS Version 20). Statistical significance of the difference between means was carried out using one-way analysis of variance (ANOVA) and unpaired t-test ($P<0.05$) were considered statistically significant as shown in Table 1.

Table 1: Prevalence of KPDM with gender of participants

	Male	Female	Total
Participants	41(59.00%)	29(41.00%)	70(100.00%)
KPDM	6(14.60%)	6(20.70)	12(17.14%)

Results are express count, N=Number participants, KPDM=ketosis-prone diabetes mellitus

3.0 Results

A population of 70 participants with 41 (59%) males and 29 (41%) females employed. The influence of demographic characteristics and biochemical parameters was analyzed with continuous variables compared using one-way analysis of variance (one-way ANOVA), unpaired t-test and presented as mean ± standard deviation (SD) while categorical data are presented in percentages and counts. The results are presented in Table 1 – 7 and Figure 1 as follow.

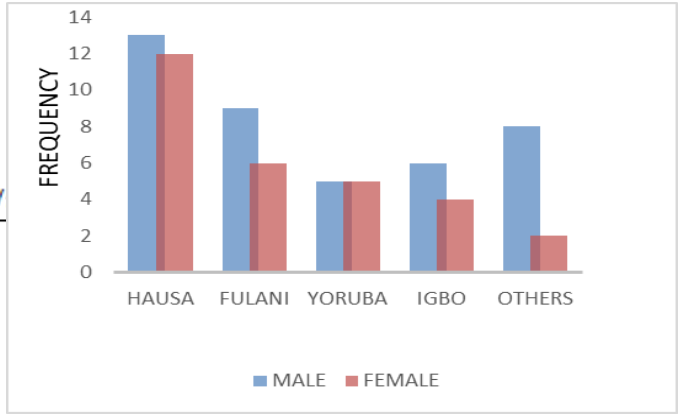

Figure 1: Distribution of participants on Ethnic groups and their frequencies in gender.

Table 2: Prevalence of KPDM participants based on Ethnic groups.

Ethnicity	Participants (N=70)	KPDM (n=12)	Participants	% Prevalence of KPDM Participants
Hausa	25	6		50.00
Fulani	15	1		8.30
Yoruba	10	3		25.00
Igbo	10	1		8.30
Others	10	1		8.30

Results are expressed as counts, prevalence as percentage, N=Total number of participants, n=total number of KPDM participant, KPDM=ketosis-prone diabetes mellitus, % prevalence is obtained by dividing each count in tribe with KPDM by total number of KPDM.

Table 3: Gender influence on demography of the study population

Demography	Study population (N=70)	Male(N=41)	Female (N=29)
Age (Years)	49.6 ± 11.95	53.46 ± 10.56*	44.77 ± 12.00
Duration of diabetes (Years)	5.19 ± 5.19	5.1 ± 5.30	5.20 ± 5.14
Family history of diabetes:			
History	(48.57%)	(51.20%)	(48.28%)
No history	(51.43%)	(48.80%)	(51.72%)
Smoking habit:			
Smoked	(7.15%)	(21.20%)	(0%)
Not Smoked	(92.85%)	(78.80%)	(100%)
Location of residence:			
Rural	(12.85%)	(14.63%)	(10.35%)
Sub-Urban	(12.86%)	(17.08%)	(6.90%)
Urban	(74.29)	(68.29%)	(82.75%)
Body mass index (BMI)(Kg/M^2)	26.05 ± 5.84	25.22 ± 5.49	27.09 ± 6.18
GAD (+/-)	70 (5/65)	41 (1/40)	29 (4/25)

Results are presented as Mean ± Standard deviation value with superscript* is statistically significant p<0.05 using unpaired t-test. GAD=Glutamic Acid Decarboxylase, N=Number of Participants, **(+/-) =**Number of GAD positive subjects/ Number of GAD negative subjects

Table 4: Gender influence on biochemical parameters of the study population

Biochemical parameter	Study population (N=70)	Male (N=41)	Female(N=29)
FPG (mmol/L)	7.78 ± 4.00	6.47 ± 2.42	9.44 ± 4.49*
PK (mmol/L)	0.35 ± 0.28	0.31 ± 0.21	0.40 ± 0.35
HbA1c (%)	7.58 ± 3.00	6.96 ± 2.14	8.37 ± 3.71
Insulin (µIU/ml)	12.08±6.27	12.17 ± 6.08	11.97 ± 6.59
HOMA-IR	4.51 ± 3.91	3.59 ± 2.95	5.67 ± 4.65*
HOMA- β	5.30 ± 5.18	4.07 ± 3.73	6.85 ± 6.29*

Results are presented as Mean ± Standard deviation value with superscript* is statistically significant p<0.05 using unpaired t-test. FPG=Fasting Plasma Glucose, PK= Plasma Ketones HbA1C=Glycated hemoglobin, N=number of populations

HOMA-IR=homeostasis model of assessment for insulin resistance, HOMA-**β**= homeostasis model of assessment of β-cell function

Table 5 Comparison of demography of KPDM between ethnic groups

	KPDM (N=12)	**Hausa (N=19)**	**Fulani (N=14)**	**Yoruba (N=7)**	**Igbo (N=9)**	**Others (N=9)**
Gender (%):						
Male	(50%)	(52.63%)	(57.14%)	(42.86%)	(66.67%)	(88.89%)
Female	(50%)	(47.37%)	(42.86%)	(57.14%)	(33.33%)	(11.11%)
Age (Years):	45.67 ± 11.43	48.11 ± 13.77	47.64 ± 9.22	54.43 ± 9.22	51.78 ± 12.00	66.22 ± 8.69
Duration of diabetes (Years)	7.33 ± 6.54	3.89 ± 4.20	4.00 ± 3.76	5.14 ± 4.53	7.22 ± 5.61	4.89 ± 6.79
Family history of diabetes (%):	(66.67%)	(57.89%)	(35.71%)	(57.14%)	(44.44%)	(66.67%)
History	(33.33%)	(42.11%)	(64.29%)	(42.86%)	(55.56%)	(33.33%)
No history						
Smoking habit (%):						
Smoked	(0.00%)	(10.53%)	(14.29%)	(0.00%)	(0.00%)	(11.11%)
Not smoked	(100.00%)	(89.47%)	(85.71%)	(100.00%)	(100.00%)	(88.89%)
Location of Residence (%):						
Rural	(8.33%)	(15.79%)	(21.43%)	(0.00%)	(0.00%)	(0.00%)
Sub-Urban	(25.00%)	(5.26%)	(14.29%)	(28.57%)	(11.11%)	(22.22%)
Urban	(66.67%)	(78.85%)	(64.29%)	(71.43%)	(88.89%)	(77.78%)
Body Mass Index (BMI)	27.84 ± 6.03	25.74 ± 5.47	23.72 ± 6.65	26.52 ± 5.86	25.08 ± 6.80	28.51 ± 3.05
(Kg/M^2)	12 (0/12)	19 (3/16)	14 (1/13)	7 (0/7)	9 (0/9)	9 (0/9)
GAD (+/-)						

Results are presented as Mean ± Standard deviation BMI=body mass index, KPDM=ketosis-prone diabetes mellitus, N=number of populations.

Table 6: Comparisons of PK, FPG, HbA1c of KPDM between ethnic groups

	KPDM (N=12)	**Hausa (N=19)**	**Fulani (N=14)**	**Yoruba (N=7)**	**Igbo (N=9)**	**Others (N=9)**
PK (mmol/L)	0.82 ± 0.30*	0.25 ± 0.17	0.25 ± 0.16	0.29 ± 0.12	0.17 ± 0.10	0.30 ± 0.18
FPG (mmol/L)	13.89 ± 4.65*	8.02 ± 3.12	6.05 ± 1.90	5.67 ± 1.62	5.78 ± 1.29	5.53 ± 0.85
HbA1C (%)	9.04 ± 5.49	7.18 ± 2.16	7.29 ± 1.89	6.88 ± 1.30	8.90 ± 3.05	6.21 ± 1.05

Results are presented as Mean ± Standard deviation, value with superscript* is statistically significant $p<0.05$ using one-way Anova and Tukey multiple comparison. PK=plasma ketones, FPG=Fasting plasma glucose, HbA1c=Glycated hemoglobin, N=Number of populations.

Table 7: Comparison of Insulin, HOMA-IR and HOMA-β of KPDM group between the ethnic groups

	KPDM (N=12)	**Hausa (N=19)**	**Fulani (N=14)**	**Yoruba (N=7)**	**Igbo (N=9)**	**Others (N=9)**
Insulin (µIU/ml)	15.13 ± 7.85	12.82 ± 6.54	11.53 ± 4.44	9.97 ± 5.63	10.81 ±6.50	10.24 ± 5.85
HOMA-IR	9.29 ± 5.05*	5.16 ± 4.04	2.95 ± 1.18	2.44 ± 1.25	2.79 ± 1.78	2.54 ± 1.44
HOMA-β	11.49 ± 7.23*	6.01 ± 5.27	2.97 ± 1.22	2.62 ± 1.10	3.40 ± 2.66	3.20 ± 1.76

Results are presented as Mean ± Standard deviation value with superscript* is statistically significant p<0.05 using one-way Anova and tukey multiple comparison. HOMA-IR=homeostasis model of assessment for insulin resistance, HOMA-**β**= homeostasis model of assessment of β-cell function, KPDM=ketosis-prone diabetes mellitus, N=number of populations.

4.0 Discussions

This research intended to estimate the prevalence of ketosis-prone diabetes mellitus in a specialist hospital, in Sokoto metropolis, Sokoto State, Nigeria. The hospital is a tertiary health care centre with patients mostly Hausa, Fulani and a few other ethnic groups like Yoruba, Igbo and other minor ethnic groups. A total of 70 confirmed diabetes participants were consented and randomly recruited for this study. Aβ classification system approach (Maldonado et al., 2003) was applied in identifying ketosis-prone diabetes mellitus (KPDM) participants, which is based on whether markers autoimmune of type 1 diabetes are present or absent and or beta-cell cell function. All KPDM participants in this study showed negative GAD autoantibody in **Table 1** and remarkably preserved Beta-cell function as indicated in **Table 2**. Ketosis-prone diabetes was found in **17.14%** of the participants and it's consistently higher when compared with **5.7%** in Benin, **7.0%** in Abidjan among patients with type 2 diabetes and **28.3%** in people admitted with a hyperglycemic crisis in Cameroon (Lontchi-Yimagou et al., 2017). Hausa ethnic group have the highest prevalence of KPDM 50% (6:12) followed by Yoruba with 25% (3:12), Fulani, Igbo and others have 8.3% (1:12) each (**Table 2**).

The demographic data of interest such as gender, age, years with diabetes, ethnicity, area of residence and smoking habit of the participants population was taken and recorded with the consent of the research volunteers. The duration of diabetes recorded as 5 years which is in line with previous findings by (Liu et al., (2013) and Sabir et al., (2017).

Ketosis-prone diabetes group was compared within the majority ethnic groups (i.e., Hausa, Fulani, Yoruba, Igbo and others comprising some minor ethnicities) among patients attending the facility. **Figure 1** shows the distribution of the participants in the study population based on gender with Hausa 35.70% tribe having the highest frequency followed by Fulani 21.40%, Yoruba 14.30%, Igbo 14.3% and Other Tribes 14.30%. The mean age of all participants was recorded as 49.6 ± 11.95 Yrs. There was a significant difference statistically between gender with the male having higher mean age (53.46 ± 10.56 Yrs.) than females (44.77 ± 12.00 Yrs.) as indicated (T**able 3**). The mean age of KPDM and the five (5) groups was compared, and no statistically significant difference in age was observed among the groups as shown in **Table 1** at p>0.05. The mean duration of diabetes among participants is 5.19 ± 5.19 (range 1≤x≤20 Yrs.) for all participants, 5.10 ± 5.30 (range 1≤x≤20 Yrs.) for men and 5.29 ± 5.19 (range 1≤x≤17) Yrs. (p=0.89). No significant difference in the duration of diabetes between the KPDM group when compared with the groups as depicted in T**able 1**.

Place of residence was also found to have a significant impact on the number of diabetes patients in the population. Urban dwellers are usually working-class individuals in the population. They don't normally engage in physical activities like farming, walking the street, or doing exercises, unlike rural settlers. They normally walk on bikes, cars, buses, trains, and planes. Physical inactivity and sedentary lifestyles contribute to ailments such as obesity, insulin resistance, heart diseases, prediabetes and so on. A score of 72.29% of the study population lived in the urban area with 12.68% in both suburban and rural settlers. In males, 68.29%, 17.08% and 14.63% are found in urban, suburban and rural areas respectively. The females also have 82.76% in urban, 6.90% in sub-urban and 10.35% in rural areas which is consistent with results of 80.38% in urban, 19.62% in rural, 82.56% in urban 17.44% in rural and 75% in urban and 25% in rural areas.

The smoking habits of all participants were recorded as 92.85% do not smoke, 12.19% of the male participants smoked and females record no smoking habit. Though no smoking habit among KPDM, Yoruba and Igbo groups Hausa, Fulani and Others have a smoking record of 10.53%, 14.29% and 11.11% respectively among their groups.

The anthropometric data of interest (weight and height) of the participants were measured and recorded. KPDM patients are Overweight, and obesity is usually associated with type 2 and ketosis-prone diabetes. The body mass index of participants was 26.05 ± 5.84 kg/m^2 with mean values of 25.22 ± 5.49 kg/m^2 in males and 27.09 ± 6.18 kg/m^2 in females, this is consistence with 27.7 ± 13.4 reported previously (Yimagou *et al*., 2016). Though there is no significant difference in the BMI of the participants between males and females with the total population of participants, the mean BMI showed that participants are over-weighted or obese ie BMI ≥ 25 Kg/M^2 (Sabir et al., 2017) and inferentially resulted in insulin resistance while the patients have a preserved beta-cell function. The BMI of the KPDM group in comparison with the groups has no statistically significant difference as indicated in **Table 1**.

Biochemical parameters in the diagnosis of diabetes such as fasting plasma glucose (FPG), glycated haemoglobin (HbA1c) and plasma ketones PK) were also measured. The fasting plasma glucose of the participants is 7.78 ± 4.00 mmol/l with 6.47 ±

2.42 mmol/l and 9.44 ± 4.49 mmol/l for males and females respectively. The FBG of females is statistically higher than that of the males among the participants (**Table 4)**. The results was found to be contrary to the study conducted by (Vellanki & Umpierrez, 2017) where they reported high prevalence of KPDM in males. KPDM was in 1984 recognized as a clinical entity among African America people and is now identified to be prevalent in sub-Saharan African population (H. E. Lebovitz & M. A. J. C. d. r. Banerji, 2018). Therefore, it is obvious that the KPDM group has higher fasting plasma glucose values as reported in the literature when compared with type 2 diabetes subjects. In this study, we also found a significantly higher ($p<0.05$) HOMA – IR and HOMA – β values in females when compare with men (**Table** 4) among the ethnic. (Ülgen et al., 2010) reported higher HOMA – IR values for male than females.

Hyperglycemia leads to the glycosylation of many amino groups in different body proteins including the RBCs. Generation of the glycated end product in the form of HbA1c gives us a clue of the long-term status of blood glucose level and HbA1c ≥6.5% is considered for diabetes (Lekkala et al., 2023). Glycated haemoglobin (HbA1c) levels of all the participants are 7.58% ± 3.00%. The male participants have HbA1c of 6.96% ± 2.14% and the female recorded 8.37% ± 3.71%. Glycated haemoglobin of the KPDM group and ethnic groups are compared, and no significant difference was statistically observed at $p<0.05$ (**Table 4**). This finding is consistent with the results of (Lontchi-Yimagou et al., 2017) for non-ketotic phase ketosis-prone diabetes.

Ketosis-prone diabetes is marked by the elevation of plasma ketones as in type 1 diabetes though there is no evidence of autoimmunity. Plasma ketones levels are considered normal at less than <0.6 mmol/L value. The average or mean plasma ketones of all participants is 0.35 ± 0.21 mmol/L, male participants recorded 0.31 ± 0.21 mmol/L and female participants recorded 0.40 ± 0.31 mmol/L. There was no significant difference in gender, male and female in our study population. KPDM patients have elevated plasma ketones as indicated (**Table 5)** and significance at $p<0.05$ in comparison with diabetes type 2 in the study groups under different ethnicities.

The insulin level and marker of autoimmunity (GAD65) of the participants were immunologically assayed using ELISA techniques. 7.2% of all participants are positive for the marker of autoimmunity (GAD 65) but all KPDM participants are GAD65 negative. Insulin secretion and insulin action are inversely related. Thus, in ketosis-prone diabetes and type 2 diabetes, there is an appreciably normal secretion of insulin as evident in previous studies (Lontchi-Yimagou et al., 2017). In this study, the insulin level of the total study participants is within the normal range (0.6-25 mIU/ml for diabetes) and no statically significant difference between the mean insulin values of participants on their gender. The insulin levels in KPDM patients (15.13 ± 7.81) are more than Hausa (12.82 ± 6.54), Fulani (11.53 ± 4.44), Yoruba (9.97 ± 5.63), Igbo (10.81 ± 6.50) and Other Tribes (10.24 ± 5.85) respectively (**Table 6&7**). This rise in insulin level was also reported in patients with non-ketotic phase ketosis-prone diabetes by (Lontchi-Yimagou et al., 2017) and could be a source of hint to the marked insulin resistance and or beta-cell function in KPDM patients.

Insulin resistance is a state in which the available insulin in the blood did not provide effective glycemic control and prevention of ketosis (Yuan et al., 2020). Insulin resistance index (HOMA-IR) is a characteristic of both type 2 and ketosis-prone diabetes. The insulin resistance index of the participant was assessed (homeostasis model; HOMA-IR <2.5) as 4.51 ± 3.91 for all participants, 3.59 ± 2.95 and 5.67 ± 4.65 in male and female participants respectively (**Table 7**). Insulin resistance indexes of participants have a significant difference at $p<0.05$ in gender among the population. The KPDM group also showed a significant difference in insulin resistance index when compared to other ethnic groups as depicted in **Table 5.** Insulin resistance was detected in patients with ketosis-prone diabetes as reported by (Mauvais-Jarvis, Smith, et al., 2004).

Beta cell function is preserved in our study participants at 5.30 ± 5.18, the male has 4.07 ± 3.73 and the female also has 6.83 ± 6.29 and there is a significant difference between the beta cell function of males and females with the total population of the participants. The results also showed a significant difference between the KPDM group and ethnic groups at $p<0.05$ level. This study showed that there was enough secretion of insulin by the beta cells, but insulin resistance and over-weight observed are characteristics of mild ketosis seen in ketosis-prone patients. In the studies conducted on ketosis-prone diabetes at the ketoacidosis phase, there was not enough insulin secretion but it returns to normal after the normoglycemic treatment (H. E. Lebovitz & M. A. Banerji, 2018)

5.0 Conclusions

In conclusion, it was estimated that the prevalence of ketosis-prone diabetes among patients in specialist hospital Sokoto with type II diabetes mellitus was 17.14%. The frequency of the disease is more in Hausa ethnic group with an estimated prevalence of 50 % (6:12), followed by Yoruba 25% (3:12), than 8.3% (1:12) each for Fulani, Igbo and other tribes. KPDM appears to be a more complicated disorder than type 2 diabetes mellitus, as evidenced by the increased plasma ketones level, glycated haemoglobin, fasting plasma glucose, insulin resistance and beta cell function. The existence of KPDM was established

among the diabetes patients of the hospital. Thus, early diagnosis of the disease is necessary to increase the chances of living of the affected subjects.

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Declaration of conflict of interest

The authors declare that there is no conflict of interest.

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