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Association of Undiagnosed Pre-Diabetes and Type-2 Diabetes Mellitus with Interleukin-2 mRNA Expression Among Adults in Bayelsa State, Nigeria

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ABSTRACT

Many cases of hyperglycemia are undiagnosed in Nigeria due to poverty and inadequate access to health care. Although hyperglycemia and type 2 diabetes mellitus (T2DM) are inflammatory conditions, however, the role of inflammatory mediators such as interleukin-2 in hyperglycemia and T2DM is not fully understood. The aim of the present study was to evaluate the prevalence and association of undiagnosed pre-diabetes and T2DM with IL-2 mRNA level among adults in Bayelsa State, Nigeria. A cross sectional design was employed to randomly select 130 adults and they were grouped into three: normal control, prediabetes, and T2DM. Fasting blood glucose concentration was measured using Accu-answer glucometer, while mRNA level of IL-2 was quantified using real time PCR method. The prevalence of undiagnosed pre-diabetes was 43.07% (males, 19.23%; females, 23.85%) while undiagnosed T2DM was 16.15% (males, 6.92%; females 9.23%). The expression of IL-2 mRNA was significantly upregulated in both pre-diabetes and T2DM subjects compared with normal control ($p < 0.05$), but was significantly higher among T2DM subjects. Age ≥ 50 years, physical inactivity and marriage was significantly associated with hyperglycemia and T2DM ($p < 0.05$). The present study found a high prevalence of undiagnosed pre-diabetes and T2DM among adults in Bayelsa State, Nigeria. Association of prediabetes and T2DM with IL-2 mRNA expression was found. IL-2 mRNA level might be a useful biomarker for monitoring diabetes mellitus progression. Directing diabetes survey and interventions to rural communities is highly recommended.

Keywords: [Undiagnosed Diabetes, pre-diabetes, IL-2, rural communities, Bayelsa State, Nigeria]

INTRODUCTION

Type 2 diabetes mellitus characterized by hyperglycemia, results from the body's ineffective use of insulin. Over 95% of people with diabetes mellitus have type 2 diabetes [1]. The global prevalence of type 2 diabetes mellitus (T2DM) in adults was 536.6 million people (10.5%) in 2021 [2]. The disease may be diagnosed several years after onset and after complications have already arisen [1]. Although several clinical medications and plant treatment have been recommended for the treatment of diabetes mellitus [3 - 4], yet, between 2000 and 2016, there was a 5% increase in premature mortality rates due to diabetes mellitus. The premature mortality rate due to diabetes decreased from 2000 to 2010 in high in-come countries but then increased from 2010 – 2016 in lower middle in-come countries [5].

Over one in two (54%) people living with diabetes in Africa are undiagnosed and 416 thousand deaths were due to diabetes in 2021, while one in eight live births are affected by hyperglycaemia during pregnancy [6]. When hyperglycemia is left untreated, it can lead to many serious life-threatening complications that include

damage to the eye, kidneys, nerves, heart and peripheral vascular system [1, 4, 7]. Factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization and increased glucose production [1, 7].

Interleukin (IL)-2 is a pleiotropic cytokine that is secreted by T cells during an immune response [8]. Interleukin-2 therapy of autoimmunity in diabetes at low dose has been reported in a previous study [9]. A study carried out by Dilara *et al.*, [10], showed that 13 – 20% of patients with metastatic renal cell carcinoma and metastatic melanoma receiving high-dose IL-2 treatment showed objective clinical responses, some enduring for up to 20 years and more.

Many cases of diabetes are undiagnosed in Nigeria due to poverty and inadequate access to health care [11]. Given the rising trend in diabetes [1], routine survey of people with diabetes as well as screening for asymptomatic hyperglycemic adults (pre-diabetes) may allow earlier detection, diagnosis, treatment and improvement in health outcomes [12]. Sparse data exist on diabetes related community based survey in Nigeria. In the quest for the cure for diabetes mellitus, research on relevant molecular biomarkers that could be the ideal candidate for drug target is actively ongoing [13]. Thus the aim of the present study was to determine the association of with IL-2 mRNA expression level among adults in Bayelsa State, Nigeria as well as the community based prevalence of undiagnosed pre-diabetes and type 2 diabetes mellitus among adults in Bayelsa State, Nigeria.

MATERIALS & METHODS

Study location

The study location was Bayelsa State, Nigeria. The State is located on latitude 5.152239 and longitude 6.192479. Bayelsa is a state in the southern part of Nigeria, located in the heart of the Niger Delta region in the southern part of Nigeria. It was created in the year 1996 on the 1st day of October. Bayelsa State is largely a riverine settlement with fishing and farming as the major occupation of the indigenes. The state is made up of eight Local Government Areas (LGA) with over four different languages spoken amongst the indigenes. However, the major language is Ijaw [14]. Two LGAs in Bayelsa State were randomly selected for the study, they were Sagbama and Yenagoa LGAs. Sagbama LGA is situated on the left bank of Forcados river, while Yenagoa is the capital city of Bayelsa State [15-16].

Study design and population

A descriptive cross sectional study design was employed to randomly select the study population and they were divided into three groups. Group 1: subjects with normal fasting blood glucose concentration (between 70 mg/dL (3.9 mmol/L) and 100 mg/dL (5.6 mmol/L). Group 2: pre-diabetes subjects with fasting blood glucose concentration between 100 to 125 mg/dL (5.6 to 6.9 mmol/L). Group 3: diabetes mellitus subjects with fasting blood glucose concentration greater than or equal to 126 mg/dL (7 mmol/L) on two separate tests [17]. A structured questionnaire was used to obtain socio-demographic variables [18] and sample size was determined using the formular below [19].

$$n_0 = \frac{Z^2 pq}{e^2}$$

Where n_0 is the sample size; e is the desired level of precision (i.e. the margin of error), 0.05; *P* is the expected prevalence or proportion, which was estimated from a previous study as 0.088 [20]; *q* is 1 – *P* which is 0.912; Z-value was found in a Z table, Z-value at 95% confidence interval was 1.96.

$n_0 = ((1.96)^2 (0.088) (0.9122)) / (0.05)^2 = 3.84 \times 0.0803 / 0.0025 = 0.3083 / 0.0025 = 123.30$

Assuming a non-response rate of 6%, the sample size was made up to 130.

Ethical approval and voluntary informed consent

Ethical approval for the study was obtained from the Bayelsa State Primary Health Care Ethics Committees in each selected LGA and voluntary informed consent was obtained from each participant.

Determination of blood glucose concentration

Blood glucose concentration estimation was carried out using the manufacturer`s protocol for Accu-answer blood glucose monitoring system (ZH-G01, Guilin Zhonghui Technology Co., Ltd, China).

Extraction of RNA from human peripheral blood mononuclear cells (PBMC)

RNA was extracted from PBMC following the manufacturer`s protocol for total RNA mini kit.

Complementary DNA (cDNA) synthesis

The extracted RNA was reverse transcribed into complementary DNA (cDNA) following the manufacturer`s protocol for FireScript Reverse Transcriptase cDNA synthesis kit (Solis BioDyne, Tartu Estonia).

IL-2 gene expression determination

Custom designed primers synthesized by Genewiz (Genewiz, South Plainfield, New Jersey) was used to amplify the target sequence GAPDH forward and reverse primer sequences were: 5`GTCTCCTCTGACTTCAA-3` and 5`ACCACCCTGTTGCTGTA-3`, while IL-2 forward and reverse primer sequences were: 5`AAGAATCCCAAACTAACCAGGAT3` and 5`TCTAGAC ATGAAGATGTTTCAGTTCTC3` respectively. The reaction mixture contained 4 µl of PCR supermix, 1 µl primer mix, 4 µl of cDNA template, and 11 µl of water. Real time PCR cycling conditions were: initial activation at 95^0C for 12 mins, followed by 40 cycles of denaturation at 95^0C for 15 s, annealing at 53^0C for 30 s and elongation at 72^0C for 30s.

STATISTICAL ANALYSIS

SPSS version 24 was used for data analysis. Descriptive parameters were presented as percentages. Chi square test was used to test for significant association between categorical variables. $2^{-\Delta\Delta Ct}$ method was used to quantify IL-2 gene expression [21]. T-test was used to test for significant difference between means and significant level was set at ($p < 0.05$).

RESULT

Prevalence of undiagnosed pre-diabetes and diabetes in relation to sociodemographic variables

Table 1 shows the prevalence of undiagnosed pre-diabetes and diabetes in relation to sociodemographic variables. The prevalence of undiagnosed pre-diabetes and diabetes were 43.07% and 16.15% respectively. Association of age, marriage life and physical inactivity with pre-diabetes and diabetes was found ($p < 0.05$).

Table 1 Prevalence of undiagnosed pre-diabetes and diabetes in relation to sociodemographic variables

Fasting blood glucose concentration (mg/dl)					
	Undiagnosed				
	Normal	**Pre-diabetes**	**T2DM**		
	≤ 99	**100 – 125**	**≥ 126**		**X^2 *p* value (< 0.05)**
Variable	**N (%)**	**N (%)**	**N (%)**	**Normal vs PD**	**Normal vs T2DM**
Age (years)					
30-49	**44(33.8)**	**19(14.6)**	**5(3.8)**	< 0.001	0.001
≥50	**9(6.9)**	**37(28.5)**	**16(12.3)**		
Gender					
Male	**20(15.4)**	**25(19.2)**	**9(6.92)**	0.464	0.684
Female	**33(25.4)**	**31(23.8)**	**12(9.23)**		
Marital Status					
Single	5 (3.8)	16 (12.3)	2 (1.5)	0.011	0.011
Married	48 (36.9)	40 (30.8)	19 (14.5)		
Occupation					
Farming / Fishing	22 (16.9)	22 (16.9)	10 (7.7)	0.715	0.866
SMEs	21 (16.2)	26 (20.0)	7 (5.4)		
Professional job	10 (7.7)	8 (6.2)	4 (3.1)		
Physical activity					
Regular	45 (34.6)	10 (7.7)	3 (2.3)	0.001	0.001
Not regular	8 (6.2)	46 (35.4)	18 (13.8)		

SME: small and medium enterprises; PD: pre-diabetes; T2DM: type 2 diabetes mellitus.

Association of undiagnosed pre-diabetes and diabetes with interleukin-2 gene expression among adults in Bayelsa State, Nigeria

Result for the characterization of interleukin-2 mRNA expression level among adults with undiagnosed pre-diabetes and diabetes in Bayelsa State is shown in Figure 1. Association of IL-2 with undiagnosed pre-diabetes and type 2 diabetes mellitus was found ($p < 0.05$).

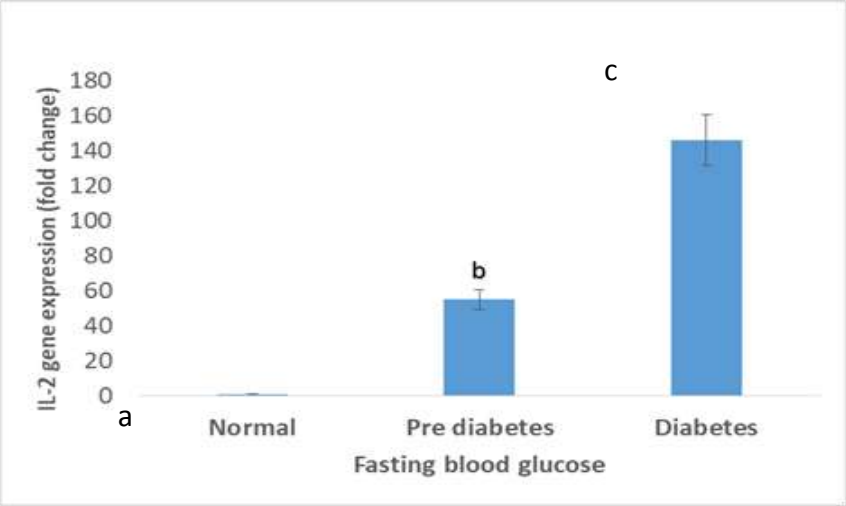

Figure 1 Association of interleukin-2 mRNA expression with pre-diabetes and type 2 diabetes mellitus.

Result is presented as mean fold change ± standard error of mean (SEM), Bars with different superscript are significantly different, $p < 0.05$.

DISCUSSION

Diabetes mellitus type-2 is an age long disease that has attracted the attention of several health organizations like the World Health Organization (WHO, 2022). However, limited community-based survey exists to unravel the magnitude of diabetes mellitus type 2 in Nigeria. Most reported studies on type 2 diabetes mellitus are hospital based. However, hospital based study has a major limitation of capturing only patients that present at the hospital and as such are not representatives of real time glycemia and diabetes distribution within a community [22-24]. The present community-based study however, has helped to fill this research gap.

Using the glucose oxidase on spot analysis method, a high prevalence of undiagnosed pre-diabetes and type 2 diabetes mellitus was found in the present study and was significantly higher among the elderly age group compared with the younger age group. Previous studies carried out by Mordarska *et al*. [25] and Roh *et al*. [26] also found a significantly higher prevalence of diabetes mellitus type-2 among the elderly age group. This suggests the need for active screening of type 2 diabetes mellitus among the elderly.

In addition, the present study found a higher prevalence of diabetes mellitus among females compared with males. On the contrary, a previous study carried out by Tomas *et al*.,\ [27] found that the prevalence of diabetes was higher among males compared with females. However, in agreement with the present study, another study carried out by Nordström *et al*. [28] found a higher prevalence of diabetes among females compared with males (14.1%). This shows that sex distribution of diabetes varies from one location to another.

Furthermore, a higher prevalence of diabetes mellitus type 2 was found among married participants in the present study. This is in disagreement with findings of previous studies where marriage was found to protect against type 2 diabetes mellitus [29]. Further study is needed to fully unravel the relationship between marital status and type 2 diabetes mellitus. Physical inactivity was associated with pre-diabetes and diabetes in the present study. Physical inactivity has also

been reported as a risk factor for diabetes mellitus in previous studies [30], [31]. The effectiveness of regular exercise therapy in improving blood glucose control was reemphasized in the present study. Further research on the most appropriate exercise for type 2 diabetes mellitus subjects is suggested.

Diabetes has long been recognized as an inflammatory disease. A previous study showed that components of the immune system are altered in type two diabetes [32]. IL-2 produced by $CD4^+$ T lymphocytes has been hypothesized to be linked to diabetes mellitus, but a verification of this claim is limited in literature. The present study has helped to fill this research gap. The present study revealed that the expression of IL-2 mRNA was significantly ($p < 0.05$) upregulated in type 2 diabetes mellitus and pre-diabetes groups compared to the normal group ($p< 0.05$). This suggests that IL-2 gene expression plays a pivotal role in the molecular mechanism that leads to the etiology of diabetes mellitus type 2 [33].

CONCLUSION

The present research clearly showed that the level of IL-2 mRNA gene expression was statistically higher among undiagnosed pre-diabetes and type 2 diabetes mellitus group than in the normal group. This suggests that IL-2 plays a major role in the molecular mechanism that leads to the progression of type 2 diabetes mellitus. Therefore, an upregulation of IL-2 gene expression can be a marker of an increased risk for type 2 diabetes mellitus. Interventions to reduce the high prevalence of undiagnosed type 2 diabetes mellitus in Bayelsa State is implicated in the present study.

Declaration by Authors

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