Original Article

Xanthine oxidase activity in type 2 diabetic Nigerians

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ABSTRACT

Aim: This study evaluated the activity of xanthine oxidase in Nigerians with type 2 diabetic mellitus as well as its relationship with lipid peroxidation, inflammatory bio markers and glycemic control indices.
Methods: Two hundred and thirty seven (237) subjects, comprising of one hundred and fifty seven (157) DM subjects and eighty (80) aged matched controls participated in this study. Blood samples were collected from the participants for the estimations of xanthine oxidase activity, uric acid, malon dia-dehyde (MDA), erythrocyte sedimentation rate (ESR), high sensitive c − reactive protein (hs CRP), glucose, fructosamine and glycosylated hemoglobin by standard methods.
Results: The results of this study showed a significantly increased activity of xanthine oxidase in DM $(0.044 \pm 0.05 \mu/mg)$ compared with apparently healthy controls $(0.028 \pm 0.00 \ \mu/mg)$. The mean plasma levels of MDA $(42.40 \pm 2.50 \mu mol/l)$ and uric acid $(7.22 \pm 0.20 \ mg/dl)$ in DM were significantly higher $(p \leq 0.05)$ than healthy non DM group. The mean levels of hs CRP in DM $(4.09 \pm 0.91 \mu g/ml)$ was significantly higher than controls $(1.30 \pm 0.50 \mu g/ml, p = 0.009)$. While no association of xanthine oxidase was observed with glycemic control indices and hs CRP, a negative association of xanthine oxidase was observed with MDA $(r = -0.514, p = 0.000)$.
Conclusion: Increased activity of xanthine oxidase in DM was associated with increased lipid peroxidation and could be a salient entity towards the onset on complications.

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1. Introduction

Diabetes mellitus (DM) is a syndrome characterized by severe morbidity and mortality. Proper clinical management is essential to prevent the occurrence of severe vascular complications. The mechanisms of diabetic complications are poorly understood, though may be multifactorial. Amongst several mechanisms suggested, the concept oxidative stress has received so much attention [1]. It is unclear if oxidants trigger the disease or they are produced as a consequence of the disease and cause disease symptoms. Hypothetically, it would be expected that free radicals generated as a result of hyperglycemia in DM will lead to an overwhelming burden on the antioxidants. Researchers have reported the major sources of these free radicals to include the electron transport chain and the reactions catalyzed by few oxidases, such as xanthine oxidase [2]. Xanthine oxidase (XO) is an enzyme which converts hypoxanthine/xanthine to uric acid in a reaction which liberates superoxide. The role of increased uric acid levels or hyperuricemia in diabetes mellitus has been a subject of much debate as some studies report it to be a resultant effect of diabetes mellitus while others have reported it to be a risk factor for the development of type 2 diabetes mellitus (T2DM) [3,4]. In contrast, high activity of xanthine oxidase has been observed in many pathological conditions; which include, myocardial infarction, hepatic disease and asthma [5,6]. Although, increased xanthine oxidase activity in DM has been reported in literature, its role in vascular complications and associated risk factors have not been extensively studied in sub Saharan Africa. Few studies conducted elsewhere have provided link between xanthine oxidase and diabetic cataract [7], as well as metabolic syndrome and its cardiovascular complications [8]. Furthermore, a possible role of xanthine oxidase in peripheral neuropathy was

proposed in experimental model [9] and this was attested to in a recent study by Minic et al., which documented a causal role in the development of peripheral neuropathy in DM [10].

In view of the fact that peroxynitrite generated from xanthine oxidase derived superoxide leads to oxidative and nitrosative injuries to proteins, lipids and DNA; an event that occurs within vicinity of xanthine oxidase in vascular compartment, there is a high tendency for endothelial dysfunction. This may be attributed to the consequential effect of high xanthine oxidase activity, serving as a depot of free radicals. On this premise, assessment of xanthine oxidase activity could presumably serve as a useful marker of early complications in DM as well as a possible therapeutic target.

It is also worthy of note that inflammation is inevitably linked with free radical generation as a result of hyperglycemia. The synergy created could mark the herald of onset of complications. Meticulous and attainment of good glycemic control has been advocated and proven to reduce cardiovascular events by the Diabetes Control and Complications Trial Research Group (DCCT). Many important biochemical mechanisms are activated in the presence of high glucose, which occur in diabetes. It is widely accepted that hyperglycemic conditions induced an enhancement of oxidative stress in a variety of tissues and cells [11,12,13]. It is instructive to note however that the free radicals generated as a result of hyperglycemia in DM are extremely reactive and consequently short lived, therefore, their activity is usually assessed by indirect methods. Xanthine oxidase activity will enable an indirect assessment of free radical load on diabetic subjects due to hyperglycemia. This study will in addition evaluate the activity of xanthine oxidase in type 2 diabetic Nigerians, with a view of exploring a possible association, if any with glycemic control indices (using fasting plasma glucose, fructosamine and glycosylated hemoglobin as indices of short, medium and long term assessment), lipid peroxidation and inflammatory biomarkers.

2. Materials and methods

This was a cross sectional analytical study conducted between February–September 2016 at the Diabetes Unit of Lagos University Teaching Hospital, a tertiary hospital in Lagos, Nigeria. Informed written consent was obtained from the study subjects and ethical approval was given by the Research and Ethics Committee of College of Medicine, of the University of Lagos (CMUL/HREC/04/17/119).Consenting patients were recruited consecutively over a 3-month period. The inclusion criteria include DM subjects between the ages of 40–75 years, and those on oral hypoglycaemic agents. Those excluded include subjects on immune suppressive drugs, those with malignancies, gouty arthritis, heart failure, renal failure and those on uric acid lowering drugs. 5.0 ml of fasting venous blood samples were collected aseptically from each subject in a sitting position after an overnight fast (10–14 h) into fluoride oxalate, lithium heparin, and ethylene diamine tetra acetic acid (EDTA) bottles for the estimations of plasma glucose, fructosamine and glycosylated hemoglobin (HbA1c) respectively. Plasma and whole blood samples for glucose, erythrocyte sedimentation rate and HbA1c estimations were analysed immediately while samples for other biochemical parameters (uric acid, malonldialdehyde, high sensitive C reactive protein, were analysed one week after storage of plasma at $-20\,°C$.

3. Biochemical analyses

3.1. Xanthine oxidase activity

Assay of XO activity Assay of XO activity was done by the method of Haidari et al. [14] with few modifications. The activity of XO was assayed by monitoring the production of uric acid from hypoxanthine. The reaction mixture consisted of 1.0 ml of hypoxanthine (20 μg/ml), 1.9 ml of phosphate buffer (0.5 M, pH 7.5) and 0.1 ml of whole blood. After 30 min, the reaction was arrested by the addition of 0.5 ml HCl (0.6 M). The reaction mixture was centrifuged and the supernatant was harvested. To the supernatant, 0.6 ml of phosphotungstic acid and 0.6 ml of sodium carbonate was added and the tubes were read at 640 nm after 20 min.

3.1.1. Malonaldehyde

Malondialdehyde (MDA) was determined using the method of Buege and Aust [15]. 1.0 ml of the supernatant (protein free filtrate from plasma) was added to 2 ml of TCA-TBA-HCl (1:1:1 ratio) reagent (thiobarbituric acid 0.37%, 0.24 N HCl and 15% TCA) tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent, boiled at 100 °C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance of the pink colour produced was read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA-complex of $1.56 \times 10^5\,M^{-1}CM^{-1}$.

3.1.2. Glucose

Blood Glucose was estimated using glucose oxidase method. Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts under catalysis of peroxidase with phenol and 4 amino phenazone to form a red − violet quinoneime dye colour [16]. The manufacturer of the kit used was Randox (UK).

3.1.3. Glycosylated hemoglobin

Glycosylated haemoglobin was estimated using chromatographic − spectrophotometric ion exchange method [17].

3.1.4. Fructosamine

This colorimetric assay is based on the ability of ketoamines to reduce nitrotetrazolium blue (NB) to formazan in an alkaline solution. The rate of formation of formazan is directly proportional to the concentration of fructosamine.

3.1.5. Uric acid

Uric acid was determined using the uricase method. The principle is based on the conversion of uric acid to allantoin and hydrogen peroxide by uricase and also under the catalytic influence of peroxidase oxidizes 3,5 − dicloro 2- hydroxybenzene sulfonic acid to 4 aminophenazone to form a red − violer quinine imine compound [18].

3.1.6. High sensitive C − reactive protein estimation (HsCRP)

This was estimated using the enzyme linked immunosorbent assay (Elisa) method, using Accu bind kit (USA).

3.1.7. Erythrocyte sedimentation rate (ESR)

ESR was estimated by the Westergreen method.

3.1.8. Statistical analysis

The results were subjected to statistical analysis. Data were analysed using SPSS software version 15. Continuous variables was analysed by the student t-test. Pearson correlation coefficient was used to evaluate for the degree of association. Quantitative data was expressed as mean±SEM. Probability values less than 0.05 was considered statistically significant ($p \leq 0.05$).

4. Results

The mean age $\pm$ SEM of the DM subjects (57.7 ± 0.76 years) and controls (59.6 ± 0.89 years) was comparable ($p = 0.8$) and the female to male ratio in both was 2:1. The pattern of diabetes treatment employed was such that majority of the subjects − 86% were on oral hypoglycemic agents, while 6 and 8% used insulin and a combination of insulin and oral hypoglycemic agents respectively. The mean plasma levels of uric acid, high sensitive CRP, ESR, and xanthine oxidase activity were significantly higher in DM when compared with healthy control group ($p \leq 0.05$). These and other results are shown in Table 1. The correlation studies between xanthine oxidative with glycemic control indices, inflammatory biomarkers and malondialdehye.are presented in Table 2. A positive correlation was observed ($r = 0.514$, $p = 0.000$) between malondiadehyde and xanthine oxidase, while no significant correlation was observed between the inflammatory biomarkers (hsCRP and ESR) and Glycaemic control indices (fasting plasma glucose, fructosamine and glycosylated hemoglobin) with xanthine oxidase ($p \geq 0.05$).

5. Discussion

This study evaluated the activity of xanthine oxidase in diabetes mellitus as well its association with malon dialdehyde (a marker of lipid peroxidation), inflammatory biomarkers and glycemic control indices. A significant elevation of plasma xanthine oxidase activity was observed in Nigerians with T2DM and this entity correlated significantly with lipid peroxidation. Increased activity of xanthine oxidase observed in diabetic Nigerians is in consonance with other reported findings elsewhere. The increase in xanthine oxidase activity in diabetes could be attributed to the increased release of xanthine oxidase from the liver; the major source of vascular xanthine oxidase, nonspecifically released into the blood, particularly under hyperglycemic conditions [19]. This suggests that glycemic control may play a role in modulating xanthine oxidase presence or its activity within vascular compartment. Surprisingly, data from our study showed no association with glycemic control, when indices of short, medium and long term assessments were used.This is at variance with a previous study by Kupusamy et al. [20]. A plausible explanation to these findings could be attributed to the fact that hyperglycemic-induced endothelial dysfunction is chronic and may adversely affect the blood vessels. In view of the fact that the endothelial cells lining the blood vessels do not depend on insulin, the cells take in more glucose than normal and cause the basement membrane to grow thicker and weaker; a scenario that may hinder the usefulness of insulin therapy. Additionally, the non-homogenous nature in the treatment regimen of the study participants may also be a contributing factor. It is pertinent to note that 6% - 8% of our participants used insulin and a combination of insulin and oral hypoglycemic agents respectively.

Considering the lack of association observed between xanthine

Table 2
Pearson correlation coefficient showing the relationship between xanthine oxidase activity with inflammatory biomarkers, lipid peroxidation and glycemic control indices.

Variables	Xanthine Oxidase r	P
Malondiadehyde	0.514	0.000*
Uric acid	0.076	0.346
High sensitive C − reactive protein	−0.136	0.098
Erythrocyte sedimentation Rate	−0.093	0.249
Fasting plasma glucose	−0.072	0.612
Fructosamine	−0.021	0.790
Glycosylated Hemoglobin	−0.044	0.762

Values are expressed as Mean $\pm$ S.E.M. The statistical evaluation of data was performed using Pearson's correlation coefficient. A value of p less than 0.05 was accepted as significant.

oxidase activity and glycemic control indices; a readily modifiable risk factor to DM complications pointed out by findings from the UK Prospective Diabetes Study (UKPDS) [21]; perhaps, the role of xanthine oxidase in contributing to DM complications may be via an alternate pathway or preferentially linked to lipid peroxidation, consequent to oxidative damage. This was evident in our study where a significant association of malondialdehyde was observed with xanthine oxidase activity. In like manner, formation of reactive oxygen species or free radical generation was specifically increased in diabetic peripheral neuropathy in a similar study [22]. The central theme of xanthine oxidase as a rate-limiting enzyme of purine catabolism to uric acid, further eliciting excessive free radicals is well documented. It is apparent from our report, which is also consistent with other findings that increased free radical load, via high activity of xanthine oxidase [23], was concomitantly linked with higher concentration of serum uric acid level. Higher uric acid levels observed in DM in this study is in consonance with a previous study by Ogbera and Azenabor [24]. Hyperuricemia is a cardiovascular risk factor that has been found to play a role in the development of renal and metabolic diseases [25]. The increased level of serum uric acid in DM observed is an attestation to this fact and could also be attributed to such complications inherent in such individuals. These excess uric acid could either be permissive to or supports the increased activity of xanthine oxidase observed in DM. This results to the degradation of endogenously synthesized purine nucleotides from ingested DNA and RNA. Accordingly, the close association of uric acid to cardiovascular events [26], could be pointer to excess xanthine oxidase activity, since xanthine oxidase is a metabolic pathway for uric acid generation.

Of particular interest also are our findings of lack of correlation between xanthine oxidase activity and uric acid levels. This observation is in agreement with a previous Japanese report in which xanthine oxidase activity showed correlation with insulin resistance and not with serum uric acid [27]. The lack of correlation between uric acid and xanthine oxidase could be due to other factors influencing plasma uric acid levels. These includes dehydration, purine or fructose rich foods, alcohol and urinary sugars −

Table 1
Levels (mean $\pm$ SEM) of Xanthine Oxidase, Uric Acid, Malon aldehyde, Inflammatory and Glycemic Control Indices in DM and controls.

Biochemical Variables	DM n = 157	Controls n = 80	t values	p values
Xanthine Oxidase (μ/mg) Uric acid (mg/dl)	0.044 ± 0.005 7.22 ± 0.02	0.028 ± 0.003 4.82 ± 0.16	1.943 8.02	0.014[a] 0.000[a]
Malondiadehyde (μmol/l)	42.40 ± 2.50	38.20 ± 3.30	0.868	0.008[a]
Fasting Plasma Glucose (mg/dl)	163.73 ± 5.00	92.59 ± 1.44	9.852	0.000[a]
Fructosamine (μmol/l)	334.91 ± 11.42	225.0 ± 5.02	6.662	0.000[a]
High sensitive C− reactive Protein (μg/ml)	4.09 ± 0.91	1.30 ± 0.50	2.690	0.009[a]
Erythrocyte Sedimentation Rate (mm/hr)	21.50 ± 1.0	11 ± 0.90	1.879	0.045[a]

Values are presented as mean $\pm$ S.E.M. A statistical evaluation of data was performed using Student's t-test. A value of less than 0.05 was accepted as significant.
[a] significant, n = number of subjects, DM − Diabetes mellitus.

associated excretion of urate [28]. In view of the aforementioned, DM patients with increased xanthine oxidase activity in the presence of reduced or normal uric acid levels may require close monitoring.

Given the notion that xanthine oxidase is activated by hypoxia and inflammation [29,30]; this study further explored the association between xanthine oxidase with some inflammatory biomarkers (erythrocyte sedimentation rate and high sensitive C—reactive protein), and did not observe any. Conversely, a previous report associated plasma xanthine oxidase activity with familial hyperlipidemia with notable inflammatory biomarkers such as nuclear factor KB and high sensitive C— reactive protein.

It is pertinent to state that risk factors to DM complications were not extensively sought in this study. Nevertheless, this has provided a thrust for further prospective studies.

6. Conclusion

Increased xanthine oxidase activity in Nigerians with type 2 Diabetes mellitus could be a salient entity towards the onset on complications and a novel therapeutic target.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.04.022.

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