Evaluation of Uric Acid as a Biomarker for Cardiovascular Disease Risk Stratification among Patients with Type 2 Diabetes Mellitus

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Abstract

Background: Biomarkers may be needed to aid in the screening of cardiovascular disease (CVD) in diabetes mellitus (DM) to enhance early detection and foster early intervention in the management of chronic complications in DM. Hyperuricemia has been correlated with diabetic cardiovascular complication (CVC) and has been implicated in the development and manifestation of CVDs. Hence, this study intends to evaluate its role in CVD risk stratification among patients DM. Aim: Evaluation of the value of uric acid (UA) in CVDs risk stratification among type 2 diabetics. Patients, Materials and Methods: This is a cross-sectional study made up of 101 type 2 DM and control participants. The diabetics were classified into two groups: depending on the presence or absence of CVCs. The CVC observed were diabetic retinopathy, peripheral neuropathy, cardiovascular accident, and ischemic heart diseases. Blood samples were collected for the determination of glycated hemoglobin and UA. Results: UA increased significantly in diabetics with CVCs and especially among those with peripheral neuropathy and poorly controlled glycemic level. However, the diagnostic measures of UA has a poor ability to distinguish between patients with and without CVD. Conclusion: UA may not be diagnostically relevant in screening for CVDs in the bid to ease early diagnosis among diabetes patients despite its comparative increase among those with CVCs.

Keywords: Cardiovascular diseases, diabetes mellitus, Makurdi, uric acid

Introduction

Diabetes mellitus (DM) poses a serious financial stress on patients, households, communities, and health-care systems.¹ The threat is growing with an increasing number of people, families, and countries afflicted. This trend is fuelled by destitution which impedes the economic growth of several nations. Diabetes causes an enormous economic loss, and the burden is heavy as its entire health expense in the country was approximately N745 billion in 2021.²⁻³ Universally, approximately 32.2% of all persons with type 2 diabetes mellitus (T2DM) are affected by cardiovascular disease (CVD). It is a significant contributor to hospitalisation among people with diabetes hence, leading to global economic loss. CVDs are four-fold more common in people with diabetes and half of the diabetic population present with CVD at the time of their first diagnosis.⁴⁻⁵

Despite several efforts, DM is yet to have a defined cure; hence, prevention plays a key role in attempts to revise or delay cardiovascular complications (CVCs). Recently, several biomarkers have been in the purview of researches to understand their role and contributions in the early detection and treatment of CVC of DM. One of these biomarkers that have gained interest is uric acid (UA).

Uric Acid and Cardiovascular Diseases

The end product of purine metabolism is UA. A positive correlation has been found with it and various CVDs.
Hyperuricemia has been implicated in the promotion of the incidence as well as the development of CVDs by various pathological mechanisms such as inflammatory response, oxidative stress, insulin resistance/diabetes, endoplasmic reticulum stress, and endothelial dysfunction. Serum UA plays a significant role both as antioxidant and pro-oxidant. As an antioxidant, it tends to react directly with hydroxyl radicals, peroxynitrite, nitric oxide, and hydrogen peroxide, etc., leading to the formation of stable intermediates; it also cooperates with superoxide dismutase to scavenge oxygen radicals, chelates with metal ions and inhibits the peroxynitrite-induced protein nitrification, protein, and lipid peroxidation.[6]

UA promotes pro-oxidation activity in cells, which has been related to the production of reactive oxygen species (ROS). This includes the reduction of nitric oxide production in arterial endothelial cells, thus inhibiting vasodilation;[7] inhibiting adiponectin synthesis by adipocytes; damaging the tricarboxylic acid cycle and fatty acid β oxidation; activating the renin-angiotensin system, stimulating the proliferation of vascular smooth muscle cells and the production of angiotensin II (Ang II); and thus, stimulating a state of chronic inflammation.[8,9]

Endothelium stress has been found to be induced by free radicals from hyperuricemia leading to endothelium reticulum stress which triggers activation of the protein kinase C pathway in the human model of umbilical vein endothelial cells, leading to endothelium dysfunction.[10] Endothelial cells secrete a variety of vasoactive substances, including vasodilators (nitric oxide, prostaglandin I₂, endothelium-derived hyperpolarising factor, etc.) and vasoconstrictors (endothelin-1, thrombin A2, and Ang II, etc.).[11,12] Therefore, hyperuricemia in cells directly combines with nitric oxide, which results in the decrease of nitric oxide bioavailability and the increase of peroxynitrite (ONOO⁻).[12] This leads to an imbalance between vasodilators and vasoconstrictors as the generation of ONOO⁻ which is a strong oxidant, that has the capacity to cause DNA damage, cell death, and lipid peroxidation.[12] They all collectively resulted to dysfunctional endothelium triggering inflammatory cascade which is pertinent in the atherosclerosis formation, inadvertently causing CVCs. Many experimental studies show that hyperuricemia plays a significant role in endothelial dysfunction development.[13−16]

UA has also been found to play pro-inflammatory role implicated in the pathophysiology of CVD. Increased intracellular UA concentrations via activating mitogen-activated protein kinases promote the expression of inflammatory markers, such as nuclear factor κB, growth factors, vasoconstrictive substances (Ang II, thromboxane, and endothelin-1), and chemokines.[13,14] In addition, hyperuricemia was observed to promote macrophage M1/M2 polarisation.[17] Hence, tends to enhance the pro-inflammatory response of M1 and inhibit the anti-inflammatory response of M2, leading to insulin resistance and cardiac dysfunction. Current studies confirm that oxidative stress and inflammation may be the pathophysiological basis of insulin resistance.[18] Therefore, hyperuricemia induced-increase in ROS level can induce insulin resistance.

On the other hand, UA possess antioxidative characteristics and have been found to have the ability to scavenge ROS. Being one of the important endogenous antioxidants in the human body, up to 60% of the total antioxidant capacity of the plasma is contributed by UA. Therefore, it can protect cells from oxidative stress.[19,20] Kellogg and Fridovich, originally discovered the urate oxygen radicals scavenging ability and its ability to safeguard the lipid membrane of red blood cells from lipid oxidation.[21] However, it only serves as a powerful antioxidant in the hydrophilic environment but is powerless to lipophilic radicals, hence, cannot attenuate the lipid plasma membranes’ radical chain propagation. Despite its limitation, the central nervous system is the major site of the proposed antioxidant effect of UA. It has been found to reverse acute brain injury and decrease severity in acute stroke and experimental allergic encephalitis, respectively, in experimental rats. However, the same effects were not observed in chronic elevation of UA.[22,23]

UA antioxidant capacity is finite in limiting the action of some highly diffusible peroxynitrous radicals, and the hydrophobic environment is also preferentially favors tyrosine nitration.[24−26] Thus, these physicochemical findings may clarify explicitly the limitation of its antioxidant ability as it can only be effective in the hydrophilic environment of body fluids. Even in the plasma, urate can only prevent lipid peroxidation in the presence of ascorbic acid.[25,26] Therefore, agreement is yet to be reached on whether it is a protective or a risk factor. Nevertheless, it seems that acute rise may be protective, whereas a long-standing increase can be a predisposing cause of disease.[27,28]

Although the causal relationship of hyperuricemia to CVDs remains controversial. Recent studies have centered on the evaluation of UA as biomarkers of CVDs, and so far, several conflicting results have emerged. In a study, a direct correlation of UA and some surrogate biomarkers of diabetic CVD was observed.[29,30] Many corroborative proofs have shown its the correlation to the underlying cause of metabolic and vascular disorders. Similarly, some researchers suggested it as only a marker of cardiovascular injury. Therefore, this study will assess the role of UA in the diagnosis of diabetic CVCs to determine its capacity to serve as a screening tool for disease stratification.

Patients, Materials and Methods

This observational cross-sectional study was carried out in the endocrinology unit of Federal Medical Centre and Benue State University Teaching Hospitals located at the heart of the Metropolitan town of Makurdi. Sample size determination was done using Cochrane’s formula, with a 95% confidence interval, 0.05 precision, and 5.5% prevalence rate[6]

\[ N = Z^2p(1-p)/d^2 \]
n = Sample size

Z = Standard normal deviation at 95% confidence interval, which is 1.96

d = Degree of precision (taken as 0.05)

p = Proportion of the target population (estimated at 5.5%, which is 5.5/100 = 0.055)

Q = Alternate proportion (1 − p) which is 1 − 0.039 = 0.961

N = \frac{(1.96)^2(0.055)(0.945)}{(0.05)^2} = 80

Adjusting the sample size for anticipated 10% attrition = 80 + 8 = 88

One hundred and one diabetic participants were recruited, with 100 controls. Sixty-two people with diabetes had CVCs while 38 had no CVCs. The recruitment of participants for the study was done using simple random sampling. Data were collected using research proforma. T2DM within the age 20–79 years attending the medical outpatient clinic of the hospitals that gave their consent in writing were recruited for the study. Those suffering from other diseases that were not complications of diabetes, pregnant women, and nonconsenting patients were excluded from the study.

Experimental design

The diabetes participants were subdivided into two groups: those with CVC and those without. CVCs found on screening the participants include macrovascular complications (ischemic heart diseases, and stroke) and microvascular complications (peripheral neuropathy and retinopathy). Physical examination was done to elicit signs of chronic complications such as neurological, peripheral pulses, and fundal examinations. Michigan neuropathy screening instrument (MNSI) was administered to assess peripheral neuropathy; electrocardiography was used to evaluate for ischemic heart disease. History of diabetes-related CVCs in the last three months was further confirmed with the aid of their medical records. 4 ml of fasting blood samples were collected from the participants in lithium heparin for UA and glycated hemoglobin (HBA1c), respectively.

Criteria for Diagnosis of Cardiovascular Complications

Assessment of diabetic peripheral neuropathy

MNSI was used to assess peripheral neuropathy in people with diabetes. A score of ≥7.0 was considered abnormal for the 15-item self-administered questionnaire, while ≥2.0 was classified as abnormal in the physical assessment conducted for lower extremities “deformity.”

Assessment of diabetic eye diseases

Diabetic retinopathy, cataract, and glaucoma were classified under this category. The assessment was made by an ophthalmologist using direct ophthalmoscopy. Fundoscopy was done following pupillary dilatation.

Assessment of stroke

Symptoms and signs of stroke and radiographic evidence of stroke via computed tomography scan was taken as stroke.

Assessment of ischemic heart diseases

Patients with symptoms and signs of ischemic heart disease confirmed by the evidence of elevated or depressed ST segment with inverted T waves in electrocardiogram and/or increased cardiac troponin and creatine kinase-MB were classified as having myocardial infarction.

Criteria for Definition of Glycemic Control Status

HBA1c ≤7% was designated a good glycemic control, while those ≥7% were defined otherwise.

Ethical approval

Federal Medical Centre Ethical Committee approved this study with approval reference no FMH/FMC/MED.108/VOL.I/X. Formal consent was obtained from the participants before recruitment. Data were obtained using number codes to ensure confidentiality was maintained throughout the study.

Result

There were 201 study participants, including 66 female and 35 male diabetics, with 70 females and 30 male control participants. Table 1 shows the comparison of serum concentration of UA between diabetics and the control group as well as across the gender. In general, UA concentration in people with diabetes does not differ (P = 0.206) significantly from control, though people with diabetes had a higher value (0.24 ± 0.11 vs. 0.18 ± 0.04). However, male diabetics who were about half the number of females, the male had significantly increased (P = 0.001) concentration of UA compared to females. Similarly, male diabetic patients had a significantly (P = 0.000) more concentration of UA than the females. More so, female diabetics, in a similar manner, had a significantly higher value of UA (P = 0.000) than female control participants.
Table 2 compares serum uric acid concentration in people with diabetes with apparent CVD and those without CVD. UA was significantly more in those with CVD.

Table 3 compares the UA concentration between diabetics with peripheral neuropathy and those without it. Results showed that those that had peripheral neuropathy had significantly greater UA concentration.

UA concentration was compared based on the glycemic control status of the diabetic participants in Table 4. People with diabetes with poor glycemic control had significantly elevated serum UA than those with good glycemic control.

Table 5 indicates the receiver operator curve (ROC) indicating the diagnostic sensitivity of UA to CVD. The area under the curve for the ROC is 0.616, and it is not significant ($P = 0.051$).

Table 6 shows the logistic regression shows the slope of the regression line ($B$) to be $-4.077$; odds ratio $[\text{Exp}(B)]$ is 0.017 while the $P$ value for the regression analysis is 0.039.

Table 7 illustrates the calculated diagnostic measures of accuracy for the diagnosis of CVC by UA. The extrapolated cutoff mark from the ROC curve for diagnosis of CVC is 0.285 mmol/l. At this cutoff value, the diagnostic sensitivity is 70%, while the diagnostic specificity is 42%. The true positives were 16, true positives were 44, false negatives were 19, and false positives were 22. The positive predictive value and negative predictive value were 66.6% and 45.71%, respectively. The diagnostic accuracy or efficiency of UA to CVC is 59.4%.

**DISCUSSION**

In spite of the male diabetics being about half the number of female diabetics, the UA of the male rose significantly compared to the females. According to some studies, serum UA was similarly increased in males than in the female.[32-34] In a study, serum UA was well known to be comparatively reduced in females than in males, which has been associated with estrogen’s capacity to promote the renal excretion of UA leading to higher renal clearance in females.[35]

UA was significantly increased in diabetics with CVD than in those without CVD. CVD, such as ischemic heart diseases, congestive heart failure, hypertension, atrial fibrillation, and stroke has been associated with UA.[36] Several mechanisms have been implicated as the modality for hyperuricemia’s deleterious effects on cardiovascular tissues. These mechanisms are as follows: Elevation of oxidative stress, limitation of endothelia’s nitric oxide production and endothelial dysfunction, promotion of local and systemic inflammation, vasoconstriction mediated by renin-angiotensin system, smooth muscle cells proliferation, impaired insulin sensitivity, and metabolic dysregulation.[36] These interrelated mechanisms cumulatively underlie the atherosclerosis, which progresses if unchecked, leading to CVDs. As a result of these findings, it can be postulated that UA can serve as a screening tool for CVDs empirical diagnosis. In addition, UA increased in people with diabetes with peripheral neuropathy than those without diabetic peripheral neuropathy,[37] asserting the association of UA with CVD. However, the receiver

![ROC Curve](image-url)
Table 7: Measures of diagnostic accuracy of uric acid for cardiovascular complication in diabetics

<table>
<thead>
<tr>
<th>Diagnostic accuracy measures</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>TP</td>
<td>44</td>
</tr>
<tr>
<td>FN</td>
<td>19</td>
</tr>
<tr>
<td>TN</td>
<td>16</td>
</tr>
<tr>
<td>FP</td>
<td>22</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>70</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>42</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>66.6</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>45.7</td>
</tr>
<tr>
<td>Diagnostic accuracy/efficiency (%)</td>
<td>59.4</td>
</tr>
<tr>
<td>Diagnostic cutoff value (mmol/L)</td>
<td>0.285</td>
</tr>
</tbody>
</table>

TP: True positive, FN: False negative, TN: True negative, FP: False positive, PPV: Positive predictive value, NPV: Negative predictive value

The operating curve indicates the poor discriminative ability for CVDs diagnosis, which implies its ability to designate a person with CVDs as positive for the disease. However, this serves as a poor biomarker of CVDs since it is expected that a biomarker should have a diagnostic sensitivity of up to 80% in order to be clinically relevant in disease stratification in clinical settings. UA was able to diagnose CVC correctly in about 44 patients, while it was incorrect in 22 patients. It was also able to diagnose no CVC correctly in 16 patients out of 38 patients without CVCs. Hence, the diagnostic accuracy is 59.4%.

Further, the binary logistic analysis revealed the odds ratio that as UA increases, the odds that the CVC would occur decreases. This also indicates that the higher the UA, the lower the odds that CVC may occur. This implies that though UA plays a role in CVDs pathophysiology, it lacks the efficient discriminatory ability to distinguish the presence or absence of CVC in a clinical setting. Hence, it may not be useful in CVDs stratification among people with diabetes. This suggests that UA may not correlate with the extent of cardiovascular damage and prognosis in clinical settings.

Conclusion
UA is elevated in CVD; however, it is observed not to be a good marker of CVDs due to its poor diagnostic measures. Although, it may have a potential of being a biomarker for atherosclerosis.

Limitation
Diagnosis of CVC was carried out mostly by clinical assessment and medical record, which is examiner dependent, and more so, CVCs that are not clinically apparent may have been neglected, which could affect the diagnostic measures of the measurements.

Recommendation
Future works may use more sensitive clinical or radiological diagnostic tool that may be able to detect subclinical CVCs.

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Conflicts of interest
There is no conflict of interest.

Authors’ contributions
M. B. K wrote study design, collected data, processed samples, analysed and interpreted and as well wrote the first draft of the manuscript. I. N. Mba played a role in analysis, editing and interpretation of data and manuscript writing. J. E. O was involved in writing of manuscript, editing of the manuscript and data interpretation. M. C. O participated in manuscript drafting and result analysis. All authors read the final draft of the manuscript and gave unanimous approval before submission.

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