1. Abstract

1.1. Background: Type 2 diabetes mellitus and hypertension are two important public health challenges, and both are linked to increased risk of cardiovascular events. Hyperuricemia has recently emerged as an independent risk factor in the development of type 2 diabetes mellitus and hypertension through several proposed mechanisms. These include endothelial dysfunction leading to vascular remodeling, inhibition of proliferation and migration of endothelial cells and NO secretion, formation of peroxynitrite through uric acid depended reactive oxygen species and NO and promoting L-arginine degradation. As a result of the effects of hyperglycemia and neurohormonal activation, UA levels are independently associated with endothelial dysfunction in animals and humans, thereby promoting hypertension.[12] This study was undertaken to find out the possible association of hyperuricemia and nitric oxide on patients with diabetes and hypertension.

1.2. Methods and Materials: The study was carried in a medical college of South India with a sample size of 186 patients which were divided into 4 groups – Group 1- healthy patients, Group 2 – patients with DM, Group 3- patients with DM and HTN, and Group 4 – patients with CAD with DM. Blood samples for serum uric acid , fasting blood sugar, nitric oxide and HbA1c and anthropometric measurements ( height and weight for BMI) were taken. The data obtained was analyzed with IBM SPSS Statistics for Windows, Version 20.0., IBM Corp., Chicago, IL and t-test was used to compare the results of various parameters among the studied groups. Linear regression analysis (Person correlation coefficient, r) was performed for determining the degree of association between different parameters. All values expressed as mean±SD, and P values of <0.001 were considered to be statistically significant.

1.3. Results: The study showed statistically significant association between hyperuricemia and nitric oxide level in patients with Diabetes and Hypertension. As HbA1C % increases from group 1 to Group 4, the levels of serum uric acid also increases from 3.51 ± 1.6 to 8.25 ± 1.54. There is a decline in nitric oxide levels from 93.40 ± 4.96 to 44.89 ± 5.64 with simultaneous rise in serum uric acid levels. There is also considerable difference between the levels of nitric oxide and uric acid of participants that are known case of coronary artery disease with DM and the other three remaining groups with uric acid levels being higher and nitric oxide levels lower in group 4 compared to other 3 groups. However, the study failed to find correlation between serum uric acid levels and sex.

1.4. Conclusion: It is evident that diabetics and hypertensive are at increased risk of developing hyperuricemia, SUA are inverse-ly related to vascular Nitric oxide levels. The combine effect of hyperuricemia, depleted NO levels and hyperglycemia along with hypertension is endothelial dysfunction. Endothelial dysfunction provides a priming bed for atheromatous plaque deposition, stiffen-ing of vessel walls, inadequate vasodilation, higher systolic blood pressure and restrictive ventricular filling pattern or diastol-ic dysfunction [18, 23-24]. As a result incidence of CAD increases along with associated co morbidities.
2. Introduction
Diabetes is pandemic in both developed and developing countries. By 2030, the projected estimate of diabetes is 354 million people with diabetes worldwide while in India alone, diabetes is expected to increase to 79.4 million by 2030 [1]. India has a high prevalence of diabetes mellitus and the numbers are increasing at an alarming rate. Diabetes is the third major contributor and Hypertension is one of the commonest non-communicable diseases and overall mortality in India. Globally, an estimated 26% of the world’s population (972 million people) has hypertension, whereas overall prevalence in India is of 29.8% [2]. Type 2 diabetes mellitus (T2D) and hypertension, the 2 leading components of the global burden of disease. The coexistence of hypertension and diabetes in a large population of patients is not coincidental; individuals with T2DM often display a constellation of metabolic derangements termed the cardiometabolic or cardiorenal metabolic syndrome [3, 4].

The prevalence of both of these conditions is likely to continue increasing in the near future. Rapidly aging and urbanization of India’s population, as well improving the standard of living results in obesity and its associated cardiovascular disease (CVD) risk factors, including diabetes and hypertension [5].

Type 2 diabetes mellitus and hypertension are two important public health challenges, and both are linked to increased risk of cardiovascular events. Hyperuricemia has recently emerged as an independent risk factor in the development of type 2 diabetes mellitus and hypertension through several proposed mechanisms. SUA definitely should be considered as one of the multiple injurious stimuli to the arterial vessel wall and capillary, which may contribute to endothelial dysfunction and arterial – capillary vessel wall remodeling through oxidative – redox stress [6,7,8]. There are multiple injurious stimuli to the endothelium and arterial vessel wall in the accelerated atherosclerosis associated with Metabolic Syndrome and T2DM (atheroscleropathy). Elevated UA reduces endothelial NO bioavailability in humans [9] Uric acid inhibits proliferation and migration of endothelial cells and NO secretion [10].Uric acid inhibits proliferation and migration of endothelial cells and NO secretion [20]. UA can react with NO to form 6-aminouracil, UA-dependent ROS reacts with NO to form peroxynitrite, and UA can hold back L-arginine uptake and stimulate L-arginine degradation [11]. As a result of the effects of hyperglycemia and neurohormonal activation, UA levels are independently associated with endothelial dysfunction in animals and humans, thereby promoting hypertension [12]. This study was undertaken to find out the possible association of hyperuricemia and nitric oxide on patients with diabetes and hypertension.

3. Aims & Objectives
1. To know the serum uric acid and nitric oxide level in patients with Type 2 Diabetes mellitus and hypertension.
2. To correlate anthropometric measurements, serum uric acid level and Nitric oxide in patients with Type 2 Diabetes mellitus and hypertension.
3. To correlate serum uric acid and nitric oxide levels and cardiovascular diseases in Type 2 Diabetes Mellitus and hypertension.

4. Material & Methods
This study was carried out in Medical College Hospital which is a 550 bedded multi disciplinary centre serving the rural population in South India. Study was undertaken to determine the possible association of hyperuricemia and nitric oxide on patients with diabetes and hypertension.

After obtaining approval from ethical committee, the aim of the study was explained, and informed consent was obtained. A total 186 patients with type 2 Diabetes mellitus and hypertension from the out-patient Diabetic clinic were included in this study.

The inclusion criteria included the following: patients with Type 2 diabetes mellitus and Hypertension attending at Medical college hospital outpatient clinic for treatment. Patients were excluded from the study if they suffered from other chronic systemic inflammatory or autoimmune disease or malignancy, type 1 diabetes patients, the pregnant women, patients with other causes of CKD were excluded, patients receiving medications for hyperuricemia or drugs known to influence both uric acid levels were also excluded.

Sample of 186 patients, including men and women aged 35–65 years, was taken and were categorized into four groups.

**Group I:** control group was selected of healthy 60 healthy and had no prior history of Type 2 diabetes mellitus, hypertension, coronary artery diseases or any other cardiovascular diseases, and were not taking medication for any chronic medical condition. Fasting blood glucose, HbA1c and blood chemistry were normal.

**Group II:** including 78 type 2 diabetic patients, with HbA1c levels ≥ 6.5% as per American Diabetes Association (ADA) guidelines, with proven history of Type 2 diabetes mellitus but no other complications.

**Group III:** including 72 type 2 diabetic patients with hypertension. Patients with HbA1c levels ≥6.5% and systolic blood pressure ≥140 mm of Hg and diastolic blood pressure ≥90 mm of Hg, with history of Type 2 diabetes mellitus as well as hypertension.

**Group IV:** including 36 patients Coronary artery disease with Type 2 diabetes mellitus. Patients with narrowing or blockage of one or more epicardial coronary artery with greater than 25% stenosis shown in coronary angiography and diagnosed by cardiologists and had HbA1c levels ≥6.5% and prior history of Type 2 diabetes mellitus.

Among diabetic patients 109 on oral hypoglycemic drugs, and the remaining 77 on insulin therapy.

Coronary artery disease patients were identified in the Cardiac unit. After coronary angiogram, all patients were evaluated by cardiologists in the inpatient setting. If patients were found to have any evidence of CAD, demographic, clinical, and angiographic data were collected from all such patients. Fasting sample were collected prior to the percutaneous coronary intervention.

4.2. Sample collection process

Clinical history and complete physical examination including measurements of blood pressure, was collected and conducted for all the participants. Blood samples were collected by venipuncture after an overnight fast, in a Red colour coded Vacutainer and blood was allowed to clot by leaving at room temperature for 15 min. After centrifugation at 1500 g for 15 min at 4°C, serum was collected in 1.2 ml cryo vials and immediately stored at -80°C until testing.

4.3. Anthropometric measurement and biochemical parameters

Height and weight were obtained using standardised techniques and instruments. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters \([\text{weight (kg)}/\text{Height (m}^2)\].

Both SBP and DBP were measured twice on the right arm by trained medical staff using a non-invasive automated sphygmomanometer (OMRON, Japan) with subjects in a sitting position in a quiet environment. Hypertension was defined as SBP ≥ 140 or DBP ≥ 90 mm Hg or history of intake of antihypertensive medication.

Fasting blood glucose levels and Uric acid was measured in the routine laboratory by the glucose oxidase method and URICASE/POD on Coralyzer 200 (Tulip Diagnostics (P) Ltd method implemented in an autoanalyzer.

The Nitric Oxide Assay determines nitric oxide composition through measurement of nitrate (NO₃) and nitrite (NO₂) levels in subjects involves the spectrophotometric measurement by the Griess reaction to form a coloured azo dye product.

4.4. Statistical Analysis

The data obtained were entered in MS Excel Sheet and data analysis done with IBM SPSS Statistics for Windows, Version 20.0., IBM Corp., Chicago, IL. t-test was used to compare the results of various parameters among the studied groups. Linear regression analysis (Person correlation coefficient, r) was performed for determining the degree of association between different parameters. All values expressed as mean ± SD, and P values of <0.001 were considered to be statistically significant.

5. Results

Table 1

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Age</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30-40</td>
<td>22</td>
<td>11.82</td>
</tr>
<tr>
<td>2</td>
<td>41-50</td>
<td>35</td>
<td>18.81</td>
</tr>
<tr>
<td>3</td>
<td>51-60</td>
<td>54</td>
<td>29.03</td>
</tr>
<tr>
<td>4</td>
<td>61-70</td>
<td>59</td>
<td>31.72</td>
</tr>
<tr>
<td>5</td>
<td>71-80</td>
<td>16</td>
<td>8.6</td>
</tr>
</tbody>
</table>
Table 2: Demographic and laboratory characteristics of the study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group- I Control (n=60)</th>
<th>Group- II Type 2 diabetic (n=78)</th>
<th>Group- III Type 2 diabetic patients with hypertension (n=72)</th>
<th>Group- IV Coronary artery disease with Type 2 diabetes mellitus (n=36)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.5 ± 10.13</td>
<td>50.72 ± 11.07</td>
<td>51.38 ± 11.38</td>
<td>51.8 ± 4.89</td>
<td>0.782</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.73 ± 2.86</td>
<td>74.03 ± 3.62</td>
<td>76.80 ± 3.50</td>
<td>78.80 ± 3.25</td>
<td>0.916</td>
</tr>
<tr>
<td>Height</td>
<td>154.80 ± 2.99</td>
<td>155.43 ± 2.80</td>
<td>154.73 ± 2.66</td>
<td>154.73 ± 2.54</td>
<td>0.894</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>30.14 ± 1.01</td>
<td>31.59 ± 1.10</td>
<td>32.21 ± 1.12</td>
<td>32.33 ± 1.12</td>
<td>0.61</td>
</tr>
<tr>
<td>Smoking:</td>
<td>64.35%</td>
<td>85.17%</td>
<td>82.24%</td>
<td>84.27%</td>
<td>0.543</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>119.59 ± 11.46</td>
<td>124.88 ± 14.95</td>
<td>141 ± 18.72</td>
<td>142 ± 26.72</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>75.36 ± 9.54</td>
<td>79.75 ± 5.04</td>
<td>89.87 ± 9.19</td>
<td>90.89 ± 9.18</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting Blood Glucose mg/dl</td>
<td>89.48 ± 10.46</td>
<td>149.03 ± 21.25</td>
<td>175.60 ± 19.18</td>
<td>188.53 ± 21.25*</td>
<td>0.001</td>
</tr>
<tr>
<td>HbA1C %</td>
<td>5.22 ± 0.56</td>
<td>7.62 ± 1.34</td>
<td>8.43 ± 1.99</td>
<td>8.53 ± 1.56</td>
<td>0.001</td>
</tr>
<tr>
<td>Uric acid: (mg/dl)</td>
<td>3.51 ± 1.6</td>
<td>5.36 ± 1.4</td>
<td>7.40 ± 1.32</td>
<td>8.25 ± 1.54</td>
<td>0.001</td>
</tr>
<tr>
<td>Nitric oxide µmol/l</td>
<td>93.40 ± 4.96</td>
<td>87.51 ± 3.26</td>
<td>48.79 ± 5.34</td>
<td>44.89 ± 5.64</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Figure 2: Distribution of study participants according to their gender (N=186)
6. Discussion

Type 2 Diabetes Mellitus and Hypertension are two of the most prevalent non-communicable diseases in India which are risk factors in itself for a variety of ailments such as cardiovascular diseases including atherosclerosis, angina, Myocardial Infarction; Chronic Kidney disease, cerebrovascular accidents such as ischemic stroke. Moreover, both of them are classical examples of diseases showing iceberg phenomenon in which for every one diagnosed and sufficiently treated person there are numerous undiagnosed or under treated individuals. These diseases are multifactorial with Hyperuricemia to be one of emerging culprit. The association between serum uric acid levels and high blood pressure in humans is well established.

In our study we found relationship between serum uric acid and Type 2 DM and hypertension is statistically significant. Our findings were consistent with other studies which reported that higher UA concentrations were independently associated with increased odds of developing hypertension [13]. Potential mechanisms behind the link between hyperuricemia and the development of hypertension have included nitric oxide and RAAS pathways. UA could lead to endothelial cell dysfunction via nitric oxide synthetase and stimulate vascular smooth muscle cell proliferation [14]. High UA levels have been associated with organ damage in hypertensive patients and are considered an integral part of the biochemical alterations that compound the metabolic syndrome. Indeed, serum UA is higher in hypertensive patients with target organ damage [9].

Our study found Uric acid is significantly associated with diabetes, as HbA1C % increases from group 1 to group 4, the levels of serum uric acid also increases. Potential mechanisms that link hyperuricemia to DM include, UA directly inhibits the trigger of insulin signaling pathway by an ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) recruitment at the receptor level [15]. All factors interference with glucose homeostasis and insulin sensitivity promotes the development of diabetes [16-17]. Similarly there is corresponding rise in uric acid levels with elevated systolic and diastolic blood pressures from 1 to 4 groups. This is mainly due to reduced renal blood flow favoring uric acid reabsorption, microvascular disease leading to increased urate production from adenosine breakdown and reduced excretion as lactate competes with urate transporter in proximal tubule [18].

We believe that uric acid is closely related to diabetes. Poor lipid metabolism in individuals with higher UA levels may lead to increased fasting and postprandial insulin levels, high-sensitivity C-reactive protein, hepatic insulin resistance index, and decreased glomerular filtration rate and skeletal muscle insulin sensitivity; high levels of SUA may impair liver insulin sensitivity and insulin clearance [19].

Our study shows drastic decline in nitric oxide levels with simultaneous rise in serum uric acid levels. The exact mechanism by which uric acid attenuates nitric oxide levels is unknown, however it is postulated that uric acid activates NADPH oxidase resulting in decreasing bioavailability of NO and simultaneously increasing protein nitration [20-21].

This study also shows considerable difference between the levels of nitric oxide and uric acid of participants that are known case of coronary artery disease with DM and the other three remaining groups. Uric acid levels are higher and nitric oxide levels are lower in group 4 compared to other 3 groups. This clearly shows that there is a probable causal association between SUA, NO levels and CAD. Endothelial dysfunction is precursor for development of CAD, is seen in both DM and Hypertension. Hyperuricemia
which may be one of the factors in endothelial dysfunction is associated with uncoupling of eNOS enzyme via decreased NO production in patients with type 2 diabetes mellitus. The enzymatic reaction of eNOS is of utmost importance to the normal functioning of the endothelial cell and the intimal interstitium. When this enzyme system uncouples, the endothelium becomes a net producer of superoxide and reactive oxygen species instead of the usual production of the protective antioxidant, NO, thus making uric acid a pro-oxidant. There are multiple causes for endothelial uncoupling of eNOS enzyme, but primary causes are HUA and the antioxidant–pro-oxidant-urate redox shuttle. This shuttle seems to rely heavily on many factors such as timing (early or late disease process), location of the tissue, its pH, the surrounding oxidant milieu and depletion of other local antioxidants. In addition to HUA, glucotoxicity also places an additional redox stress on the arterial vessel wall and capillary endothelium. This in turn also contributes to the switch of uric acid from antioxidant to pro-oxidant state, leading to endothelial dysfunction [22]. Endothelial dysfunction provides a priming bed for atheromatous plaque deposition, stiffening of vessel walls, inadequate vasodilatation, higher systolic blood pressure and restrictive ventricular filling pattern or diastolic dysfunction [18,23-24]. As a result incidence of CAD increases along with associated co morbidities. On top of it, diabetic and hypertensive patients with hyperuricemia are at a higher risk of developing CKD, PAD peripheral artery disease, and retinopathies. This may again lead to a vicious cycle of hyperuricemia and endothelial dysfunction.

Our study failed to find a correlation between SUA levels and sex; possibly may be due to most of women were menopausal over the age 50 years. Its because of impact of oestrogens on the renal tubular handling of uric acid [25]. Premenopausal levels of oestrogens in women may promote more efficient renal clearance of urate [26-27]. These findings suggest that the increase was explained by menopause and other age-related factors that are associated with hyperuricaemia. Age-related increases in serum uric acid levels among women have been reported by previous cross-sectional studies [28]. In contrast, serum urate levels did not vary significantly among men. [29-30].

7. Conclusion

It is evident that diabetics and hypertensive are at increased risk of developing hyperuricemia. SUA are inversely related to vascular Nitric oxide levels and endothelial dysfunction caused by depleted nitric oxide reserves results into increase in prevalence of CAD. The pathogenesis of T2DM is complex, involving various interacting factors. We believe that uric acid is closely related to the development of diabetes and its chronic complications. The combine effect of hyperuricemia, hyperglycemia along with hypertension is endothelial dysfunction. Changes in endothelial function may affect the coronary artery and peripheral artery circulation being unable to cope with the increased metabolism of myocardial muscles.

We call for further researches to explore the molecular mechanism, especially in the direct effect of uric acid on insulin secretion.

References

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