Assessment of Renal Damage Using Selected Biochemical Markers in Smokeless Tobacco Users

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Abstract: Usage of smokeless tobacco is a common practice among the people of Andhra Pradesh in India. The renal damage in the costal belt of Andhra Pradesh is also high. The present study was aimed at the assessment of the relation between renal failures and the usage of Gutka controlled by special and specific biochemical markers. Results have showed that the all reported markers were low in both serum and urine except B2MG. These results indicated a possible relation between serum nicotine levels and the occurrence of renal failures.

Key words: ACE · Polymorphism · Nicotine · Smokeless tobacco · Gutka

INTRODUCTION

Although dangerous effect of smoking on human health, the tobacco as drug substance has been used throughout the world [1, 2]. Nicotine is used in different forms including smoking and smokeless tobacco [1]. Most of the educated and uneducated young people are addicted to Gutka powder in India. For the preparation of Gutka nicotine tobacco was used. Tobacco leaves are powdered and mixed with lime [3]. This mixture was packed in 9 g/packet and sold in local groceries. This simply readily available powder was placed between lower labile mucosa and gingival for about 5-10 min and then spit out. The mixture was used; (7-10 times per day) 2-3 g for each time. The immediate availability and the low price give rise to high consumption of chewing tobacco. The effect of smokeless tobacco on different biochemical and hematological studies were studied, studies on its effect on renal functions were very scanty.

The routine classical evaluation of nephropathy (any type of renal problems) includes the identification of glomerular and tubular markers in the patient’s serum and urine. The normal individual doesn’t contain this content elevated in their urine or in serum samples. These glomerular and tubular markers include: transferrin, Ig G, alpha 1-antitrypsin, β-2-microglobulin and angiotensin converting enzyme (ACE). Recent studies also have demonstrated that, there were tubular components in renal complications of disease conditions as shown by the detection of renal tubular enzymes and low molecular weight proteins in the urine as well as in serum. In fact, tubular involvement may precede glomerular involvement because several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and rise in serum creatinine [4].

Hence the present investigation was conducted to evaluate a relation between ST usage and its relation towards the development of renal failures.

MATERIALS AND METHODS

Around six hundred ST consumers (n=600) were taken from three districts (200 per district) for the analysis among the three selected coastal districts with different age groups (ranging 16-40 years) who are consuming Gutka 10-12 g/day for a period of 4-5 years. Controls also found in the same selected area those who did not take any smokeless powder (n=260). They were provided with explanations for all experimental procedures and informed consent was obtained before the beginning of the study. Blood and urine samples were collected from the subjects and preceded for further hematological and biochemical analysis.

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Estimation of Serum and Urine Nicotine and Related Content: The nicotine and its other analytes have been analyzed in the serum and urine of both controls and test samples using a combination of solid phase extraction with liquid-chromatography-tandem mass spectroscopy. Qualitative identification and quantitation are accomplished by comparison of the specimen components to a standard curve of each analyte addressed in the assay [5].

Biochemical Analysis
Estimation of Serum Creatinine: Blood was collected into the sterile micro tubes and stand at room temperature to clot. Centrifuge the tube at 3000 rpm for separation of blood clot with the serum. The supernatant was collected and analyzed for the presence of creatinine. Serum creatinine levels were all assayed with the Rate-Jaffé reaction on a Hitachi 747 autoanalyzer (Roche Diagnostics Corp., Indianapolis, Indiana). This assay was calibrated daily with a Cfas calibrator (Roche Diagnostics Corp.) by using the uncompensated method during the study period.

Estimation of Random Blood Sugar: Random blood glucose was measured routinely by using “One Touch Ultra Blood Glucose Meter” (AccuChekGluco Meter, USA).

Estimation of Glomerular and Tubular Markers:
Estimation of transferrin: The amount of transferrin was estimated using SRID kit in the first sample was 7.5 g. We used 10.5mg Fe and 11.44 anionic bicarbonate.

Estimation of IgG: The serum and urine IgGs were by Sandwich Elisa with one week storage using the reagents supplied by Genei, Bangalore, India.

Estimation of Antitrypsin: Oxidative antitrypsin (AT) was estimated using Elisa with a monoclonal antibody against oxidized alpha 1 antitrypsin in which chloramine T oxidized alpha 1 antitrypsin was the antigen [6]. The sensitivity of oxAT measurement was 1.0ng/ml with an inter CV of <6.7%.

Beta 2 Microglobulin Assay: The samples were analyzed by using Enzyme linked immunosorbent assay (B2-microglobulin EIA kit, Immunotech, France). 2.4mg/L was used as upper limit, when 97% of normal values are below this cutoff value.

Estimation of Serum and Urinary ACE Levels: Serum or urine Ace levels were measured by a colorimetric method (Colorimetric assay Kit, Fujizoki assay, Tokyo, Japan) using p-hydroxyhippuryl L-histdyl-L-leucine as the substrate [7].

Statistical Analysis: Statistical work was carried out by using SPSS software for Windows 10.0 (SPSS Inc., Chicago, II, USA) and Microsoft Excel. Values were reported as mean+/−standard deviation. SD was not more than 10%.

RESULTS AND DISCUSSION

The analysis of serum and urinary nicotine analytes have revealed that all the three analytes were found to be increased than the controls to greater extent both in serum and urine samples of the ST users than the controls (Table 1).

Blood glucose level (fasting and post prandial) was assayed with the help of one pick glucometer. These results showed that the values are not significant (p<0.001) and there was not much change when compared to that of control value. Mean values of both fasting and post prandial glucose levels are within the normal range both in control and test samples, which indicates that the selected population did not have history of diabetes or hypertension (Figure 1).

Control subjects showed the creatinine content of 1.43 mg/dl, whereas the ST consumers showed a significant (p<0.05) increase of 2.75 mg/dl, which shows a drastic increase in the serum creatinine value and the loss of renal function. Studies were also conducted to evaluate the glomerular and tubular marker in urine as well as serum of the control and ST consuming people. Transferrin is a plasma protein that transports iron through the blood to the liver, spleen and bone marrow.

Table 1: Analysis of serum nicotine and its metabolites

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Control (Serum)</th>
<th>Test (Serum)</th>
<th>Control (Urine)</th>
<th>Test (Urine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine (ng/ml)</td>
<td>2.7</td>
<td>39.4</td>
<td>3.1</td>
<td>3730</td>
</tr>
<tr>
<td>Cotinine (ng/ml)</td>
<td>1.9</td>
<td>403</td>
<td>2.2</td>
<td>5024</td>
</tr>
<tr>
<td>OH-Cotinine (ng/ml)</td>
<td>2</td>
<td>153</td>
<td>2.7</td>
<td>7789</td>
</tr>
</tbody>
</table>
Here in this present study the level of transferrin seems to be low when compared to that of control. That indicates the chance of anemia due to ST toxicity. Low levels of IgG occur in macroglobulinemia. In this disease, the high levels of IgM antibodies suppress the growth of cells that produce IgG. Other conditions that can result in low levels of IgG include some types of leukemia and a type of kidney damage (nephrotic syndrome). Herein the present study we can find the low levels of serum IgG, but within the normal range indicating the altered renal function.

Alpha 1-antitrysin (A1AT) is produced in the liver. Accumulation of this in liver causes lower levels of A1AT in blood results in the development of liver cirrhosis. Excessive excretion of A1AT through urine indicates the loss of renal function. In present case there is no difference with the control value. It seems to be almost equal, that indicates the normal functioning of liver. Beta 2-microglobulin is a protein found on the surface of many cells. Testing is done primarily when evaluating a person for certain kinds of cancer affecting white blood cells including chronic lymphocytic leukemia, non-Hodgkin's lymphoma and multiple myeloma or kidney disease. In our study it was very interestingly rapid enhancement of B2M was noticed.

The control subjects showed 3.03 mg/L, whereas the ST consuming people showed a maximum increase of B2M to 10.60 mg/L. That shows a drastic increase which indicates the altered renal activity in the test people. There was a significant (P<0.05) change was noticed compared to the normal (Figure 4). This altered range is more supportive for further analysis for the ST mediated renal damage. ACE test analysis was used to measure the blood level of angiotensin-convert enzyme, which converts angiotensin I to angiotensin II and controls blood pressure. Angiotensin-convert enzyme and ACE2 are highly expressed in the kidney. The role of ACE in the development of renal damage is generally accepted. Here in the present study the ACE level seems to be decreased when compared to that of control individuals. Control individuals showed a concentration of 44.97 and ST consuming people showed a concentration of 37.07 indicating a significant (P<0.05) decrease. This indicates the accumulation of angiotensin I (Figure 2).

After identifying the glomerular and tubular markers in the serum, studies has made to know the status of the same markers in the urine to confirm the ST mediated renal failures. From the study it was clear that transferrin levels were hiked in the ST consumers. Control people showed
a value of 195.50 and the ST affected are showing 221.43. Similar results were found in the case of IgG in the urine as well as in serum. Here also we can find the decreased concentration of serum IgG and increased levels of urinary IgG indicates the renal alterations. The control urinary IgG seems to be 34.54 and in the ST threaten people it reaches to 45.41. The results has showed significant (P<0.05) increase. But in the case of AIAT it was changed, where the serum AIAT did not have any significant change. But the study showed altered values of AIAT (Figure 3).

The control individuals have showed 36.61 AIAT, where the ST consumers showed an increased value of 39.96 proved the increase in the excretion of AIAT due to renal failure. Here B2M hasshowed similar pattern of over excretion. Here we can find 3.64 in the ST consuming people where the control value is 1.24. It was found to be drastically increased in the serum as well as in urine of the affected people (Figure 4). The same was also found with ACE levels here the control value is 11.46 and the treated people are showing 13.77, which means over excretion indicates the renal problems (Figure 3). An increase level of B2MG as well as ACE were indicated monitoring the altered kidney function.

Increased urinary levels were found in people with kidney damage and caused by high exposure to the heavy metals cadmium and mercury. Periodic testing of workers exposed to these metals helping in the detection and beginning of kidney damage. B2M is normally cleared by the kidneys at a rate comparable to GFR [8,9], then reabsorbed and catabolized in the tubules and serum levels are inversely related to GFR [10]. Clearance by conventional dialyzers is negligible as these membranes are impermeable to β2m. Production of β2m in normals is 9 mg/ hr/70 kg [8]. Production may be increased in proliferative disorders and rheumatoid arthritis [11] as indicated by high serum levels in the presence of normal renal function.

1. Angiotensin-converting enzyme and ACE2 are highly expressed in the kidney (Figure 3). The role of ACE in the development of renal damage is generally accepted [12]. Individual differences in renal ACE activity predict the susceptibility for proteinuria-associated renal damage in experimental conditions [13, 14]. Furthermore, Ang II is increased in damaged tubules and is suggested to be a possible mediator of renal damage in experimental and human renal disorders [15, 16]. Blockade of the actions of Ang II by ACE inhibitors or AT1 receptor blockers has been proven to effectively reduce blood pressure and proteinuria [17], thereby providing renoprotection. A disrupted balance between intrarenal ACE and ACE2 with consequent low levels of Ang II and high levels of Ang might contribute to the renoprotective mechanisms of ACE inhibitors [18,19].

REFERENCES


