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Status of some selected renal function indicators in cigarette smokers

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ABSTRACT

In recent years, it has become apparent that smoking has a negative impact on renal function, being one of the most important avoidable renal risk factors. This study was aimed at assessing the renal function of cigarette smokers. The study was carried out on one hundred and sixty (160) subjects comprising of one hundred (100) cigarette smokers and sixty (60) apparently healthy non-smokers serving as control. Renal function was assessed by measuring the levels of plasma urea, creatinine, sodium, potassium, chloride and bicarbonate using standard colorimetric methods as indicators. In this study, it was observed that there was no significant difference in the levels of urea, creatinine, chloride and bicarbonate when compared with control. There was significant increase ($p < 0.05$) in sodium level (143.76±5.45) when compared with control (139.85±4.03) ($p<0.05$). There was a significant decrease in potassium level (3.86±0.54) when compared with control (4.08±0.49) ($p<0.05$). Conclusively, Smoking has an acute increasing effect on plasma sodium levels and at the same time decreasing the plasma potassium level. We therefore suggest that clearance test be done on smokers for more detail assessment of their renal function.

1. Introduction

The kidneys have as their function, excretion of waste products of metabolism and play essential homeostatic role in maintenance of the interior of the body by adjusting or controlling the excretion of water and different plasma constituents (Evans, 1978). Electrolytes are minerals found in the blood stream (plasma) and other body fluids that carry electric charges. Electrolyte balance is necessary for normal functioning of cells and organs. They exist in the blood as acids, bases and salts such as sodium, calcium, potassium, chloride, magnesium and bicarbonate.

In recent years, it has become apparent that smoking has a negative impact on renal function, being one of the most important remediable renal risk factors. It has been shown clearly that the risk for high-normal urinary albumin excretion and microalbuminuria increases in smoking compared with non-smoking subjects of the general population. Data from the Multiple Risk Factor Intervention Trial (MRFIT) indicates that at least in men, smoking increases the risk to reach end-stage renal failure (Klag et al, 1996). Smoking is particularly “nephrotoxic” in older subjects, subjects with essential hypertension, and patients with pre-existing renal disease. Of interest, the magnitude of the adverse renal effect of smoking seems to be independent of the underlying renal disease. The mechanisms of smoking-induced renal damage are only partly understood and comprise acute hemodynamic (e.g., increase in BP and presumably intra-glomerular pressure) and chronic effects (e.g., endothelial cell dysfunction).

Increasing evidence suggests that chronic smoking is a risk factor for the progression of nephropathies (Orth et al., 1997). Smoking was implicated in all aspects of the progression of renal disease in type 1 diabetics, as it increases the risk of microalbuminuria and accelerates the rate of progression from microalbuminuria to proteinuria and subsequent renal failure (Stegmay and Lithner, 1987; Orth et al., 1997). Further evidence suggests that smoking has a similar influence on the progression of nephropathies in patients with type 2 diabetes mellitus (olivarius et al., 1993) and polycystic kidney disease (chapman et al., 1994).

However, studies investigating the effect of smoking on renal function in subjects without renal disease are scarce. It was recently reported that effective renal plasma flow was lower in smokers than in non-smokers (Gambaro et al., 1998). Such findings lead to the hypothesis that smoking would not only be a factor involved in the progression of nephropathies, but could also cause renal dysfunction in subjects without documented renal disease. If true, smoking could play a prominent role in the development of renal vascular disease, a major cause of end-stage renal disease, especially in the older population (Valderrabano et al., 1995). Nevertheless, large studies demonstrating that smoking are a threat for renal function in normal subjects are lacking. A cute smoking or nicotine administration indeed reduces glomerular-filtration rate in never-smokers or occasional smokers (Ritz et al., 1998; Halimi et al., 1998), but not in habitual smokers (Halimi et al., 1998). However, chronic smoking was not associated with impaired renal function in normal subjects. In addition, the exact influence of chronic smoking on the physiologic decline in renal function with age is unclear, since longitudinal studies have not addressed this issue (Rowe et al., 1976).

Finally, it is unknown whether chronic smoking affects renal function or represents a cause of renal damage in subjects without pre-existing renal disease; it is also unclear whether the renal effects of smoking are reversible upon discontinuation of smoking (Gambaro et al., 1998). Assessment of the renal effect of smoking in normal subjects could provide valuable information regarding the relationship -yet to be elucidated- between smoking and the progression of nephropathies (Orth et al., 1997).

2. Materials and methods

2.1. Study location

The study was carried out in Ekpoma, Esan-west local government, Edo state, Nigeria. Ekpoma is located in the geographical co-ordinates 6045 N 08 E with a population of one hundred and twenty five thousand, eight hundred and forty two (125,842) (National bureau of statistics, 2006).

2.2. Selection of subjects

One hundred (100) smokers were recruited for the present study after verbal informed consent was granted and sixty (60) apparently healthy non-smokers were also included in the study to serve as control. All the subjects

recruited for this study were males and they have being smoking at least a stick of cigarette per day for the past six months.

Blood samples were collected from the antecubital vein into lithium heparin bottles for biochemical measurements. The blood samples were centrifuged and plasma was separated from cells into plain bottles and stored frozen at -200c until the period of analysis.

2.3. Biochemical analysis

2.3.1. Urea estimation

Plasma urea was estimated by the urease-Berthelots colorimetric method (Sims, 2006).

Urea in plasma is hydrolysed to ammonia in the presence of urease. The ammonia produced is then measured photometrically by berthelot's reaction.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{hypochlorite} + \text{phenol} \longrightarrow \text{indophenols (blue compound)}$$

3test tubes were set for blank, test and standard and 5µl of distilled water was added to the tube for blank. 5µl of standard was added to the tube for standard and 5µl of sample was added into the test tube for sample. 50µl of sodium nitroprusside and urease reagents was added to all the tubes. The tubes were mixed and incubated at room temperature at 370c for 10 minutes. 1.25ml of phenol was added to all the tubes after the completion of first incubation and 1.25ml of sodium hypochlorite was added to all the tubes and the tubes were mixed and incubated at 370c for 15 minutes. The absorbance of sample and standard was read against blank.

$$\text{Urea concentration} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (mg/dL).}$$

2.3.2. Creatinine estimation

Creatinine was estimated by the Jaffe's method (Toora and Rajagopal, 2002).

Creatinine reacts with picric acid in alkaline solution to form a colored compound. The rate of formation of the colored compound is proportional to the creatinine concentration when compared with a standard.

3test tubes representing blank, standard and test were used for the test.100µl of distilled water was pipetted into the test tube for blank and 100µl of standard was pipetted into the test tube for standard.100µl of sample was pipetted into the test tube for test after prior deproteinization with trichloroacetic acid. 1ml of reagent was added to all the test tubes and the tubes were mixed and absorbance was read after 30 seconds against reagent blank and 2 minutes.

$$\text{Creatinine concentration} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (mg/dL).}$$

2.3.3. Sodium estimation

Sodium estimation was carried out by colorimetric method (Goldzieher and Stone, 1948).

Sodium is precipitated as a triple salt, sodium magnesium uranyl acetate, with the excess uranium being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

2.3.3.1. Filtrate preparation

Three test tubes were labelled as blank, standard and sample. 1.0ml of filtrate reagent was pipetted to all tubes. 50µl of the sample, blank and standard were added to the corresponding tubes. All the tubes were shaken vigorously and mixed continuously for 3 minutes. The tubes were centrifuged at high speed for 10 minutes.

2.3.3.2. Color development

Test tubes were labelled as blank, standard and sample and 1.0ml of dilute acetic acid was pipetted to all the tubes. 50µl of supernatants were added to respective tubes. 50µl of colour reagent was added to all tubes and absorbance was read at 550nm.

$$\text{Sodium concentration} = \frac{\text{absorbance of blank} - \text{absorbance of Standard x conc. of standard (mmol/L)}}{\text{absorbance of test} - \text{absorbance of Standard}}$$

2.3.4. Potassium estimation

Potassium was estimated by colorimetric method (Matthieu and Jose Roberto, 1992).

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration.

Test tubes were labelled as standard, test and blank and 1.0ml of potassium reagent was pipetted to all the tubes. 0.01ml (10µl) of standard, sample and distilled water was added to respective tubes.

All the tubes were mixed and allowed to stand at room temperature for 3 minutes and absorbance was read at 500nm against blank using a spectrophotometer.

$$\text{Potassium concentration} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (mmol/L).}$$

2.3.5. Chloride estimation

Chloride was estimated by colorimetric method (Goldzieher and Stone, 1948).

$$Hg(SCN)_2 + 2Cl_2 \longrightarrow HgCl + 2SCN^-$$
$$3SCN^- + Fe^{3+} \longrightarrow 4Fe(SCN)3 \text{ red complex}$$

Chloride ions form a soluble, non-ionized compound with mercuric ions and will displace thiocyanate. The released thiocyanate ions react with ferric ions to form a colour complex that absorbs light at 480nm. The intensity of colour produced is directly proportional to the chloride concentration.

Test tubes were labelled as blank, standard and sample 1.5ml chloride reagent was pipetted into all the tubes. 0.01ml (10µl) of distilled water, standard and sample was added to respective tubes and the tubes were incubated at room temperature for 5 minutes and absorbance was read at 480nm against blank.

$$\text{Chloride concentration} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc. of standard (mmol/l)}$$

2.3.6. Bicarbonate estimation

Bicarbonate was estimated by enzymatic/kinetic method as described (Micheal et al., 1987)

$$\text{Phosphoenol pyruvate} + HCO^{-3} \xrightarrow{\text{PEPC}} \text{oxalate} + H_2PO_4$$

$$\text{Oxalate} + \text{NADH} \xrightarrow{\text{MDH}} \text{malate} + \text{NAD}$$

Phosphoenol pyruvate carboxylase (PEPC) catalyzes the reaction between phosphoenol pyruvate and carbon dioside (bicarbonate) to form oxalacetate and phosphate ion. Oxalacetate is reduced to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to NAD; the reaction is catalyzed by malate dehydrogenase (MDH). This results in a decrease in absorbance at 340nm that is directly proportional to bicarbonate concentration in the sample.

Test tubes were labelled as blank, standard and test and 1.0ml of bicarbonate reagent was pipetted into all the tubes. All the tubes were incubated for 3 minutes at 370c. 5µl of water, standard and sample was pipette into cuvettes labelled blank, standard and sample. It was mixed gently by inversion and incubated for 5 minutes and absorbance was read for all the cuvettes at 340nm.

$$\text{Bicarbonate concentration} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (mmol/l).}$$

3. Results

Table 1 showed the mean ± SD of sodium, potassium, urea, Creatinine, chloride and bicarbonate in smokers compared with control. The sodium level was significantly increased while the potassium level was significant decreased of smoker when compared with control. There was no significant difference between smokers and control for other indicators studied.

Table 1
Mean ± SD of renal function indicators of smokers compared with control.

Parameter	Smokers (mean ± SD) N = 100	Non smokers (mean ± SD) N = 60	t-value	p-value	Remark
Urea (mg/dl)	10.92±5.32	11.61±4.95	0.710	>0.05	NS
Creatinine (mg/dl)	1.09±0.29	1.02±0.20	1.569	>0.05	NS
Na^+ (mmol/l)	143.76±5.45	139.85±4.03	2.306	<0.05	S
K^+ (mmol/l)	3.86±0.54	4.08±0.49	2.142	<0.05	S
Cl^- (mmol/l)	95.23±7.04	97.23±3.75	1.760	>0.05	NS
HCO^{-3} (mmol/l)	24.18±2.76	24.51±2.79	0.623	>0.05	NS

S =Significant, NS = Non significant, N=Number

4. Discussion

Cigarette smoke consists of many chemicals, including nicotine, tar and many carcinogens and gaseous compounds (Pomerleau, 1992); some of these compounds have neuro-endocrine effects. In this study, effect of cigarette smoking on renal function was investigated in one hundred and sixty (160) subjects comprising of one hundred (100) smokers and sixty (60) apparently healthy non-smokers as control. In this study, there was no significant change in plasma urea and creatinine, the major markers of renal function. The reason for this insignificant difference may be because of the dose and duration of smoking of the subjects recruited for this study which may not be significant to cause extensive renal damage that will lead to abnormal levels of urea and creatinine. For abnormal levels of urea and creatinine to be evident, about 60% of the kidney must have been damaged (Stevens et al., 2006). Plasma sodium level was significantly increased among smokers compared to control. This may be due to cigarette smoke stimulation of the adrenal cortex, which leads to increase of circulatory cortisol (Tziomalos and Charsoulis, 2004) that increases sodium retention and urinary potassium loss and also increases the glomerular-filtration rate (GFR). In addition, cigarette smoke stimulates the adrenal medulla to secrete adrenaline that increases the GFR via its action on blood vessels and may also facilitate pituitary ACTH secretion (Reisine et al., 1984). Moreover, there was a decrease in Plasma potassium levels among smokers in comparison to non smokers (control); Cigarette smoke contains glycyrrhizinic acid that mimics aldosterone action which increases urinary potassium excretion (Philip, 1994). Longstanding intracellular potassium depletion causes extracellular alkalosis. Prolonged potassium depletion impairs the renal concentrating mechanism and may cause polyuria with potassium depletion (Philip, 1994).

In the present study no significant change was observed in plasma chloride concentration in smokers when compared to controls since people consume enough salt which is very high in chloride. There was no significant difference in bicarbonate between smokers and non smokers. The reason for this insignificant difference may be because of the dose and duration of smoking of the subjects recruited for this study which may not be significant to cause extensive renal damage that will lead to abnormal metabolism (metabolic acidosis) with consequent bicarbonate imbalance.

5. Conclusion

In conclusion, Smoking has an acute increasing effect on sodium levels and at the same time decreasing the potassium level. We therefore suggest that clearance test be done on smokers for more detail assessment of their renal function.

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References

Chapman, A.B., Johnson, A.M., Gabow, P.A., Schrier, R.W., 1994. Overt proteinuria and microalbuminuria in autosomal dominant polycystic kidney disease. J. Am. Soc. Nephrol., 5,1349–1354.

Evans, D.B., 1978. Acute Renal Failure. Br . J. Hosp. Med., 19,597-604.

Gambaro, G., Verlato, F., Budakovic, A., Casara, D., Saladini, G., Del Prete, D., Bertaglia, G., Masiero, M., Checchetto, S., Baggio, B., 1998. Renal impairment in chronic cigarette smokers. J. Am. Soc. Nephrol., 9,562–567.

Goldzierher, J.W., Stone, G., 1948. The colorimetric determination of sodium and its application to the study of sodium and chloride balance. J. Clin. Endocrinol. Metab., 8(7),620.

Halimi, J.M., Philippon, C., Mimran, A., 1998. Contrasting renal effects of nicotine in smokers and never-smokers. Nephrol Dial Transplan, 13, 940–944.

Klag, M.J., Whelton, P.K., Randall, B.L., Neaton, J.D., Brancati, F.L., Ford, C.E., Shulman, N.B., Stamler, J., 1996. Blood pressure and end-stage renal disease in men. N. Engl. J. Med., 334,13– 18.

Matthieu, T. Jose Roberto, O.T., 1992. Turbidimetric determination of potassium of potassium with tissue with sodium tetraphenylboron. Commun. Soil Sci. Plant Anal., 23(2), 123-128.

Micheal, M.B., Dereck, P.H., Brian, A.N., Fritz, L., 1987. Utilization of bicarbonate by apple fruit phosphoenolpyruvate carboxylase. Phytochem., 26(9), 2475-2476.

Olivarius, N.F., Andreasen, A.H., Keiding, N., Mogensen, C.E., 1993. Epidemiology of renal involvement in newly diagnosed middle-aged and elderly diabetic patients: Cross-sectional data from the population-based study "Diabetes Care in General Practice," Denmark. Diabetologia, 36,1007–1016.

Orth, S.R., Ritz, E., Schrier, R.W., 1997. Renal risks of smoking. Kidney Int; 51, 169–1677.

Philip, D.M., 1994. Clinical chemistry in Diagnosis and treatment, 6th Edn, ELBS Publication: Pp. 63-66.

Pomerleau, O.F., 1992. Nicotine and the central nervous system: biobehavioral effect of cigarette smoking. Am. J. Med., 93 (1A), 2S-7S.

Reisine, T.D., Mezey, E., Palkovits, M., Heisler, S., Axelrod, J., 1984. Bet adrenergic control of adrenocorticotrophic hormone release from the anterior pituitary. Catecholamines: Neuropharmacology and central nervous system. Thoeretical aspect: Alan, R. Liss, New York, Pp. 419-423.

Ritz, E., Benck, U., Franek, E., Keller, C., Seyfarth, M., Clorius, J., 1998. Effects of smoking on renal hemodynamics in healthy volunteers and in patients with glomerular disease. J. Am. Soc. Nephrol., 9,1798–1804.

Rowe, J.W., Andres, R., Tobin, J.D., Norris, A.H., Shock, N.W., 1976. The effect of age on creatinine clearance in men: A cross-sectional and longitudinal study. J. Gerontol., 31, 55–63.

Sims, G.K., 2006. Using the berthelot method for nitrites and nitrates analysis. Soil Sci. Soc. Am. J., 70(3), 1038.

Stegmay, B., Lithner, F., 1987. Tobacco and end-stage diabetic nephropathy. BMJ., 295, 581–582.

Stevens, L.A., Coresh, J., Greene, T., Levey, A.S., 2006. “Assessing kidney function in measured and estimated glomerular filtration rate”. The New England J. Med., 345 (23), 2473-2483.

Toora, B.D., Rajagopal, G., 2002. Indian J. Exp. Biol., 40(3), 352-354.

Tziomalos, K., Charsoulis, F., 2004. Endocrine effect of tobacco smoking. Clin. Endocrinol., 61(6), 664-674.

Valderrabano, F., Jones, E.H.P., Mallick, N.P., 1995. Report on management of renal failure in Europe, XXIV, 1993. Nephrol Dial Transplant., 10(5), 1–25.