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Research Article

Impact of Tobacco Smoking on Renal Function Tests in Asymptomatic Individuals

Fateheya M Metwally, Asmaa Mahmoud Mohammed*

Environmental and Occupational Medicine Department, National Research Centre.

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ABSTRACT

Objectives of this work was studying the effect of smoking on renal function tests among apparently healthy smokers. Study design: This is a cross sectional study was implemented on 53 active tobacco smokers and 72 nonsmokers (control group). Methods: we studied the effect of smoking on renal functions using both conventional renal function tests namely; serum urea, serum creatinine, estimated glomerular filtration rate and one of the urinary early renal biomarkers namely; N acetyl-B-D glucosaminidase as an indicator for renal impairment. The results revealed statistically significant higher activity of the Urinary N-Acetyl-B-D- glucosaminidase activity (NAG index), (P-value=0.001) among smokers when compared to their matched nonsmokers. The proportion of smokers having elevated NAG activity (45.2%) was significantly higher than that among non-smokers (18.4%) with statistically significant difference (p-value=0.01). Conclusion: Results of this study revealed detection of early renal effects among asymptomatic tobacco smokers which couldn't be detected by routine kidney function tests.

Keywords: N acetyl-B-D glucosaminidase, NAG index, Renal Function, Smoking.

INTRODUCTION

The burden of chronic kidney disease is growing throughout the past three decades; the incidence and prevalence of end stage renal disease (ESRD) have risen progressively[1]. It is clear that an individual's likelihood of developing progressive Chronic kidney disease results from complex interactions between multiple genetic (none modifiable factors) and environmental factors (modifiable factors)[2]. Identification of the modifiable and controllable risk factors is an important first step in understanding and hopefully, reversing the increasing incidence of such disease[3]. Since 2003, smoking as a renal risk factor has been addressed in several studies. Two studies found no effect of smoking on renal function[4]. The Multiple Risk Factor Intervention Trial (MRFIT) investigated 332,544 men and documented that smoking was significantly associated with an increased risk for ESRD[5] but the magnitude of this effect was not reported. Due to the large reserve capacity of the kidney, kidney diseases are diagnosed at a rather late stage, which can be irreversible and end up in end-stage renal failure. It is therefore important to detect kidney defects as early as possible to prevent progression of the disease[6]. The kidney function can be evaluated by a number of methods, including the assessment of urinary enzymes such as *N*-acetyl-β-D glucosaminidase. Enzyme activity is normally low in urine and may increase when renal tubular cells are injure[7]. The aim of this work is to study the impact of cigarettes smoking on renal functions, using both conventional renal function tests as well as early renal urinary sensitive biomarker *N*-acetyl-β-D glucosaminidase enzyme as an indicator for renal impairment, in asymptomatic individuals.

METHODS

Study subjects and data collection

This is a cross sectional study was implemented on 137 individual; 53 active tobacco smokers and 72 nonsmokers (control group). The control group was randomly selected and matched with the case subjects in their gender, occupational career and age group. Active smokers are defined based on self reported smoking status as those who are currently smoke at least one cigarette per day. The non smokers were defined as those who never smoke cigarette or shisha in their life. The both groups were asymptomatic and haven't any history of renal disease.

Those who were diabetic, hypertensive, exposed to nephrotoxic substances (occupationally or recreationally), receiving long term analgesics or had family history of renal disease were excluded from the study.

All participants were subjected to an interview to complete a prepared field tested questionnaire form, full clinical examination; included general and abdominal examination with emphasis on the signs of chronic renal disease. Smoking history was taken and pack-years of cigarettes was calculated individually for each smoker as the number of *cigarettes* smoked per day/20) × number of years smoked. For example, 1 pack year of cigarettes for an individual is equal to smoking 1 pack of cigarettes per day for 1 year, or 2 packs per day for half a year, and so on. The smokers then classified into two groups using 20 pack-years of cigarettes as a cut off value.

**Author for Correspondence asmamahdy@yahoo.com:*

Anthropometric measurements were performed (weight and height**)** and the body mass index (BMI) was calculated as the individual's body weight divided by the square of his height (kg/m^2)[8]. The blood pressure was measured by maintaining the arm-cuff position at the heart level during rest in seated position. Two readings were taken (1-2min. interval) and the mean value of the two measurements was used. The blood pressure was measured by auscultation method using mercury sphygmomanometer[9]. Mean arterial pressure (MAP) (the average blood pressure in single cardiac cycle in an individual) was calculated using the following formula: MAP = [(2 x diastolic) + systolic] divided by 3**.** MAP from 70 to 110 mmHg is considered normal[10].

Blood samples were collected by sterile disposable syringes. Each sample was left to clot and centrifuged. The separated serum was used for determination of conventional Kidney function tests namely; Serum urea, Creatinine and calculated glomerular filtration rate**.** The enzymatic method for determination of urea concentration in serum was used[11] with reference values 0.15-50mg/dl.Values above 50mg/dl have considered elevated. A colorimetric, alkaline picrate method (Jaffè) was used for determination of creatinine concentration in serum[12] with Reference values 0.6-1.4mg/dl for males. Values above 1.4mg/dl have considered elevated. High performance diagnostic reagent kits had been used for determination of Serum urea and creatinine in this study. The glomerular filtration rate (GFR) was calculated individually for each person using *Modification of Diet in Renal Disease (MDRD)* formula that recommended by **NKF-K/DOQI** clinical practice guidelines. This formula depends on 4 variables (serum creatinine, age, gender and race):

Estimated GFR= 186 X (serum creatinine) $^{-1.154}$ X (age) $^{-0.203}$ X (0.742 if female) X (1.212 if African American).

Urine samples were collected in sterile labeled containers in the morning from all individuals then centrifuged to remove insoluble salts and debris. Aliquots were stored at -20°C for the estimation of. *N*-acetyl-β-D glucosaminidase (NAG) using the colorimetric method[13] with normal reference values 4.2±1.2mU/mg of urinary creatinine. NAG activity is known to vary with age and diuriesis; hence, a NAG index (ratio of NAG activity to urinary creatinine) was calculated to minimize variability[14]. A commercial kits (FAR NAG kit) for colorimetric determination of N-Acetyl-B-D-Glucosaminidase in urine and in serum manufactured by FAR srl via Fermi, 12-37026 Pescantina – VERONA-ITALY.

Ethical considerations

Informed consent was taken from the participants with assured confidentiality**.** The study protocol was approved by the Ethics Committee of the national research center prior to the work.

Statistical analyses

The collected data, the clinical and laboratory results have been computerized and coded using SPSS version 18.0 soft ware (Statistical Package for Social Science) and statistically analyzed. Data were expressed as mean values ± standard deviation (SD). Ranges, frequency of distributions were estimated for quantitative variables. The mean of quantitative variables of the two comparable groups (smokers group and non smokers group) was compared using the Independent-Samples Student's t-test. The Mann-Whitney U test was used to compare between the two groups in case of skewed data. The significance of differences between proportions was tested by the Chi-square test ($\chi 2$). The Differences were considered significant with P value ≤ 0.05. The correlations between individual variables were tested using Pearson correlation coefficient (r) Values $\leq$ 0.05 were considered statistically significant. Linear regression analysis was performed to predict the effect of change in one variable based on change in other one (CI 95 %.).

RESULTS

All studied individuals of both groups were males. The smokers group consists of 53 active smokers with mean age 41.4±11 years old. The control group (non smokers) consists of 72 nonsmokers with mean age 40.2±9.9years old. They are matched with the smokers group in age, gender and occupational career. No signs of renal impairment were observed in the two groups. No statistically significant differences were observed between smokers and nonsmokers either in their mean arterial blood pressure (88.7±10.7mmHg) and (88.4± 10.7mmHg) respectively, (P-value=0.8) or BMI (27.8 ±7.3 kg/m^2) and (28.3 ±6.7 kg/m^2) respectively, (p-value=0.7).

(Table 1) Clarifies the statistically significant difference (P-values=0.005 &0.024 respectively) in mean values as well as in mean ranks of the Urinary –N-Acetyl glucosaminidase (NAG index) between smokers and non-smokers with higher values among smokers group. No statistically significant differences are observed between smokers and nonsmokers in their routine renal function tests.

Table (2) shows that the proportion of smokers having elevated NAG activity (45.2%) is higher than that among non-smokers (18.4%) with statistically significant difference (p-value=0.01).

In Fig.1 we classified the smokers group into two groups according to their pack-year of cigarettes. Statistically significant difference is observed in median values of urinary NAG index between smokers that have pack-year of cigarettes ≥ 20 and those have pack- year of cigarettes < 20 with higher NAG activity among the former group.

Figure (2) shows strong positive correlation between NAG activity and Number of pack-years of cigarettes among smokers (r=0.4 & p-value=0.02).

DISCUSSION

Smoking as a cause of renal injury is a recent perception in the history of nephrology. Studies showed that cigarette smoking is a risk factor for the development and progression of chronic kidney disease (CKD) in community[15]. In the last decade of the last century, studies demonstrated that smoking causes deterioration in the renal function of patients with kidney diseases

Table 1: Statistical comparison between the routine renal function tests of smokers and non-smokers.

Renal function test	Smokers (n=53)	Nonsmokers (n=72)	p- value
Serum urea (mg/dl)	30±8.1[a] (62.8)[b]	30.4±8.8[a](63.1)[b]	0.8
Serum Creatinine(mg/dl)	1±0.2[a](62.9)[b]	1±0.3[a] (63)[b]	0.7
Glomerular filtration rate (GFR) (ml/min/1.73m)	94±30.5[a](62.2)[b]	98±47.8[a](63.5)[b]	0.6
Urinary –N-Acetyl glucosaminidase (NAG index)(mu/mg cr)	6.7±8.2[a](47.9)[b]	2.7±3.8[a](35.8)[b]	0.024*

[a] Mean±SD, [b]Mean Rank

Table 2: Distribution of NAG activity among smokers and non-smokers.

Renal biomarker	Cut off value	Frequency of normal and abnormal values				Pearson Chi-Square test
		Smokers group (n=31)		Nonsmokers group (n=49)		
		NO.	%	NO.	%	
Urinary –N-Acetyl glucosaminidase (NAG index)	5.4 mu/mg cr.					$\chi 2$=6.6 df=1 p-value=0.01*
Normal		17	54.8	40	81.6	
Elevated		14	45.2	9	18.4	

Table 3: Regression coefficient table to predict the effect of increase in pack –year of cigarettes on NAG activity.

	Unstandardized Coefficients		Standardized Coefficients	t-value	p-value
	B	Standard Error	Beta		
Pack –year of cigarettes	0.3	0.1	0.4	2.4	0.02*

including hypertensive nephrosclerosis, glomerulonephritis and diabetic nephropathy[16]. This study was conducted on 53 active smokers and 72 matched nonsmokers to investigate the effect of active cigarette smoking on their renal function. General examination of the two groups showed absence of symptoms and signs of chronic kidney disease. Serum creatinine and serum urea or blood urea nitrogen (BUN) are commonly ordered tests to detect renal dysfunction[17]. The sensitivity of serum urea is 50% and specificity 87% as a marker of renal dysfunction[18]. Serum creatinine is a crude marker that is the most widely used to predict glomerular filtration rate (GFR)[19]. It is poor marker to detect early stages of chronic kidney disease because its levels do not significantly increase until the GFR is reduced to less than 50% of normal[20]. Based on all previous statements we could explain our findings in table (1) where no association was detected between the smoking status of studied groups and the routine conventional renal function tests namely, serum urea, serum creatinine and estimated glomerular filtration rate(P-value>0.05). Recently, there has been a great interest in identifying novel sensitive biomarkers that can be easily detected in the urine to diagnose renal injury at earliest stages[21]. N-acetyl-B-D-glucosaminidase (NAG) is especially very sensitive indicator of kidney parenchymal damage when compared to functional measurements, such as glomerular filtration rate (GFR), creatinine[22]. In this study we used urinary *N*-acetyl-β-D glucosaminidase (NAG) index as early biomarker for renal impairment. We noticed that the smokers group showed significantly higher mean values 6.7±8.2 mu/mg.cr., as well as mean ranks (47.9) of NAG index than mean values 2.7±3.8 mu/mg.cr., and mean rank (35.8mu/mg.cr) among nonsmokers group (p-value=0.024) (table1).On studying the proportion of smokers that have elevated NAG activity using cut off value 5.4mu/mg.cr which is the upper limit of the referent value, we found that 45.2% of smokers versus 18.4% of non-smokers (p-value=0.01) have had significant elevated enzyme activity (table 2).

To study the effect of intensity in cigarette smoking on NAG activity, a pack-year of cigarettes was calculated individually for each smoker, then the smokers group has been classified into two groups using cut off value 20 pack-year of cigarette. Statistically significant difference in median values of NAG index have been observed between the smokers that have pack-year of cigarettes ≥20 and those having pack-year <20, with higher values among the former group (4.2mu/mg.cr, 1.4mu/mg.cr respectively), (P-value=0.05) as shown in figure (1). Furthermore, the NAG activity was significantly directly correlated with the number of pack-year of cigarettes among smokers (r=0.4 & p-value=0.02) as illustrated in Figure (2). The linear regression analysis has significantly predicted that each increase one pack-year of cigarette for an individual smoker may increase the level of his NAG index by 0.3mu/mg.cr (Table 3), the effect that may initiate the renal impairment in healthy individuals or enhance the progression of pre-existing renal disease in renal patients then worsen the scenario if untreated. Our findings are in agreement with other studies[23,24,25]. It adds a new evidence that smoking, particularly heavy smoking (≥20 pack-year cigarette) i.e. consumption of 20 cigarrete/day or more for 20 years or 40 cigarrete or

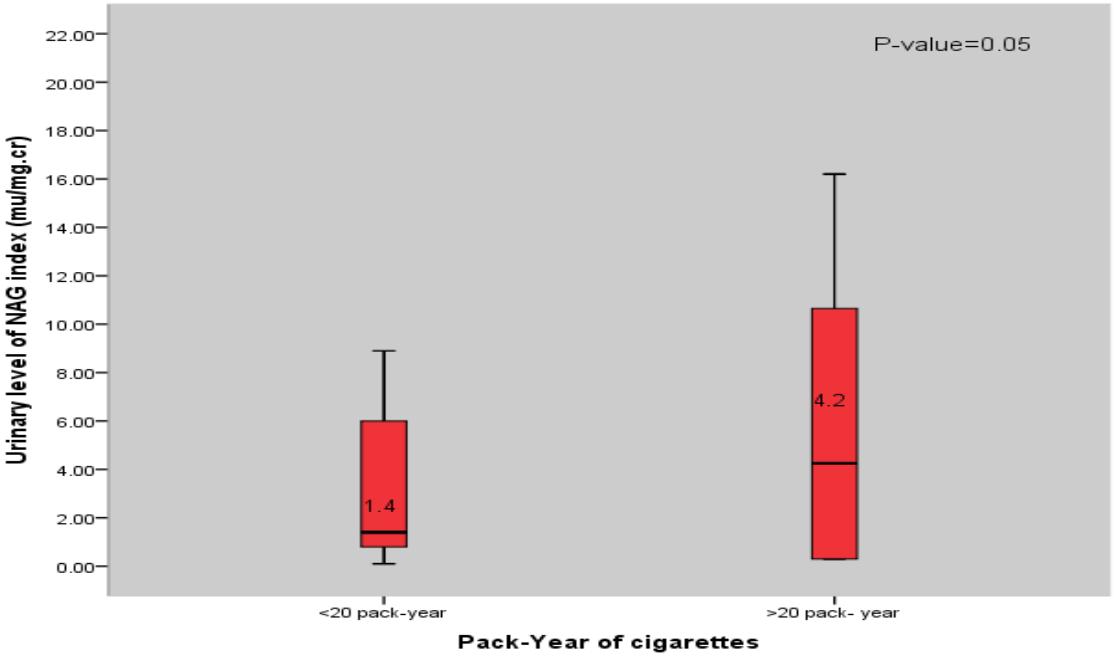

Figure 1: Box plot shows the association between the NAG activity of smokers and their pack- years of cigarettes.

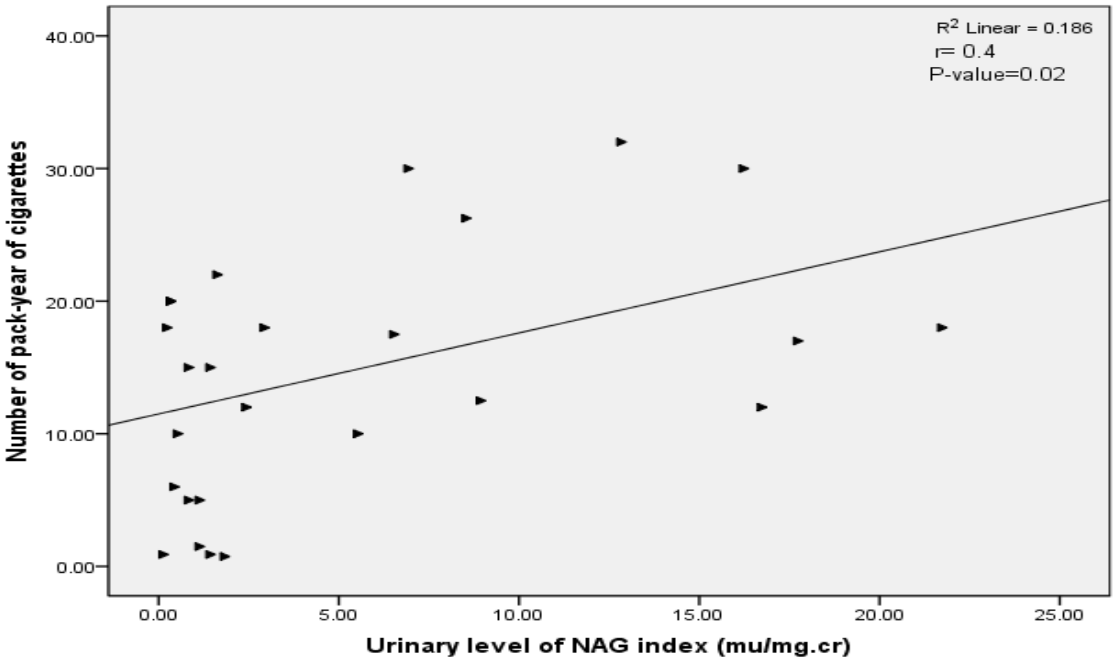

Figure 2: Scatter plot diagram shows the positive correlation between NAG activity and Number of pack-years of cigarettes among smokers.

more/day for 10 years and so on, is an important risk factor to the development of renal impairment in individuals with apparently normal kidneys. This result raises the importance of smoking cessation as a preventive measure to decrease the incidence of chronic kidney disease.

AUTHORS' CONTRIBUTIONS

AM and FM participated in the study design and selection of patients. FM was the principle investigator for the funding project.AM made statistical analysis and interpretation of data. AM and FM wrote the drafting manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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