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CLINICAL STUDY

Investigation of the Relationship between Serum Levels of Cotinine and the Renal Function in Active and Passive Smokers

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Abstract

Objective: We have investigated the effects of active and passive smoking on renal functions in terms of glomerular filtration rate, microalbuminuria, and β-2 microglobulin excretion. *Design and method*: The volunteers included in this study were classified into three groups as active smokers (n = 24), passive smokers (n = 20), and controls (n = 20). Blood and urine samples were collected from all groups. Serum glucose, urea, creatinine, and cotinine levels in the collected blood samples were measured. Also, microalbumin, β-2 microglobulin, and creatinine levels were measured in the collected urine samples. *Results*: Serum cotinine levels were found to be higher in both passive and active smokers when compared with controls ($p < 0.01$), whereas urinary microalbumin and creatinine levels were significantly higher in active smokers ($p < 0.01$). The urinary microalbumin/creatinine ratio was significantly increased in both active and passive smokers compared with controls. *Conclusion*: The kidney and the glomerular functions may be affected even by passive smoking. In addition, increased microalbumin/creatinine ratio may be a sign of increased atherosclerosis risk in these persons.

Keywords: Active smokers, passive smokers, microalbuminuria, β-2 microglobulin, cotinine

INTRODUCTION

There are numerous harmful substances found in tobacco and tobacco smoke. Nicotine is one of these substances, which may be inhaled during active and passive smoking.[1,2]

Cigarette smoking is a major risk factor for vascular diseases and various forms of cancer. The kidney is an important target for negative effects induced by smoking. Chronic cigarette smoking has serious adverse effects on renal functions of patients with primary hypertension, primary glomerular diseases, or diabetic nephropathy and on patients undergoing chronic hemodialysis. It is an independent predictor of albuminuria in patients with primary hypertension and it has been linked to the development of microalbuminuria or proteinuria and chronic renal failure in patients with diabetes. Smoking also plays a pathogenetic role in the development of idiopathic nodular glomerulosclerosis.[3]

Smoking has harmful effects on albumin excretion because it increases the risk of microalbuminuria; shortens the interval between the onset of diabetes and the start of albuminuria or proteinuria; accelerates the rate of progression from microalbuminuria to persistent proteinuria; and pathologically promotes the progression of diabetic nephropathy to end-stage renal disease.[1,4]

A quantitative measure of smoking exposure is cotinine, a nicotine metabolite, which can be measured in several body fluids, including saliva, plasma, and urine. Cotinine measurement has additional benefit to expired air carbon monoxide as an objective measure of active smoking because it has a longer half-life and represents a quantitative, daily measure of exposure to tobacco.[5,6]

Previous studies performed on cigarette and renal functions[7–10] mainly investigated the effects of smoking

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on renal damage especially in diseases such as hypertension and diabetes mellitus.

Thus, we have investigated whether early kidney damage occurs in people who have no known diseases and who are considered healthy and are exposed to cigarette smoke both actively and passively.

MATERIAL AND METHODS

The subjects of this study were selected relatives of the patients who came to our hospital due to several diseases and the volunteers frequented public places such as cafes where cigarette smoke was dense. Volunteers who had no previous medical complaints or symptoms and were not exposed to cigarette smoke were selected as control subjects.

All participants submitted informed written consents, and the study protocol was approved by the Ethics Committee of the College of Medicine of Yüzüncü Yıl University in line with the Declaration of Helsinki.

The subjects included in this study were divided into three groups as follows:

Group I (active smokers; $n = 24$): This group consisted of subjects who smoked an average of 20 cigarettes per day.

Group II (passive smokers; $n = 20$): This group consisted of subjects who did not smoke but were exposed to cigarette smoke because of the active smokers they were living with for at least 5–6 hours during the day.

Group III (control group; $n = 20$): This group consisted of healthy subjects who did not smoke and who did not encounter smokers.

A great attention was paid to the subjects in all three groups to make sure that they did not have any known diseases such as hypertension or diabetes mellitus. Fasting blood samples of the subjects were drawn for biochemical analysis. Urine samples were also collected after the first urine of the morning. Blood and urine samples were then centrifuged at 2000 rpm for 10 min in a refrigerated centrifuge to separate serum samples. Samples were stored in plastic tubes at –70°C until the day of analysis. Serum cotinine, urine microalbumin, and β-2 microglobulin levels were measured using commercial kits, which were solid-phase, two-site chemiluminescent immunometric assays (Immulite, DPC, Los Angeles, CA, USA). Urine creatinine, serum glucose, urea, and creatinine levels were determined by routine colorimetric methods on an autoanalyzer (Roche Modular Autoanalyzer; Roche, Tokyo, Japan). Glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease formula[11]:

$$\mathrm{GFR\,(mL/\,min/1.73\,m^2)} = 186 \times (\mathrm{S_{cr}})^{-1.154} \times (\mathrm{Age})^{-0.203} \times (0.742 \text{ if female})$$

Statistical Analysis

Kolmogorov–Smirnov goodness-of-fit test was used to check whether distribution of parameters is normal. Means and standard error of means were calculated, and differences between means were assessed by one-way analysis of variance. Differences among groups were measured by the post hoc Tukey test. Results were given with standard error of the mean (mean ± SE). Some parameters did not have normal distribution. Thus, groups were compared using the Kruskal–Wallis test. However, chi-square test was used to define the relationship between the categorical variations and the groups. In all statistical calculations, the level of significance was considered to be 5% and the calculations were done with SPSS (Statistical Package for the Social Sciences, version 11.5, SPSS inc., Chicago, USA).

RESULTS

The urine microalbumin levels were increased in active smokers compared with the passive smokers and controls. This difference was statistically significant compared with the control group ($p < 0.01$). The urine microalbumin/creatinine ratio was significantly higher in both active and passive smokers compared with the control group ($p < 0.01$). There were no significant differences between GFR rates of the groups ($p > 0.05$) (Table 1).

The serum cotinine levels were significantly higher in active smokers than in both passive smokers and control subjects ($p < 0.01$). However, the difference in cotinine levels between the passive smokers and the control subjects was not statistically significant ($p > 0.05$). Serum glucose levels of the active smokers were significantly lower than those of the passive smokers and the control subjects ($p < 0.05$) (Table 2).

In addition, the number of subjects with urine microalbumin/creatinine ratio above or below 30 mg/g, which is the clinical limit to confirm presence of microalbuminuria, was determined. The rate was higher than 30 mg/g in 3 of 24 active smokers and in 2 of 20 passive smokers. The rate was lower than 30 mg/g in all subjects in the control group. There was no statistically significant difference between microalbumin/creatinine ratio of the groups ($\chi^2 = 2.47$, $p = 0.294$) (Table 3).

DISCUSSION

There are limited studies in the literature that analyze the relationship between exposure to cigarette smoke and early kidney pathologies. Many previous studies have investigated the status of renal functions in existing diseases. However, this study was conducted on subjects who had no known diseases and who were healthy. In this respect, the rate of exposure to cigarette smoke was defined by observing the serum cotinine levels of the subjects in all three groups involved in this study. The serum cotinine levels in active smokers were

Table 1. The diagnostic statistical values of urine parameters in active smokers, passive smokers, and control groups and the comparative results.

		Active smokers (n = 24)	Passive smokers (n = 20)	Controls (n = 20)
Gender	Male	20	11	8
	Female	4	9	12
Age (years)		33.46 ± 6.73	33.35 ± 9.53	27.55 ± 8.59
Microalbumin (μg/mL)		**27.25 ± 12.74*,****	**14.41 ± 3.12*****	**9.03 ± 3.58**
β-2 microglobulin (ng/mL)		80.42 ± 18.78	67.30 ± 9.79	49.53 ± 7.61
Creatinine (g/L)		**0.98 ± 0.08#**	**1.00 ± 0.10#**	**0.70 ± 0.10**
Microalbumin/creatinine (mg/g)		**13.73 ± 1.93***	**11.53 ± 2.65***	**5.65 ± 0.10**
GFR (mL/min/1.73 m^2)[a]		96.54 ± 3.79	82.95 ± 3.80	91.05 ± 5.21

Notes: Data are expressed as mean ± SE.
[a]Glomerular filtration rate (GFR) has been estimated using the Modification of Diet in Renal Disease (MDRD) formula.
*,**$p < 0.01$, ***,#$p < 0.05$; *,***,#comparison with control group; **the degree of significance of comparison between active and passive groups.

Table 2. The diagnostic statistical values of serum parameters in active smokers, passive smokers, and control groups and the comparative results.

	Active smokers (n = 24)	Passive smokers (n = 20)	Controls (n = 20)
Cotinine (ng/mL)	**365.73 ± 52.1*,****	**1.92 ± 0.26**	**0.10 ± 0.02**
Glucose (mg/dL)	**84.79 ± 2.07*****	**102.20 ± 3.04**	**95.80 ± 3.10**
Urea (mg/dL)	35.34 ± 2.21	37.84 ± 2.88	34.32 ± 1.69
Creatinine (mg/dL)	0.93 ± 0.04	1.02 ± 0.05	0.94 ± 0.05

Notes: Data are expressed as mean ± SE.
*,**$p < 0.01$, ***$p < 0.05$; *,***comparison with control group; **the degree of significance of comparison between active and passive groups.

Table 3. The relation between microalbumin/creatinine ratio of the groups.

		Microalbumin/creatinine		Total
		<30	≥30	
Groups	Actives	21	3	24
	Passives	18	2	20
	Controls	20	0	20
Total		59	5	64

Note: $\chi^2 = 2.47$, $p = 0.294$.

significantly higher than that of the passive smokers and the control subjects ($p < 0.01$). However, there was no significant difference in cotinine levels between the passive smokers and the controls ($p > 0.05$).

In a study conducted by Jones-Burton et al.,[5] the relationship between urinary cotinine levels and chronic renal insufficiency was investigated. The study showed that there was a positive correlation between urinary cotinine levels, GFR, and renal damage and was dependent on the number of cigarettes smoked.

When the kidney functions are evaluated, the glomerular and tubular functions should be examined separately. Therefore, in this study, the glomerular function has been evaluated by using the urine microalbumin/creatinine ratio and by measuring the serum urea and creatinine levels. The tubular function has been evaluated by measuring urine β-2 microglobulin levels.

Hyperfiltration and albuminuria, which accelerate the loss of kidney function in patients with diabetes and in smokers, are linked to kidney function failure. A positive correlation was found between cigarette smoking and microalbuminuria in one study conducted on subjects who had no known hypertension or diabetes mellitus. Also, in that study, it was found that hyperfiltration and proteinuria caused by smoking resulted in glomerular damage in a long period of time.[12]

In the present study, we have found that urine microalbumin and creatinine levels were significantly (respectively, $p < 0.01$ and $p < 0.05$) increased in active smokers when compared with the passive smokers and control subjects. At the same time, a significant positive correlation was found between urine microalbumin and creatinine levels ($p < 0.01$).

On the other hand, there was no significant difference between urine β-2 microglobulin levels of the groups ($p > 0.05$). From these results, it can be suggested that exposure to cigarette smoke leads to more destruction in the glomerular function than in the tubular function, and the increase in urine microalbumin/creatinine ratio is an early sign of this destruction.

In hypertensive patients, cigarette smoking results in a difficulty in controlling blood pressure. Furthermore,

smoking increases the risk of damaging the target organ in hypertensive patients. In patients with essential hypertension who smoke, the frequency of microalbuminuria is nearly two times as much as that in hypertensive patients who do not smoke. In a study conducted on hypertensive patients with left ventricular hypertrophy, it was found that microalbuminuria was 1.6 times and macroalbuminuria was 3.7 times higher in those who smoke more than 20 cigarettes a day than those who did not smoke.[13]

A retrospective study conducted by Bleyer et al.[14] on 4142 patients who were not diabetic and who were over 64 years old, in order to investigate the cause of kidney function failure with age, found a positive correlation between the numbers of cigarettes smoked and the serum creatinine levels. It was reported that the factors affecting the decrease in kidney functions along with age are connected to the frequency of hypertension, smoking, and vascular diseases.

We have not investigated the correlation between the numbers of cigarettes smoked per day by the subjects and the measured parameters. However, the serum cotinine and serum and urine creatinine levels were measured in the group of active smokers who smoked an average of 20 cigarettes a day, but no significant correlation was found ($p > 0.05$).

Smoking may affect the excretion of urinary albumin and GFR in diabetic and nondiabetic persons. Ishızaka et al.[15] found in one of their studies conducted on 7078 Japanese men that there was a correlation between GFR and the number of cigarettes smoked per day in those who continued smoking. On the other hand, there was a significant decrease in the estimated GFR ratio in those who used to smoke before. In light of these data, it has been concluded that smoking may increase the frequency of albuminuria and hyperfiltration and the estimated GFR. It was shown in the same study that the increased risks decreased after giving up smoking cigarettes.

In the present study, we have found no significant difference between GFR levels of the groups ($p > 0.05$). The reason of that finding was thought to be the duration of exposure to cigarette smoke.

In healthy people, nearly 95% of β-2 microglobulin in circulation is filtered by glomeruli, and then almost all of it is reabsorbed. β-2 microglobulin is catabolized by proximal tubule cells. β-2 microglobulin penetrates into cells by means of endocytosis. Proteins are reabsorbed and then hydrolyzed to amino acids in these cells. A kidney having a normal GFR has the ability to reabsorb nearly 99% of filtered β-2 microglobulin. During any incident that impairs proximal tubule functions, there will be an increase in excretion of β-2 microglobulin along with urine depending on the decrease in the reabsorbed amount.[16]

We have found no significant difference between β-2 microglobulin levels of the groups. We have concluded that this finding could be due to fewer negative effects of cigarette smoking on tubular functions than on glomeruli.

In this study, microalbumin/creatinine ratio was found to be significantly higher in both active and passive smokers compared with the control subjects ($p < 0.01$). Since the higher microalbumin/creatinine ratio in passive smokers is a sign of cardiovascular risk factor, it shows that passive smoking may increase the risk of atherosclerosis.

In summary, we have found that urine microalbumin and creatinine levels increase in active smokers. Thus, we have concluded that glomerular functions of kidney fail due to negative effects of early smoking, and passive smoking may increase the risk of atherosclerosis. However, these findings need to be supported by studies performed on more number of subjects who are exposed to cigarette smoke for longer durations.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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