

Patterns of Cotinine Excretion among Diabetic, Cardiac Patients and Healthy Smokers in Jordan

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Abstract: Cotinine, major proximate metabolite of nicotine has been widely involved as highly specific and sensitive biomarker of exposure to tobacco and both active and secondhand tobacco smoke. To explore the biological role of the cotinine as a metabolite of nicotine among different types of smokers and to explore if cotinine level is affected by smoking related diseases as heart problems and other diseases as diabetes. This is a cross sectional study design. Study population included smokers in North of Jordan. The study sample included 400 participants visiting cardiac and diabetic clinics in King Abdulla University Teaching Hospital and Health Center. Different cafes in Irbid city were involved to collect samples from the apparently normal individuals. About 400 participants were involved in this study. About 40% of participants were smokers. About 95% of smokers were positive for cotinine. The following variables were associated significantly with cotinine: smoking status, smoking contact hours, smoking in sleeping place and smoking type. Last smoking time and inhaling of smoking were not associated significantly with cotinine ($p < 0.05$). The following smoking related variables were significantly associated with cotinine level: last 30 days cigarette frequency, daily smoking cigarette in last month, regular smoking cigarette for at least once last month ($p < 0.05$). While smoking cigarette years was not associated significantly with cotinine level ($p > 0.05$). The following water pipe smoking variables were not associated significantly with cotinine level: last 30 days water pipe frequency, smoking water pipes years, years of smoking water pipes and cigarettes ($p > 0.05$). On the other hand, regular smoking water pipe at least once in last month was associated significantly with cotinine level ($p < 0.05$). Both of heart diseases and diabetes were not associated significantly with cotinine level ($p > 0.05$). Cotinine is a sensitive biomarker to measure exposure to smoking.

Key words: Cotinine, smoking, water pipe, diabetes, heart diseases, biomarker

INTRODUCTION

Cotinine is known as the major proximate metabolite of nicotine. It has been widely involved as a biomarker of exposure to tobacco and both active and second hand tobacco smoke (Benowitz, 1996). It has also been shown that cotinine reflects the extent of exposure, not how the exposure was derived (Benowitz *et al.*, 2002).

From a chemical point of view, nicotine is an oxidizing agent. Both of nicotine and cotinine are responsible for tobacco-dependence (Velloso *et al.*, 2007). However, nicotine has been found to be the principal alkaloid in tobacco and is present as a major component of tobacco smoke. Usually, nicotine is absorbed in measurable quantities by both active and passive smokers who have

been shown to inhale an amount of nicotine proportional to the product of concentration, duration of exposure and respiration rate (Bentley *et al.*, 1999). It has been recently found that both of nicotine and cotinine are oxidized in the liver (Messina *et al.*, 1997; Nakajima *et al.*, 1996). Cotinine has been used as a marker of nicotine exposure both in smokers and in nonsmokers because it is one of the main metabolites of nicotine and has a longer half-life than its precursor (McCarthy *et al.*, 1992).

There are several factors influencing nicotine uptake and metabolism in smokers including: smoking rate; characteristics of the cigarette such as length, nicotine content and delivery systems (e.g., pH, ammonia content), freshness, filter versus nonfilter and menthol content; smoking topography including puff volume and duration,

inhalation frequency, retention time in lung, depth of inhalation and percentage of available tobacco smoked factors that determine the metabolic state such as age, gender, genetic sensitivity, physical fitness and body composition (Gilmour *et al.*, 2006). Thus, all these factors may influence the cotinine levels in body fluids.

Health effects of nicotine and/or cotinine: There are lots of adverse physical and mental health, social, developmental, cognitive and economic consequences associated with smoking (Pechacek *et al.*, 2003). Smoking related health issues include cardiovascular diseases, respiratory diseases and cancer (Benowitz, 1996). It is worth mentioning that cigarette smoking is one of the most important preventable risk factors for cardio vascular disease at a global level (Teo *et al.*, 2006). It has been shown that smokers have a three-fold increased risk of a heart attack. Even lower levels of exposure of only 8-10 cigarettes day⁻¹ doubles the risk of heart attack in smokers while smoking only 1 cigarette daily increases the risk by 6% (Leone, 1995). Epidemiological surveys suggest that diseases of the heart and blood vessels account for over one-third of deaths in cigarette smokers (Glantz and Parmley, 1991).

Furthermore, active smoking is associated with an 80% increase in Coronary Artery Disease (CAD) and passive smoking with a 30% increase. Thus, it is obvious that both active and passive smoking are undoubtedly associated with cardiovascular diseases in both men and women although, the particular component(s) of cigarette smoke or the specific mechanism(s) have not yet been fully elucidated (Law *et al.*, 1997; Chen *et al.*, 2002).

It has been found that nicotine administration may result in oxidative stress by inducing the generation of reactive oxygen species in the peripheral and central nervous system (Zevin *et al.*, 2000). It was found that cotinine has pharmacological activity so that it interferes with the release of brain neurotransmitters and affects enzymes involved in the synthesis of estrogen and testosterone, decreases vascular resistance and blood pressure in animals and has been reported to have effects on cognitive performance and to modify nicotine withdrawal symptoms in abstinent smokers (Reznick *et al.*, 2003).

It has been emphasized by different studies that exposure to tobacco is the major inducer of oral cancer. Tobacco was found to be responsible for 50-90% of cancer cases worldwide (Kelly, 2003). The association between cigarette smoking and cancer may come from the fact that cigarette smoking results in an increased cumulative exposure to ROS and because its accumulation, it would seem cigarette smokers may have

an increased requirement for antioxidant nutrients (Domino *et al.*, 1993). Nicotine in food as a source of cotinine several foods have been found to contain small amounts of nicotine (Davis *et al.*, 1991; Sheen, 1988; Idle, 1990). It has been suggested that nicotine from food might falsely indicate exposure to ETS (Nelson *et al.*, 1991; Leaderer and Hammond, 1991).

Davis *et al.* (1991) have estimated that average daily consumption of tomatoes, potatoes, cauliflower and black tea together could result in a daily intake of 8.8 µg nicotine. They estimated based on a maximum consumption of all of these particular foods on the same day that a person could ingest as much as 99.9 µg of nicotine day⁻¹ from food (Repace, 1994).

Average nicotine intake (i.e., absorbed dose) from significant ETS plus dietary exposure has been estimated to be about 80 µg. Repace (1994) used average American vegetable consumption data which included 27 g of tomatoes and 75 g of potatoes to estimate daily nicotine intake from the diet of 0.7 µg day⁻¹ (Tunstall-Pedoe *et al.*, 1991).

The impact of tea drinking on serum cotinine levels of nonsmokers has been studied in Scotland. The results revealed that no effect on plasma cotinine levels was observed with consumption levels of up to 10 cups or more of tea per day. In contrast, the same study showed a robust relationship between self-reported ETS exposure and plasma cotinine levels. Thus, nicotine in tea appears to contribute little to cotinine levels in most people and would be insignificant compared with nicotine levels from exposure from ETS (Willers *et al.*, 1995).

MATERIALS AND METHODS

Study design and population: This is a cross sectional study design. Study population included smokers in North of Jordan. The study sample included 400 participants visiting cardiac and diabetic clinics in King Abdulla University Teaching Hospital and Health Center. Different cafes in Irbid city were also involved to collect samples from the apparently normal individuals. The study included the use of a previously prepared questionnaire.

Procedure: Commercial kits for detection of cotinine were used (Nano-Ditech Corp.). This is an immunoassay test involved the use of chromatographic strips which were previously coated with the antigen of cotinine. The test is considered positive in case there is a development of one line after the sample is added to the sample part. Positive result indicates the cotinine level in urine sample exceeds 200 ng mL⁻¹.

Samples: Samples were taken from the following groups:

- Coffee shop attendees: smokers and passive smokers
- Diabetic patients from the health center clinics: diabetic smokers and diabetic nonsmokers
- Chronic disease (clinic at internal medicine clinics), King Abdulla University Teaching Hospital: smokers and nonsmokers for heart patients

RESULTS

Socio-demographic characteristics of participants: In the present study, age, sex and nationality were studied. About 51% of participants were under 40 years, 49% were ≥ 40 years. About 52% were males and about 48% were females. The majority of participants 92% were Jordanians (Table 1).

Smoking related factors: About 40% of participants reported that they are smokers while about 60% of participants reported that they are not smokers. Smoking contact hours (< 3 h) were reported by 75% of participants and ≥ 3 h were reported by the rest of participants. About

30% of participants reported the presence of smoking in their sleeping place. About 63% of participants reported last smoking time for 1-2 h before sample collection while last smoking time for > 2 h was reported by the rest of participants. Smoker population consisted of about 68% of cigarette smokers, 24% water pipe smokers and about 9% cigarette and water pipe smokers. About 40% of smokers reported always for smoking inhaling, about 51% reported mostly and about 9% reported seldom. About 38% of participants were positive for cotinine (Table 2).

Cigarette smoking related factors: About 73% of cigarette smoking participants reported that they smoked < 10 cigarettes per day during the last 30 days. About 43% of them reported smoking for < 9 days during last month. About 68% of cigarette smoking participants reported that they smoked one cigarette for at least once last month regularly. About 71% of cigarette smoking participants reported ≤ 10 years (Table 3).

Water pipe smoking related factors: About 63% of water pipe smoking participants reported water pipe smoking for < 2 days during the last month. About 40% of water pipe smoking participants reported regular smoking of water pipe at least once during last month. Of those water pipe smokers, about 54% had smoked water pipe for < 5 years. About 36% of water pipe and cigarette smoking participants reported smoking both for ≤ 7 years (Table 4).

Table 1: Socio-demographic characteristics of participants

Variables	Number (N)	Percent
Age		
<40	204	51.0
≥ 40	196	49.0
Sex		
Male	209	52.3
Female	191	47.7
Nationality		
Jordanian	368	92.0
Non Jordanian	32	8.0

Table 2: Smoking related factors

Variables	Number (N)	Percent
Smoking status		
Yes	161	40.3
No	239	59.7
Smoking contact hours		
< 3 h	300	75.0
≥ 3 h	100	25.0
Smoking in your sleeping place		
Yes	122	30.5
No	278	69.5
Last smoking time		
1-2 h	102	63.4
> 2 h	59	36.6
Smoking type		
Cigarette	109	67.7
Water pipe	38	23.6
Water pipe and cigarette	14	8.7
Inhaling of smoking		
Always	64	39.8
Mostly	82	50.9
Seldom	15	9.3
Cotinine level		
Positive	153	38.3
Negative	247	61.7

Table 3: Cigarette smoking related factors

Variables	Number (N)	Percent
Last 30 days cigarette frequency		
< 10 days	34	72.7
≥ 10 days	89	27.3
Daily smoking cigarette in last month		
< 9 days	53	43.1
≥ 9 days	70	56.9
Regular smoking cigarette for at least once last month		
Yes	84	68.3
No	39	31.7
Smoking cigarette years		
≤ 10 years	87	70.7
> 10 years	36	29.3

Table 4: Water pipe smoking related factors

Variables	Number (N)	Percent
Last 30 days water pipe frequency		
< 2 days	33	63.5
≥ 2 days	19	36.5
Regular smoking water pipe at least once in last month		
Yes	21	40.4
No	31	59.6
Smoking water pipes years		
≤ 5 years	28	53.8
> 5 years	24	46.2
Years of smoking water pipes and cigarettes		
≤ 7 years	5	35.7
> 7 years	9	64.3

The relationship between cotinine level and socio-demographic variables: In this part and the coming parts of the results, the significance level ($p \leq 0.05$) using χ^2 -test. The results showed that 47.1% of participants <40 years were positive for cotinine and 29.1% of participants ≥ 40 years were positive.

The trend observed is to have less positive results with increasing age. However, cotinine level is correlated significantly with age ($p = 0.000$). The results also showed that 65% of male participants and 18.8% of female participants were positive for cotinine. Males are more likely to be affected by smoking and to give positive results for cotinine.

Cotinine level is correlated significantly with sex ($p = 0.000$). It has also been shown that about 35% of Jordanians and 72% of non-Jordanians were positive for cotinine. However, cotinine level is associated significantly with nationality ($p = 0.000$) (Table 5).

The relationship between cotinine level and the smoking related factors: About 80% of smokers and 10% of nonsmokers were positive for cotinine. At the same time, about 20% of smokers and 90% of nonsmokers were negative for cotinine. Cotinine level is associated significantly with smoking status ($p = 0.000$).

About 22% of participants with smoking contact hours for <3 h and about 44% with smoking contact hours for ≥ 3 h were positive for cotinine. However, cotinine level is associated significantly with smoking contact hours ($p = 0.000$).

About 43% of participants with smoking in sleeping place and 27% of participants without smoking in sleeping place were positive for cotinine. Cotinine level is associated significantly with smoking in sleeping place ($p = 0.001$).

The results showed that about 85% of cigarette smokers, 80% of water pipe smokers and 50% of cigarette and water pipe smokers were positive for cotinine. These findings show that cotinine level is associated significantly with smoking type ($p = 0.007$).

Table 5: The relationship between cotinine level and socio-demographic variables

Variables	Cotinine level		p value
	Positive N (%)	Negative N (%)	
Age			
<40 years	96 (47.1)	108 (52.9)	0.000
≥ 40 years	57 (29.1)	139 (70.9)	
Sex			
Male	117 (65)	92 (44)	0.000
Female	36 (18.8)	155 (81.2)	
Nationality			
Jordanian	130 (35.3)	238 (64.7)	0.000
Non-jordanian	23 (71.9)	9 (28.1)	

The data of the present study showed that about 83% of participants who are always inhaling smoking, 83% who are mostly inhaling smoking and 60% who are seldom inhaling smoking were positive for cotinine. Anyhow, cotinine level is not associated significantly with inhaling of smoking ($p = 0.101$) (Table 6).

The relationship between the cotinine level and the cigarette smoking related factors: The results showed that about 44% of participants with <10 days and 95.5% of participants with ≥ 10 days cigarette frequency last month were positive for cotinine. Cotinine level is correlated significantly with last 30 days cigarette frequency ($p = 0.000$).

About 64% of cigarette smoking participants with <9 cigarettes day⁻¹ and about 94% of cigarette smoking participants with ≥ 9 cigarettes day⁻¹ were positive for cotinine. Cotinine level is associated significantly with daily smoking cigarettes in last month ($p = 0.000$).

About 94% of cigarette smokers who reported regular smoking cigarettes for at least once last month and about 54% of those who did not report were positive for cotinine. The results showed that cotinine level is correlated significantly with regular smoking cigarette for at least once last month ($p = 0.000$).

The results showed that about 82% of cigarette smokers with ≤ 10 years smoking and about 78% of cigarette smokers with >10 years experience were positive for cotinine. Cotinine level is not associated significantly with cigarette smoking years ($p = 0.389$) (Table 7).

Table 6: The relationship between cotinine level and the smoking related factors

Variables	Cotinine level		p value
	Positive N (%)	Negative N (%)	
Smoking status			
Yes	129 (80.1)	32 (19.9)	0.000
No	24 (10.0)	215 (90.0)	
Smoking contact hours			
<3 h	22 (22.0)	78 (78.0)	0.000
≥ 3 h	131 (43.7)	169 (56.3)	
Smoking in your sleeping place			
Yes	120 (43.2)	158 (56.8)	0.001
No	33 (27.0)	89 (73.0)	
Last smoking time			
1-2 h	80 (78.4)	22 (21.6)	0.222
>2 h	50 (84.7)	9 (15.3)	
Smoking type			
Cigarette	93 (85.3)	16 (14.7)	0.007
Water pipe	30 (78.9)	8 (21.1)	
Water pipe and cigarette	7 (50.0)	7 (50.0)	
Inhaling of smoking			
Always	53 (82.8)	11 (17.2)	0.101
Mostly	68 (82.9)	14 (17.1)	
Seldom	9 (60.0)	6 (40.0)	

Table 7: The relationship between the Cotinine level and the cigarette smoking related factors

Variables	Cotinine level		p value
	Positive N (%)	Negative N (%)	
Last 30 days cigarette frequency			
<10 days	15 (44.1)	19 (55.9)	0.000
≥10 days	85 (95.5)	4 (4.50)	
Daily smoking cigarette in last month			
<9 day ⁻¹	34 (64.2)	19 (35.8)	0.000
≥9 day ⁻¹	66 (94.3)	4 (5.7)	
Regular smoking cigarette for at least once last month			
Yes	79 (94.00)	5 (6.00)	0.000
No	21 (53.8)	18 (64.2)	
Smoking cigarette years			
≤10 years	71 (81.6)	16 (18.4)	0.389
>10 years	28 (77.8)	8 (22.2)	

Table 8: The relationship between the cotinine level and the water pipes smoking related factors variables

Variables	Cotinine level		p value
	Positive N (%)	Negative N (%)	
Last 30 days water pipe frequency			
<2 days	21 (63.6)	12 (36.4)	0.102
≥2 days	16 (84.2)	3 (15.8)	
Regular smoking water pipe at least once in last month			
Yes	19 (90.5)	2 (9.5)	0.011
No	18 (85.1)	13 (41.9)	
Smoking water pipes years			
≤5 years	21 (75.0)	7 (25.00)	0.361
>5 years	16 (66.7)	8 (33.3)	
Years of smoking water pipes and cigarettes			
≤7 years	2 (40.0)	3 (60.00)	0.500
>7 years	5 (55.6)	4 (44.4)	

The relationship between the cotinine level and the water pipe smoking related factors variables: About 64% of water pipe smokers who smoked <2 days in last 30 days and about 84% of water pipe smokers who smoked for ≥2 days in last 30 days were positive for cotinine. Cotinine level is not associated significantly with last 30 days water pipe frequency (p = 0.102).

About 90% of water pipe smokers who reported regular smoking water pipe at least once in last month and about 85% of those who did not report were positive for cotinine. The results revealed that cotinine level is associated significantly with regular smoking water pipe at least once in last month (p = 0.011).

The results indicated that 75% of water pipe smokers with ≤5 smoking years and about 67% of water pipe smokers with >5 smoking years were positive for cotinine. Cotinine level is not associated significantly with water pipe smoking years (p = 0.361).

The results showed that 40% of cigarette and water pipe smokers for ≤7 years and about 55% of those who are smokers for >7 years were positive for cotinine. Cotinine level is not associated significantly with years of smoking water pipe and cigarettes (p = 0.361) (Table 8).

Table 9: The relationship between the cotinine level and heart and diabetic diseases

Variables	Cotinine level		p value
	Positive N (%)	Negative N (%)	
Heart disease	58 (45.7)	69 (54.3)	0.141
Diabetes	37 (35.9)	66 (64.1)	-

The relationship between the cotinine level and heart and diabetic diseases: The results showed that about 46% of heart disease patients and about 36% of diabetic patients were positive for cotinine. Cotinine level is not associated significantly with heart and diabetic diseases (p = 0.141) (Table 9).

DISCUSSION

The results of the present study showed that all of the three socio-demographic variables under study, age, sex and nationality were correlated positively with cotinine level (p = 0.000 for the all).

It was observed that participants <40 years had more positive results for cotinine than who were ≥40 years. Anyhow, this finding is contradicted by the study conducted by Ann D. and his associates who reported that cotinine level increases with age (Jarvis *et al.*, 1987). Age was found one of the factors participating into the discrepancies related to cotinine concentration (Holl *et al.*, 1998). The findings may be explained by the fact that the age group among the participants in the present study is larger than the other studies previously mentioned. We have a tendency to accept the fact that among the participants in the present study, higher metabolic rates for nicotine may explain higher cotinine levels among ages <40.

The results of this study showed that sex is associated significantly with cotinine level (p = 0.000). Males were more positive for cotinine than females. This result is consistent with other studies. H.J. Glander and his associates found that males are more positive for cotinine than females. According to their study, they thought that males are more affected by fertility due to having more cotinine (Mcneill *et al.*, 1989).

The findings are also consistent with the results obtained by R. Ronchetti and his colleagues who found that active smokers are usually males with more cotinine levels (Wagenknecht *et al.*, 1992).

The results have also showed that cotinine level is associated significantly with nationality (p = 0.000). Non-Jordanians were more positive for cotinine. This result can be explained by the fact that more cotinine indicates that more exposure to nicotine (1, 2). We think that non-Jordanians as any foreigners, may have been exposed to stress factors and smoking may be a

compensating factor. Another plausible explanation is the race and ethnicity as reported by Neal L. Benowitz (1996) in a study to find the relationship between cotinine levels and distinguishing smoking status. It has been found that optimal cutpoints differed among adults by race/ethnicity (Pirkle *et al.*, 1996).

The results of the present study showed a significant correlation between smoking and cotinine ($p = 0.000$). The results showed that 10% of non smokers were positive for cotinine and about 20% of smokers were negative for cotinine. It can be concluded from the above results that smoking is the major causative agent for cotinine positivity. The positive result for cotinine among nonsmokers indicates either the participants did not tell the truth or they had been exposed to nicotine from other sources (environmentally or by food intake). The findings are in line with several reported studies on literature. These studies indicated that several daily foods as tomatoes, potatoes, tea, etc., lead to false positive cotinine and ETS is indicated as a result (Davis *et al.*, 1991; Sheen, 1988; Idle, 1990; Nelson *et al.*, 1991; Leaderer and Hammond, 1991; Repace, 1994).

The results indicated that smoking contact hours are associated significantly with cotinine level ($p = 0.000$). This finding is consistent with other studies on literature in which it has been emphasized that nicotine is absorbed in measurable quantities by both active and passive smokers who have been shown to inhale an amount of nicotine proportional to the product of concentration, duration of exposure and respiration rate (Bentley *et al.*, 1999). It has been shown by the results that smoking in sleeping place is associated significantly with cotinine level ($p = 0.001$). This result is similar to smoking contact hours and will be discussed under the same considerations. It is worth mentioning that 27% of participants who denied smoking in their sleeping place were positive for cotinine. This may be explained by the fact that other contributing factors as food intake (Jarvis *et al.*, 1987; Mcneill *et al.*, 1989; Pirkle *et al.*, 1996). Another explanation is the possibility of being water pipe smokers. However, slow metabolic rate of cotinine could explain partly why cotinine is detected after exposure to smoking by several hours (Shiono and Behrman, 1995; Haddow *et al.*, 1987).

The results of the data failed to prove a significant correlation between last smoking time and cotinine level ($p = 0.222$). This finding can be explained by taking into consideration that the metabolism of cotinine is slow and accordingly this finding is in line with other reported studies in literature (Shiono and Behrman, 1995; Haddow *et al.*, 1987; Klesges *et al.*, 1992).

The results of the data showed that smoking type is associated significantly with cotinine ($p = 0.007$). It is also shown that cigarette smokers come first followed by water pipe and both of them. The result is in line with results obtained by Digambar Behera in his study about urinary cotinine levels among different smokers. He found that nicotine levels were highest in cigarette smokers followed by hooka smokers (Behera *et al.*, 2003).

The data showed that inhaling of smoking is not associated significantly with cotinine ($p = 0.101$). This result is not in line with other reported studies in literature in which inhalation of smoking is proportional to cotinine concentration (Bentley *et al.*, 1999; Jarvis *et al.*, 1987).

In the present study, the researcher tested the relationship between several factors related to cigarette smoking and cotinine. It was shown that last 30 days cigarette frequency is associated significantly with cotinine ($p = 0.000$).

Daily smoking cigarette in last month was also associated significantly with cotinine level ($p = 0.000$). Regular smoking cigarette for at least once last month was also associated with cotinine level ($p = 0.000$). Smoking cigarette years was not associated significantly with cotinine ($p = 0.389$). The previous factors indicated that exposure to cigarette smoking led to positive cotinine level.

The findings are in line with other reported studies in which it is reported that cotinine is a good indicator for nicotine exposure (Benowitz, 1996; Benowitz *et al.*, 2002). Because cotinine has a longer half-life than nicotine, cotinine has been used as a marker of nicotine exposure, both in smokers and in nonsmokers (McCarthy *et al.*, 1992).

Smoking years are not associated significantly with cotinine. This may reflect the fact that the metabolism of nicotine to cotinine needs about 20 h (McCarthy *et al.*, 1992).

The results also showed that regular smoking water pipe at least once in last month is associated significantly with cotinine ($p = 0.011$). This result is in line with other reported studies in literature in which it is indicated that cotinine is an indicator of tobacco exposure (Bentley *et al.*, 1999; Messina *et al.*, 1997; Gilmour *et al.*, 2006).

The results of the present study showed that last 30 days water pipe frequency is not associated significantly with cotinine level ($p = 0.102$) and those smoking water pipe years are not also associated significantly with cotinine ($p = 0.361$). The results also showed those years of smoking water pipes and cigarettes are not associated significantly with cotinine level ($p = 0.500$). The only

variable with significant association with cotinine is regular smoking water pipe at least once in last month ($p = 0.011$). These findings can be explained in view of metabolic aspects of cotinine. As previously mentioned, nicotine has a half time about 20 h (Benowitz, 1996; Benowitz *et al.*, 2002). During that time, it can be detected in different biological fluids as saliva, serum and urine (Willers *et al.*, 1995; Jarvis *et al.*, 1987; Holl *et al.*, 1998).

Finally, the results of the data showed that cotinine level is not associated significantly with diabetes and heart diseases ($p = 0.141$). About 36% of diabetic participants were positive to cotinine. This finding is in line with other reported studies in literature in which it was found that the frequency of smoking has been found in adult patients with type 1 diabetes, to be 27% which is not different from 26% in nondiabetic control subjects (Willers *et al.*, 1995).

About 46% of patients with heart diseases were positive for cotinine. Other studies reported that there was no consistent association between cotinine concentration and risk of stroke. It was also found that studies based on reports of smoking in a partner alone seem to underestimate the risks of exposure to passive smoking. Further prospective studies relating biomarkers of passive smoking to risk of coronary heart disease are needed (Law *et al.*, 1997; He *et al.*, 1999; Bonita *et al.*, 1999).

Anyhow, it is worth mentioning that the non significant association between heart diseases and diabetes with cotinine is due to the fact that all participants in this study are under treatment.

CONCLUSION

Cotinine can be used to detect exposure to tobacco smoking irrespective to the way of exposure. There is a good correlation between smoking self reporting and cotinine results among participants in the study.

Cotinine excretion was not affected by heart diseases and diabetes in the present study which may due to either the small number of participants with both diseases or because the patients were under treatment.

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