

QUANTITATIVE RELATIONSHIPS OF URINARY COTININE LEVELS IN SMOKING DIABETIC PATIENTS

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Manuscript received: December 2014

Abstract

Nowadays, it is recognized the fact that cotinine can serve as a quantification marker of nicotine addiction, as well as for passive smoking. Several studies reported in the recent literature have emphasized that, in patients with different pathologies, objective assessment of tobacco use is required, based on biomarkers (e.g. determining cotinine levels in blood or urine) and not on self-reporting of estimated consumption. To highlight the possible correlations between the concentration of cotinine and various parameters, such as socio-demographic or smoking related diseases, we have evaluated the urinary cotinine levels in smoking diabetic patients. The study group consisted of twenty two smoking diabetic patients, evaluated comparatively with a group of healthy active smokers in terms of age, gender, education level, marital status, occupation, comorbidities, glycated haemoglobin values, urinary cotinine concentration, nicotine dependence score, duration of tobacco use, number and characteristics of the smoked cigarettes. Urinary cotinine concentration was determined by an ELISA (enzyme-linked immunosorbent assay) method using a commercial kit. Quantitative relationships such as concentration of cotinine in urine vs. dependence score and duration of tobacco use; vs. dependence score and comorbidities or vs. dependence score, glycated haemoglobin, and comorbidities were obtained. The predictive power of the proposed mathematical models is high as the correlation coefficients obtained have values close to unity ($R > 0.90$). The quantitative relationships between urinary cotinine concentrations and different parameters evaluated in the study, established using various combinations of these parameters can predict the cotinine levels in urine, reflecting the biotransformation and urinary excretion of nicotine as this metabolite.

Rezumat

În prezent, cotinina urinară este recunoscută drept *biomarker* pentru evaluarea dependenței de nicotină, precum și pentru evaluarea nivelului fumatului pasiv. Mai multe studii raportate în literatura recentă au subliniat că, la pacienții cu diferite patologii, este necesară evaluarea obiectivă a consumului de tutun, bazată pe *biomarkeri* (de exemplu, determinarea nivelurilor de cotinină în sânge sau urină) și nu pe auto-raportări estimative ale consumului. Pentru a evidenția posibilele corelații între concentrația de cotinină și diverși parametri, cum ar fi cei socio-demografici sau asociați fumatului și patologiei, am evaluat nivelurile de cotinină urinară la pacienți cu diabet zaharat, fumători. Lotul de studiu a fost constituit din douăzeci și doi de pacienți fumători, evaluați comparativ cu un grup de fumători activi sănătoși, asemănători în ceea ce privește vârsta, sexul, nivelul de școlarizare, starea civilă, ocupația, comorbiditățile, valorile hemoglobinei glicozilate, concentrația urinară de cotinină, scorul dependenței de nicotină, vechimea fumatului, numărul de țigări fumate zilnic, caracteristicile țigărilor. Concentrația urinară de cotinină a fost determinată prin metoda *ELISA* folosind un *kit* comercial. Relațiile cantitative între concentrațiile urinare de cotinină și diferiți parametri analizați în studiu au fost realizate folosind software-ul StatisticaTM StatSoft 6.0, subrutina "*piecewise linear regression with breakpoint*" (regresie liniară prin iterații succesive). Au fost obținute relații cantitative, cum ar fi: concentrația de cotinină în urină vs. scorul dependenței și vechimea fumatului; vs. scorul dependenței și comorbidități sau vs. scorul dependenței, hemoglobina glicozilată și comorbidități. Puterea predictivă a modelelor matematice propuse este mare, deoarece coeficienții de corelație obținuți au valori apropiate de unitate ($R > 0,90$). Relațiile cantitative între concentrațiile de cotinină urinară și diferiți parametri evaluați în cadrul studiului, stabilite folosind diferite combinații ale acestor parametri pot predicționa nivelul de cotinină din urină, reflectând biotransformarea și excreția urinară a nicotinei sub forma acestui metabolit.

Keywords: urinary cotinine, diabetic patients, quantitative relationships

Introduction

Cotinine, a metabolite of nicotine, is commonly used as a biomarker for the evaluation of smoking, as well as the exposure to tobacco smoke and passive smoking. The level of cotinine in the blood is proportional to the degree of the exposure to tobacco, thus making it a valuable indicator on smoking, including the passive one [7]. Existing evidence suggests that there is an association between the salivary cotinine levels and passive smoking [15].

Cotinine has a half-life of about 20 hours, and is usually detectable for several days (up to a week) after the tobacco consumption.

Blood cotinine levels below 10 ng/mL are related with passive smoking. Values of 10 ng/mL - 100 ng/mL are associated with light smoking or moderate passive exposure, and levels of 300 ng/mL are noticed in heavy smokers (more than 20 cigarettes per day). In urine, levels from 11 ng/mL to 30 ng/mL may be associated with light smoking or moderate passive exposure, while the levels in active smokers typically reach 500 ng/mL or more. Cotinine quantification thus provides an objective quantitative measure which is more reliable than the smoking history or counting the number of cigarettes smoked per day. Cotinine also allows the measurement of secondary exposure to tobacco smoke (passive smoking).

In literature, there are reported several studies that have highlighted the need for an objective evaluation of tobacco consumption, based on the determination of cotinine levels in blood or urine, and not on estimative self-reporting of consumption in patients with various pathologies (e.g. psychiatric disorder, heart disease) [1, 2, 6, 16, 19]. Thereby, it is recognized that smoking is a major risk factor in the development of coronary heart disease and chronic heart failure, but the value of self-reporting of smoking behaviour has not been validated in patients with chronic heart failure. The study of N. Ebner et al. showed that determination of the serum concentration of cotinine provides a mean to quantify objectively the smoking behaviour in patients with chronic heart failure [6]. In addition, the quantitative determination of cotinine in serum can provide data on distorted self-reports in daily clinical routine.

Investigations suggest that smoking is associated with an increased risk of developing type 2 diabetes [19]. Lately, evidence has been accumulated regarding the synergistic effect of smoking in diabetes mellitus type 1 and 2 and increased morbidity and mortality in smoking patients with diabetes [5, 8, 11-13].

However, the prevalence of smoking among patients with diabetes was estimated to be almost the same as in the general population [12].

The effects of smoking on the risk of diabetes are usually attributed to nicotine. On short term, nicotine is known to cause increases in blood glucose concentration [9]. It is unclear whether this effect is transient or if it leads to persistent increased blood glucose.

Several studies have shown that smoking is associated with elevated levels of glycosylated haemoglobin (HbA1c), an objective parameter of glucose levels for long term, but that relationship may be affected in the case where smokers do not correctly report their smoking status [16, 17].

It was suggested that a more accurate assessment of the relationship between tobacco use and HbA1c should be achieved by using a biological marker for smoking, such as cotinine, the most important metabolite of nicotine.

In this context, we considered useful an assessment study of cotinine levels in smoking diabetic patients to highlight the possible correlations between urinary cotinine concentration and various socio-demographic parameters, associated to the pathology or associated with smoking.

In order to compare the urinary cotinine levels, we included in the study a group of active healthy smokers and also a group of non-smoking persons, exposed to passive smoking.

Materials and Methods

Three study groups were established as it follows:

- ✓ smoking diabetic patients group (n = 22) undergoing treatment at a clinical centre in Bucharest, Romania;
- ✓ active smoking subjects group (n = 23); they participated voluntarily in the study;
- ✓ non-smoking subjects group (n = 12), exposed to passive smoking. They have volunteered in the study.

Variables / parameters analysed in the study:

- ✓ socio-demographic parameters: age, sex, education level, marital status, occupation / work;
- ✓ parameters associated with the pathology: comorbidities, treatment, glycosylated haemoglobin;
- ✓ parameters of smoking: duration of smoking, nicotine dependence score, number of cigarettes / day, characteristics of the smoked cigarettes (amount of tar, nicotine quantity, amount of carbon monoxide); it is recognized that smokers can vary the intake of smoke to satisfy their needs by adjusting several features: the brands of cigarettes selected, number of cigarettes smoked, and number of puffs per cigarette, depth and duration of inhalation [18];
- ✓ urinary cotinine concentration.

All patients and subjects included in the study signed an informed consent for the participation in the study. The study was approved by the UMF Carol Davila ethics committee.

The score of nicotine dependence was assessed by using the Fagerström test. Urinary cotinine concentration was determined by an ELISA method, using a commercial kit (Abnova, Catalog Number KA1416). All the determinations were performed on a Chemwell 2010 ELISA system (Awareness Technology INC, USA).

Socio-demographic parameters, associated with pathology and associated with the smoking habit were determined based on clinical records and questionnaires applied.

Statistics

Descriptive statistics and the frequencies distribution were performed with SPSS Statistics ver. 21; data for different parameters are presented as the mean $\pm$ standard deviation (SD). Comparison between groups was achieved by applying Student t-test. The correlations between the investigated parameters have been evaluated using correlation coefficients (Pearson, Kendall and Sperman). All tests were considered statistically significant at threshold $p_1 = 0.05$ and $p_2 = 0.01$. Quantitative relations were investigated between urinary

cotinine and various parameters analysed in the study, using StatSoft 6.0 Statistica™ program; the graphics of quantitative correlations were drawn using the same program.

Results and Discussion

Smoking diabetic patients group

In the study group consisting of 22 smoking diabetic patients, the predominance of male (64% men, 36% women) was showed, the ratio between men and women being 1.75 (Table I). The average age was 50.68 years (range 28-74 years). It is noticed the predominance of the age category 41-50 years in the group of patients studied. Most patients were married, had secondary education (95.45%, 21/22 cases) and 59% (13/22 cases) of patients were employed (Table I).

All patients were diagnosed with type 2 diabetes mellitus and most of them received treatment with oral antidiabetic agents; in four cases insulin was also administrated. Most of the patients presented comorbidities such as dyslipidaemia, hypertension stage 2, ischemic heart disease (IHD).

Approximately 40% of patients did not have other diseases associated with diabetes (Table I).

Table I

Socio-demographic characteristics, comorbidity, treatment and parameters evaluated in the study in smoking diabetic patients group

Number of patients	22
Distribution by sex	14 men, 8 women
Ratio men:women	1.75
Average age ($\pm$ SD)	50.68 $\pm$ 10.46 years (range 28 – 74 years, median 48.5)
Education	Secondary education: 21/22 cases; higher education: 1/22 cases
Employment	Employees: 13/22; retired: 6/22; unemployed: 3/22
Marital status	Married: 15/22 cases; unmarried: 7/22 cases
Diagnostic	Diabetes mellitus type 2 (22/22 cases)
Comorbidities	Dyslipidaemia: 8/22 cases (36.36%); Hypertension (stage 2): 4/22 cases (18.18%); coronary artery disease: 1/22 cases (4.54%); without comorbidities: 9/22 cases (40.9%)
Medication	Oral hypoglycaemic agents: 18/22 cases; Oral hypoglycaemic agents + insulin: 4/22 cases
Dependence score	5.90 $\pm$ 2.32 (range 1 – 9)
Cotinine concentration in urine (ng / mL)	247.30 $\pm$ 254.97 (range 22.6 – 266.66)
Glycosylated haemoglobin	8.35 $\pm$ 0.50 (range 7.90 – 8.90)
Smoking duration	30.27 $\pm$ 10.25 (range 10 – 50)
Tar in smoked cigarettes	7.86 $\pm$ 1.78 (range 4 – 10)
Nicotine in smoked cigarettes	0.68 $\pm$ 1.32 (range 0.40 – 1.00)
Carbon monoxide in smoked cigarettes	8.36 $\pm$ 1.89 (range 5 – 10)
Number of cigarettes per day	18.45 $\pm$ 2.95 (range 15 – 26)

Duration of smoking is long in the studied group, with an average of 30.27 years (range 10-50 years); most of the patients have a long smoking history, between 25-35 years.

Regarding the dependence score, the average score (5.90) indicates medium to high dependence. The

distribution of the dependence score shows that most of the smoking diabetic patients, have high nicotine dependence (score above 6).

Urinary cotinine levels have varied within very wide limits, from concentrations close to those characteristic for the passive smoking to very high

levels characteristic for the heavy smoking. The average concentration of cotinine in urine was 247.3 ng/mL, but most of the values were in the range 102-550 ng/mL.

Simultaneous graphical representation of the concentration of urinary cotinine and smoking

dependence score suggests a possible correlation between the two parameters (Figure 1).

The Nicotine dependence score has been evaluated by applying the Fagerström scale [20].

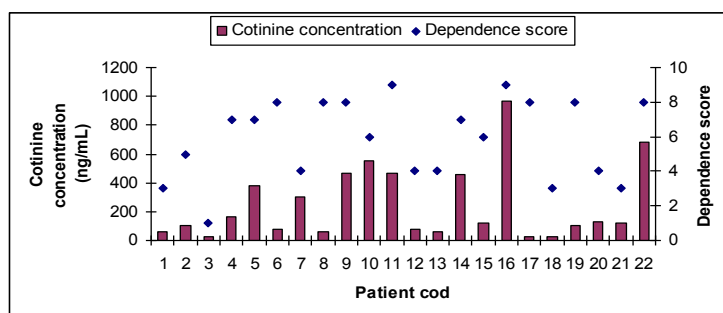


Figure 1.

Distribution of smoking dependence score and urinary cotinine concentrations in smoking diabetic patients

Regarding the characteristics of cigarettes, it was observed that most patients smoke cigarettes with a medium amount of nicotine (0.7 mg), and the number of cigarettes / day is about 20 (most patients smoke approximately 1 pack of cigarettes / day).

Glycosylated haemoglobin values are high in the study group, indicating the severity of the basic disease, type 2 diabetes.

Healthy active smoking and passive smoking groups

In the group of healthy active smokers ($n = 23$), men were also predominant (65%, Table II); the ratio between men and women was 1.85. The average age was 34 years (range 19-58 years). The age category 21-30 years was predominant in the group of healthy active smokers.

Most subjects are married (56.52%), almost half of them have secondary education (47.82%, 11/23 subjects) and 39% (9/23 subjects) of the subjects were students (Table II). In order to compare the urinary cotinine levels in active smokers with those due to passive exposure to tobacco smoke we have established a group of passive smokers ($n = 12$).

Socio-demographic characteristics and parameters evaluated in healthy active smoking and passive smoking groups are summarized in Table II.

In line with the lower average age than in the case of the group of diabetic patients, the average duration of smoking was of approximately 16 years (range 3-37 years); however, most of the subjects have a medium – long smoking history, in the range 4-24 years.

Table II

Socio-demographic characteristics and parameters evaluated in the study in healthy active smoking and passive smoking groups

	Healthy active smokers	Passive smokers
Number of subjects	23	12
Distribution by sex	14 men, 8 women	3 men, 9 women
Ratio men:women	1.85	0.33
Average age ($\pm$ SD)	34.04 $\pm$ 13.56 years (range 19 – 58 years, median 27)	23.81 $\pm$ 0.87 years (range 23 – 25 years, median 24)
Education	Bellow secondary education: 2/23 subjects; secondary education: 11/23 subjects; high education: 1/23 subjects; students: 9/23 subjects	High education: 2/12 subjects; students 10/12 subjects.
Employment	Employees: 14/23 subjects; students: 9/23 subjects	Employees: 2/12 cases; students: 10/12 subjects
Marital status	Married: 13/23 subjects; unmarried: 9/23 subjects	Unmarried: 12/12 subjects
Nicotine dependence score	5.90 $\pm$ 2.32 (range 1 – 9)	
Cotinine concentration in urine (ng/ mL)	794.27 $\pm$ 158.54 (range 425.18 – 1066.98)	13.78 $\pm$ 3.06 ng/mL (range 4.45 – 38.78 ng/mL)
Smoking duration	15.95 $\pm$ 12.03 (range 3 – 37)	
Tar in smoked cigarettes	9.08 $\pm$ 1.08 (range 7 – 10)	
Nicotine in cigarettes	0.79 $\pm$ 0.13 (range 0.70 – 1.00)	
Carbon monoxide in cigarettes	9.26 $\pm$ 1.13 (range 7 – 10)	
Number of cigarettes per day	19.52 $\pm$ 3.76 (range 15 – 26)	

Regarding the nicotine dependence score, the average score (5.26) indicates medium to high dependence. The distribution of the dependence score (Table II) indicates that approximately half of active smokers have a high dependence (score above 6).

Cotinine levels in urine varied in a wide range, but certainly hovering in the characteristic concentrations of heavy smokers (425.18 - 1066.98 ng/mL). The average concentration of cotinine in urine was 794.27 ± 158.54 ng/mL. The majority of the values were within the range of 600-930 ng/mL.

Regarding the characteristics of smoked cigarettes, it was noticed that most of the subjects smoke cigarettes with an average content of nicotine (0.7 mg) and the number of cigarettes / day is about 20.

The concentrations of cotinine in the urine in the group of non-smoking exposed to passive smoking are clearly different from the levels registered for the active smokers, the results being consistent with the literature data. Thus, the mean value was 13.78 ± 3.06 ng/mL (range 4.45 - 38.78 ng/mL, median 8.77 ng/mL). Most of the subjects exposed to passive smoke have cotinine levels in the urine between 5-20 ng/mL.

Correlations between the parameters analysed in the study

The analysis of correlations between the investigated parameters, using SPSS program, highlighted Pearson correlations, statistically significant ($p < 0.05$) between parameters: cotinine concentration in urine - dependence score; cotinine concentration in urine - the number of smoked cigarettes / day.

High statistically significant correlations ($p < 0.01$) were obtained between the parameters: age - duration of smoking; score of dependence - number of smoked cigarettes / day; quantity of tar in smoked cigarettes - amount of nicotine in smoked cigarettes; quantity of tar in smoked cigarettes - amount of CO smoked in cigarettes.

Quantitative relationships between the concentration of cotinine in urine and different parameters evaluated in the study

Considering the results obtained in the study, as well as their statistics evaluations, which do not always stand predictable correlation between cotinine concentration and the analysed variables, we expected that the combined effect of these variables may reflect the levels of cotinine in urine.

Therefore, we undertook a study to develop several mathematical models for predicting the levels of cotinine depending on different influencing parameters evaluated in this study.

Thus, the study performed in order to establish of quantitative relations between cotinine concentrations in urine and influencing factors, was based on a number of combinations of these factors that could describe a certain level of concentration of cotinine in urine, reflecting possible biotransformation and urinary elimination of nicotine as its metabolite, cotinine.

The influencing parameters considered were: nicotine dependence score, number of smoked cigarettes per day, duration (age) of cigarettes use (smoking), comorbidities, and glycosylated haemoglobin value (Table III).

Table III

Defining variables for assessing the quantitative relationships

Dependent variable (Y)	Independent variable ($X_1 - X_n$)
Concentration of cotinine in urine (ng/mL)	Nicotine dependence score (NDS) Duration of smoking (AS) Number of cigarettes smoked/day (NT) Amount of nicotine in cigarettes (CN) Glycosylated haemoglobin (HbA1c) Comorbidities (CMB)

The quantitative relationships between the above mentioned parameters and the concentration of cotinine in urine were determined using the nonlinear correlation model, the subroutine "piecewise linear regression with breakpoint" (linear regression through successive iterations) of 6.0 StatSoft STATISTICA software.

The correlation model is such as:

$$Y_i(X_1, X_2) = [a_0 + a_1x_1 + a_2x_2] \text{ breakpoint } [b_0 + b_2x_2 + b_1x_1]_2$$

and consists in a discontinuous regression relation, applied when the nature of the relationship between independent variables and the dependent variable changes over the range of the independent variable.

Thus, at some point there can be a discontinuity or a gap between the two types of variables. This discontinuity is quantified by the so-called „point of discontinuity" ("breakpoint" or "jump" of the regression line) [14].

The following quantitative relationships have been achieved (Table IV): Cotinine (C) vs nicotine dependence score (NDS) and duration of smoking (AS); Cotinine (C) vs nicotine dependence score (NDS) and amount of nicotine in smoked cigarettes (AN); Cotinine (C) vs nicotine dependence score (NDS) and glycosylated haemoglobin (HbA1c); Cotinine (C) vs nicotine dependence score (NDS) and comorbidities (CMB); Cotinine (C) vs nicotine dependence score (NDS), glycosylated haemoglobin

(HbA1c) and comorbidities(CMB); Cotinine (C) vs nicotine dependence score (NDS), duration (age) of

smoking (AS), amount of nicotine in smoked cigarettes (AN) and comorbidities (CMB).

Table IV

Mathematical equations on the correlations of urinary cotinine concentration (C) with different influencing parameters analysed in the study, in diabetic patients; *breakpoint* at C = 247.3 ng/mL

No.crt.	Quantitative relationship	R
1	$C = f(SD, AS) = [8.36 + 5.83SD + 1.39AS]_1$ <i>breakpoint</i> $[-55.59 + 74.04SD + 1.96AS]_2$	0.9219
2	$C = f(SD, CN) = [52.02 + 4.38SD + 12.69CN]_1$ <i>breakpoint</i> $[-88.00 + 72.17SD + 133.19CN]_2$	0.9280
3	$C = f(SD, HbA1c) = [318.98 + 5.74SD - 32.46 HbA1c]_1$ <i>breakpoint</i> $[311.47 + 78.67SD - 40.08 HbA1c]_2$	0.9216
4	$C = f(SD, CMB) = [71.062 + 4.74SD - 8.04CMB]_1$ <i>breakpoint</i> $[-512.43 + 104.97SD + 176.31CMB]_2$	0.9360
5	$C = f(SD, HbA1c, CMB) = [345.96 + 5.92SD - 33.72 HbA1c - 11.15CMB]_1$ <i>breakpoint</i> $[-762.22 + 104.58SD + 27.28HbA1c + 186.54CMB]_2$	0.9376
6	$C = f(SD, AS, NT, CMB) = [-2.25 + 4.35SD + 1.37AS + 1.549NT - 5.04CMB]_1$ <i>breakpoint</i> $[176.53 + 138.88SD - 5.71AS - 40.97NT + 220.57CMB]_2$	0.9555

R=correlation coefficient

Since the correlation coefficients obtained for all mathematical equations achieved values closed to unity, it can be concluded that the predictive power of the proposed mathematical models is high (Table IV). We observed a positive correlation for the concentration of cotinine in urine with dependence score and the duration of smoking, also with the dependence score and the amount of nicotine in the smoked cigarettes, both before and after the point of discontinuity, reached at a concentration of 247.3 ng cotinine/mL (Equations 1 and 2).

Regarding the value of glycosylated haemoglobin, an indicator of the gravity of the diabetic disease, in association with the nicotine dependence score, the mathematical model proposed is not eloquent, obviously highlighting a negative correlation with glycosylated haemoglobin, both before and after the breakpoint (Equation 3).

There is a negative correlation between the concentration of cotinine in the urine and comorbidities in association with the nicotine dependence score before the breakpoint and a positive one after the breakpoint (Equation 4).

These variations could be related to individual variability, in the context of the basic disease associated with other pathologies that could affect the biotransformation of nicotine and elimination of cotinine in urine.

Several studies have shown that smoking is associated with elevated levels of glycosylated haemoglobin (HbA1c) [16, 17], an objective index of the chronic hyperglycaemia, but the relationship may be affected when the smokers do not report correctly their smoking status. The study conducted by Clair et al. shows that the level of cotinine is associated with increased levels of HbA1c in a representative sample of the US population without diabetes mellitus [4]. Masson et al. showed no correlations between cotinine levels and glycosylated haemoglobin values in young patients with diabetes [10].

In the study carried out by Boran et al., in smoking patients with diabetes, the following variables were

significantly associated with the levels of cotinine: smoking status, hours of contact with smoking, smoking in the bedroom, type of smoking [3]. Duration of smoking was not significantly correlated with cotinine. Both heart disease and diabetes were not significantly associated with the levels of cotinine.

The results of the mathematical modelling based on nonlinear correlations with breakpoint have been completed by the implementation of the response surface methodology (RSM), an effective tool that can estimate the main effects and the interaction of the influencing parameters selected over the levels of cotinine in urine. Therefore, the relationships between the dependent variable (the concentration of cotinine in urine) and the influencing factors (parameters) evaluated in the study illustrated by using the RSM allow to visualize the effects of these factors in the three-dimensional space [14].

By applying the RSM the following correlations were obtained and the graphics were drawn (Figure 2):

- cotinine (C) vs. nicotine dependence score (NDS), duration (age) of smoking (AS): a positive correlation C – NDS was observed (low concentrations of cotinine, low dependence score);
- cotinine (C) vs. nicotine dependence score (NDS) and number of smoked cigarettes (NC): a positive correlation C - NDS, NC was observed (low concentrations of cotinine, low dependence score and number of smoked cigarettes);
- cotinine (C) vs. nicotine dependence score (NDS) and the amount of nicotine in smoked cigarettes (AN): a positive correlation C - DS, AN was observed (low concentrations of cotinine, low dependence score and amount of nicotine in cigarettes);
- cotinine (C) vs. nicotine dependence score (NDS) and glycosylated haemoglobin (HbA1c): a positive correlation C - DS was observed (low concentrations of cotinine, low dependence score, but the lack of influence of the value of glycosylated haemoglobin).

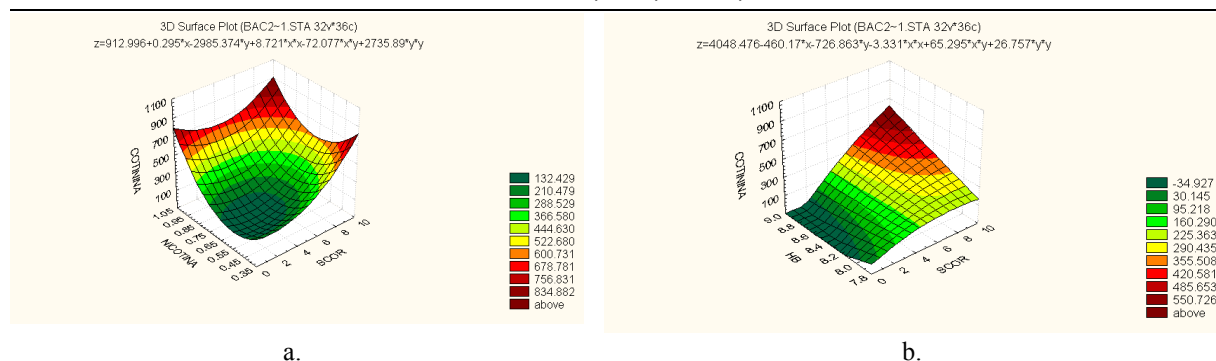


Figure 2.

Three-dimensional representations of the concentration of cotinine in urine vs. dependence score and the amount of nicotine in the smoked cigarettes (a), and vs. dependence score, and glycosylated haemoglobin (b), in smoking diabetic patients

Conclusions

A profile of smoking diabetic patients was achieved, in terms of socio-demographic characteristics, comorbidities and treatment. Nicotine dependence score, evaluated on the Fagerström scale reflects a high dependence, registering similar values as those of healthy active smokers.

Cotinine levels in urine varied within very wide limits, from concentrations close to those characteristic to passive smoking, to very high levels, characteristic of heavy smokers. The studied patients group differs statistically significant from the passive smoking group, in terms of urinary cotinine concentration. However, the levels of cotinine in urine were significantly different in diabetic patients compared to healthy active smokers, being in a range of concentration considerably lower, and suggesting the likely influence of comorbidities and treatment administered; these variables can cause changes in the metabolism of nicotine to cotinine and urinary elimination of this metabolite.

Statistically significant Pearson correlations were revealed between the parameters: age – duration of smoking, nicotine dependence score - number of smoked cigarettes / day, the concentration of cotinine in urine – nicotine dependence score, amount of tar in smoked cigarettes, amount of nicotine in smoked cigarettes, smoked cigarettes CO quantity.

Several mathematical models with a high predictive power (correlation coefficients with values close to unity) for cotinine levels depending on the influencing factors (nicotine dependence score, number of smoked cigarettes per day, duration of smoking, comorbidities, glycosylated haemoglobin) have been established. The patterns of correlations obtained consist in discontinuous regression relationships applied when the nature of the relationship between independent variables and the

dependent variable changes over the range of the independent variable (correlation with breakpoint).

The quantitative relationships can predict cotinine levels in urine, reflecting the possible biotransformation and urinary excretion of nicotine as this metabolite.

Acknowledgements

This paper was co-financed from the European Social Fund, through the Sectorial Operational Programme Human Resources Development 2007-2013, project number POSDRU/159/1.5/S/138907 "Excellence in scientific interdisciplinary research, doctoral and postdoctoral, in the economic, social and medical fields -EXCELIS", coordinator The Bucharest University of Economic Studies.

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