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Evaluation of Serum Biochemical Parameters Primarily Liver Functions in Smokers: A Case-control Study

Sigara İçenlerde Karaciğer Fonksiyonları Başta Olmak Üzere Serum Biyokimyasal Parametrelerinin Değerlendirilmesi: Bir Vaka-kontrol Çalışması

Yılmaz SEZGİN[1], Sinan BECEL[2], Abdurrahman POLAT[3]

ABSTRACT

We investigated the effects of smoking on serum biochemical parameters primarily liver functions and metabolism.

This is a case-control study. The case and control groups were formed by clinical randomization by using the data obtained from the hospital information system and patient records, including age, gender, height, and weight. Smokers were identified as the case group, while non-smokers were identified as the control group. In the comparisons of rates, Chi-square tests were used and in the comparisons of averages, independent sample t and MANCOVA tests were used.

When covariance factors such as age, gender, body mass index, and alcohol use were taken into consideration, it was found that AST, ALT, and GGT were higher in smokers, whereas vitamin D, vitamin B12, and TSH were higher in non-smokers.

We found that smoking has a negative effect on liver and bile functions, and vitamin D values are affected secondary to this negative effect

Keywords: Biochemical parameters, Hepatotoxicity, Metabolism, Smoking.

ÖZ

Sigaranın karaciğer fonksiyonları ve metabolizma başta olmak üzere serum biyokimyasal parametreleri üzerine etkilerini araştırdık.

Bu bir vaka kontrol çalışmasıdır. Olgu ve kontrol grupları hastane bilgi sistemi ve hasta kayıtlarından elde edilen yaş, cinsiyet, boy, kilo gibi veriler kullanılarak klinik randomizasyonla oluşturuldu. Sigara içenler vaka grubu, içmeyenler ise kontrol grubu olarak belirlendi. Oranların karşılaştırılmasında Ki-kare testi, ortalamaların karşılaştırılmasında ise bağımsız örneklem t ve MANCOVA testleri kullanıldı.

Yaş, cinsiyet, vücut kitle indeksi ve alkol kullanımı gibi kovaryans faktörleri dikkate alındığında sigara içenlerde AST, ALT ve GGT'nin, sigara içmeyenlerde ise D vitamini, B12 vitamini ve TSH'nin daha yüksek olduğu görüldü.

Sigaranın karaciğer ve safra fonksiyonlarını olumsuz etkilediğini, bu olumsuz etkiye ikincil olarak D vitamini değerlerinin de etkilendiğini tespit ettik.

Anahtar Kelimeler: Biyokimyasal parametreler, Hepatotoksisite, Metabolizma, Sigara içmek.

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[1] Dr. Öğr. Üyesi, Yılmaz SEZGİN, Aile Hekimliği, SBÜ Trabzon Tıp Fakültesi, drysezgin@gmail.com, ORCID: 0000-0002-3626-0264
[2] Uz. Dr.,Sinan BECEL, Acil Tıp, İstanbul Florence Nightingale Hastanesi, sinanbecel@gmail.com, ORCID: 0000-0003-4269-9879
[3] Uz. Dr., Abdurrahman POLAT, Aile Hekimliği, Bahçelievler Devlet Hastanesi, a_polat21@hotmail.com, ORCID: 0009-0003-6317-7102

İletişim / Corresponding Author: Yılmaz SEZGİN
e-posta/e-mail: drysezgin@gmail.com

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INTRODUCTION

More than 5,000 chemical compounds have been identified in tobacco and tobacco smoke.[1] The most well-known of these substances is nicotine and identified with cigarettes. Nicotine is responsible for the addictive effect of smoking.[2] Smoking is one of the most important risk factors for chronic obstructive pulmonary disease.[3, 4] Smoking is accepted to increase the risk of cardiovascular diseases.[5, 6]

Studies investigating the effects of smoking are mostly focused on the pulmonary and cardiovascular systems. The number of studies on the effects of smoking on other systems such as the gastrointestinal system and urinary system than the pulmonary and cardiovascular systems is not sufficient to establish a consensus on the effects of cigaret on these systems. Recently, the number of studies investigating the relationship between smoking and obesity, and diabetes has been observed to be increased.[7, 8] We investigated the effects of smoking on several serum biochemical parameters and metabolism.

MATERIALS AND METHODS

Study Design

This was designed to be a case-control study.

Population Selection

The sample size was calculated as a minimum of 42 and a maximum of 70, taking into account the high effect size value of 0.80 (from Cohen's table) for the independent sample t-test, 0.05 for the margin of error, and 0.80-0.95 for the statistical power in the G-power program.

The case and control groups were formed by clinical randomization using data obtained from the hospital information system and patient records, including age, gender, height, and weight. The case group included 30 individuals with smoking habit. The control group consisted of 30 individuals with non-smokers, matching with the case group in terms of number, gender, age, and body mass index (BMI). Patients with any known disease and those using medications were not included in the study. Laboratory data of individuals who met the inclusion criteria among patients who admitted to the family medicine outpatient clinic during the last three months were evaluated for analysis.

Laboratory Testing

The blood samples of the participants were collected at the time of admission. A total of 19 parameters, including creatinine, urea, AST, ALT, GGT, sodium, potassium, albumin, cholesterol, triglyceride, HDL-C, LDL-C, HbA1C, glucose, insulin, vitamin D, vitamin B12, TSH, and free T4 were measured simultaneously for the analysis.

Statistical Analysis

All statistical analyses were performed by using IBM SPSS software (V25). A value of $P < .05$ was considered statistically significant. Categorical data were expressed by numbers and percentages. In the comparison of categorical variables chi-square test was used. Numerical data were expressed by mean values. In the comparison of the means, independent sample t-tests and MANCOVA tests were used. The compliance of the variances with normal distribution was tested by using Box's and Levene's tests.

RESULTS AND DISCUSSION

There was no difference between the case and control groups in terms of gender and BMI. However, the rate of alcohol use was lower in the case group compared to the control group. The mean age was higher in the case group compared to the control group (Table 1).

Table 1. Comparison of the age, BMI, gender and using alcohol in between case and control groups

Characteristics of participants		Case (n=30)	Control
Age: Mean (SD)*		25.80 (3.84)	22.30 (5.54)
BMI: Mean (SD)		23.21 (2.73)	22.66 (2.69)
Gender: n (%)	Female	15 (25)	15 (25)
	Male	15 (25)	15 (25)
Alcohol: n (%)*	Yes	7 (11.7)	14 (23.3)
	No	23 (38.3)	16 (26.7)

SD: Standard Deviation; *The statistical significant different was accepted as $P < 0.05$ level (2-tailed)

According to independent sample t test analysis between smokers and non-smokers; were found significant differences in terms of serum AST, ALT, GGT, LDL-C, vitamin D, vitamin B12 and TSH levels. We found that AST, ALT GGT, and LDL-C levels were higher in the case group, whereas vitamin D, vitamin B12 and TSH levels were higher in the control group (Table 2).

Tablo 2. Comparison of the serum biochemical parameters in between case and control groups

	Case (n=30)	Control (n=30)	t	P value
	Mean (SD)			
Creatinine (mg/dL)	0.71 (0.27)	0.69 (0.26)	0.15	.881
Urea (mg/dL)	27.63 (8.35)	24.20 (5.30)	1.90	.062
AST (U/L)	25.90 (10.84)	17.70 (5.42)	3.70	.001*
ALT (U/L)	22.50 (10.01)	15.30 (6.04)	3.37	.001*
GGT (U/L)	19.80 (7.07)	15.06 (5.31)	2.92	.005*
Sodium (mmol/L)	140.60 (3.94)	140.23 (2.69)	0.42	.676
Potassium (mmol/L)	4.18 (0.29)	4.22 (0.38)	-0.41	.684
Albumin (g/L)	4.38 (0.35)	4.34 (0.22)	0.40	.688
Cholesterol (mg/dL)	157.50 (24.03)	155.46 (23.09)	0.33	.740
Triglycerides (mg/dL)	84.10 (26.37)	73.16 (34.15)	1.38	.171
HDL-C (mg/dL)	55.66 (12.48)	53.26 (10.92)	0.79	.431
LDL-C (mg/dL)	90.43 (18.33)	78.96 (21.70)	2.21	.031*
HbA1C (%)	5.17 (0.41)	5.29 (0.21)	-1.41	.163
Glucose (mg/dL)	87.20 (6.89)	84.96 (8.60)	1.10	.272
Insulin (µU/mL)	9.09 (5.98)	7.95 (7.39)	0.65	.512
Vitamin D (ng/mL)	14.50 (3.34)	18.76 (9.01)	-2.42	.019*
Vitamin B12 (ng/L)	110.30 (52.03)	167.66 (78.85)	-3.32	.002*
TSH (mIU/L)	1.27 (0.47)	1.93 (0.86)	-3.62	.001*
Free T4 (ng/dL)	0.81 (0.15)	0.82 (0.14)	-0.16	.869
HOMA-IR	1.97 (1.34)	1.75 (2.04)	0.47	.635

SD: Standard Deviation; *İndependent simple t test is significant at the $P < .05$ level (2-tailed).

According to one-way MANCOVA analysis performed by taking into consideration covariance factors including age, gender, BMI and alcohol use between smokers and non-smokers were found significant differences in terms of serum biochemical parameters. The assumption of variance equality could not be met, since the value of P obtained from the box's test result

was less than .05 (P = .001). In this case, pillai's trace results were taken into account for MANCOVA test statistics (pillai's trace = 0.57, F[20, 35] = 2.36, P = .013). When the covariance factors were considered, we found that the difference between the two groups continued for AST, ALT, GGT, vitamin D, vitamin B12, and TSH, whereas the difference did not continued for LDL-C (Table 3).

There was no significant difference between the case group and the control group in terms of glycemic parameters. However, HOMA-IR values were higher in the case group while HbA1C values were higher in the control group (Table 3).

Table 3 **Comparison of serum biochemical parameters between case and control groups, taking into account covariate factors**

	Case (n=30)	Control (n=30)	Levene's	F	*P* value
	Mean[a] (SE)				
Creatinine (mg/dL)	0.69 (0.04)	0.71 (0.04)	0.901	0.13	.713
Urea (mg/dL)	27.46 (1.23)	24.37 (1.23)	0.001	2,85	.097
AST (U/L)	25.97 (1.65)	17.62 (1.65)	0.040	11.67	.001*
ALT (U/L)	22.73 (1.59)	15.06 (1.59)	0.002	10.63	.002*
GGT (U/L)	19.51 (1.08)	15.34 (1.08)	0.532	6.72	.012*
Sodium (mmol/L)	140.68 (0.60)	140.15 (0.60)	0.085	0.34	.558
Potassium (mmol/L)	4.20 (0.06)	4.19 (0.06)	0.053	0.01	.916
Albumin (g/L)	4.38 (0.05)	4.34 (0.05)	0.392	0.22	.641
Cholesterol (mg/dL)	156.99 (4.54)	155.97 (4.54)	0.842	0.02	.880
Triglycerides (mg/dL)	83.98 (5.85)	73.28 (5.85)	0.134	1.52	.222
HDL-C (mg/dL)	55.48 (0.05)	53.45 (0.05)	0.803	0.36	.547
LDL-C (mg/dL)	89.44 (3.90)	79.95 (3.90)	0.463	2.70	.106
HbA1C (%)	5.17 (0.05)	5.30 (0.05)	0.024	2.43	.124
Glucose (mg/dL)	87.37 (1.53)	84.78 (1.53)	0.147	1.30	.259
Insulin (µU/mL)	9.38 (1.29)	7.66 (1.29)	0.843	0.80	.373
Vitamin D (ng/mL)	14.74 (1.23)	18.52 (1.23)	0.070	4.29	.043*
Vitamin B12 (ng/L)	105.80 (12.37)	172.16 (12.37)	0.045	13.18	.001*
TSH (mIU/L)	1.30 (1.12)	1.89 (1.12)	0.006	10.91	.002*
Free T4 (ng/dL)	0.80 (0.02)	0.82 (0.02)	0.777	0.23	.629
HOMA-IR	2.03 (0.33)	1.69 (0.33)	0.853	0.46	.496

SE: Standard Error; *One-way MANCOVA is significant at the *P* < .05 level (2-tailed). a: Covariates appearing in the model are evaluated at the following values; age = 24.05, BMI = 22.94, gender = 1.50, alcohol = 1.35

The fact that the alcohol use rate was lower and the average age was higher in the case group in comparison to the control group, indicates that randomization was not performed very well. However, this limitation has been minimized by one-way MANCOVA analyzes performed by considering covariance factors such as age, gender, BMI and alcohol use.

In our study, the higher values of serum AST, ALT, and GGT observed in the case group indicates that smoking affects liver and bile functions, negatively. In the literature

there are studies that support our results. In a study conducted on males, GGT was higher in smokers in comparison to non-smokers.[9] It has also been suggested that smoking causes cellular damage by causing oxidative stress and ultrastructural changes on hepatocytes.[10]

In our study, the lower serum vitamin D values in the case group compared to the control group may be caused by a defect in the vitamin D synthesis in the liver and / or an absorption disorder. Cholecalciferol synthesized in the epidermis or taken with diet turns into 25-hydroxycholecalcidiol in the liver and then to 1,25-hydroxycholecalcitriol as the active form in the kidney.[11] The 25-hydroxycholecalcidiol transformation that takes place in the liver may be impaired due to the negative effects of smoking on hepatocytes. In addition, absorption of Vitamin D, a fat-soluble molecule, may be impaired due to the negative effects of smoking on the biliary system. In the literature, there are a few studies supporting our results, related to vitamin D in our study. However, the reasons for vitamin D deficiency in smokers have not been fully clarified in these studies.[12]

We found that serum vitamin B12 values were lower in the case group compared to the control group. These results are in line with the knowledge in the literature. There are studies suggesting that smoking, which is a source of free radicals, causes serum vitamin B12 levels to decrease.[13-15] The use of tobacco causes the serum cyanide level to increase due to the cyanide it contains. It has been shown that high cyanide levels increase the excretion of thiocyanate from the kidneys, which is associated with a decrease in serum vitamin B12 level.[16]

We found that serum TSH levels were lower in the case group compared to the control group. In the literature, it has been reported that the effects of smoking on thyroid tissue are complex, while smoking generally increases susceptibility to hypertroidism.[17, 18] This relationship between smoking and thyroid functions may be due to vitamin B12 deficiency seen in smokers. Although there is a widespread opinion in the literature that vitamin B12 deficiency and increased levels of homocysteine increase susceptibility to hypothyroidism and autoimmune thyroid diseases, studies on animals suggest contradictory evidence.[19-21] In addition, S adenosyl methionine, a product of the homocysteine methionine cycle with additive vitamin B12 cofactor stimulates TRH and therefore TSH release and TSH receptor interaction.[22]

In our study, we found no difference in kidney function, between smokers and non-smokers. In the literature, it has been reported that kidney functions are affected by smoking due to the increase in nicotine-induced adrenergic activity.[23]

In our study, no significant difference was found between smokers and non-smokers in terms of serum glycemic index and lipid profile. However, HOMA-IR values were higher in the case group while HbA1C values were higher in the control group. HOMA-IR values show the momentary state, while HbA1C shows the last three-month period. These findings indicate that there was a predisposition to hypoglycemia in the smoker group in the past. In a study conducted on obese people, HOMA-IR was found to be higher in smokers compared to non-smokers, but there was no significant difference in serum glucose, insulin, total cholesterol, triglyceride, HDL-C, and LDL-C levels.[24] Nicotine causes hyperglycemia by increasing gluconeogenesis by stimulating catecholamine-mediated glucagon release from the adrenergic medulla.[8] It is plausible that the relationship between smoking and insulin resistance, which is frequently emphasized in the literature, maybe a result rather than a cause. Given the assumption that hyperinsulinemia-induced hypoglycemia episodes seen at the onset of type 2 diabetes can be prevented by increasing gluconeogenesis, nicotine may be preferred due to avoidance behavior.

CONCLUSION AND RECOMMENDATIONS

In conclusion, we suggest that smoking has a negative effect on liver and bile functions, and vitamin D values are affected secondary to this negative effect. In addition, the relationship between smoking and thyroid functions may be due to vitamin B12 deficiency seen in smokers.

Abbreviations

BMI: Bbody mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TSH: Thyroid stimulating hormone; HbA1C: Glycosylated hemoglobin; HOMA-IR: Homeostatic model assessment for insulin resistance; TRH: Thyrotropin releasing hormone

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None

Authors' contributions

The concept and design of the study: Y.S. and S.B.; Data acquisition: Y.S., S.B., and A.P.; Statistical analysis: Y. S.; Analyzed the data and drafted the manuscript: Y.S., S.B. and A.P. All authors read and approved the final version of the manuscript

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Availability of data and materials

The dataset is available from the corresponding author on reasonable request.

Declarations

Ethical approval

The study was approved by the Istanbul Training Research Hospital Clinical Research Ethics Committee (Decision no: 2153). Informed consent was not obtained because of the retrospective study.

Consent for publication

Not applicable.

Conflicts of Interest

The authors declare that no conflicts of interest.

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