THE TURKISH JOURNAL ON ADDICTIONS
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ORIGINAL ARTICLE

Evaluation of Plasma Heat Shock Protein Levels in Smokers

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Main Points

- Smoking significantly increased plasma heat shock protein (HSP) 90, HSP70, and HSP40 levels.
- There is no significant correlation between plasma HSPs and the number of cigarettes smoked daily, the number of cigarettes smoked so far, duration, and the number of cigarettes smoked.
- There is a significant positive correlation between carbon monoxide and HSP levels.

Abstract

This study aims to evaluate the effects of smoking on extracellular heat shock protein levels. Heat shock proteins have been associated with numerous diseases. Studies on the relationship between smoking exposure and heat shock proteins were mostly evaluated with intracellular heat shock proteins. This cross-sectional case – control study was conducted on patients who applied to outpatient clinics for smoking cessation and routine control in our faculty hospital. Of the smoking men, 75 between the ages of 30 and 65 years and 82 nonsmoking men were included in the study. Heat shock protein levels in plasma samples were determined using ELX 800 plate reader and Human HSP ELISA kit. Plasma heat shock protein levels were found to be significantly higher in smokers. There is no significant relationship between all three heat shock proteins and age, the number of cigarettes smoked daily, and the number of cigarettes smoked so far (package/year) ($p > .050$). Smoking significantly increases plasma heat shock protein 90, heat shock protein 70, and heat shock protein 40 levels.

Keywords: Chaperone proteins, heat shock protein 40, heat shock protein 70, heat shock protein 90, smoking

Introduction

Smoking is one of the leading causes of preventable morbidity and mortality worldwide, as is known. Cigarette smoke is considered to be one of the most important risk factors in diseases such as carcinogenesis, cardiovascular disease, and chronic obstructive pulmonary disease (COPD) (Repine et al., 1997). Tobacco smoke consists of more than 7000 chemicals, more than 70 of which are known to be carcinogens ("Harmful Chemicals in Tobacco Products," 2020). Smoking accounts for approximately 30% of all cancer deaths in the USA ("Harmful Chemicals in Tobacco Products," 2020).

Heat shock proteins (HSPs) are polypeptide proteins and perform the most basic functions. Their roles as molecular chaperones are the most famous of their functions (Lianos et al., 2015). Heat shock proteins are synthesized by the cell in response to stress conditions such as hypoxia, hyperoxia, exposure to chemicals and ultraviolet light, surgical stress, viral agents, and nutritional deficiencies (Vidyasagar et al., 2012). HSPs are classified as small HSPs that include HSP90, HSP70, HSP60, HSP40, and HSP27 according to their size (Lianos et al., 2015; Vidyasagar et al., 2012). Furthermore, HSPs have been shown to engage in various important processes such as protein combination, secretion, exchange, protein degradation, and regulation

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Received: March 26, 2022
Accepted: September 15, 2022
Publication Date: December 19, 2022

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Cite this article as: Kızmaz, M., Marakoğlu, K., Saraçlıgil, B., & Vatansev, H. (2022). Evaluation of plasma heat shock protein levels in smokers. *Addicta: The Turkish Journal on Addictions*, 9(3), 252-257.

DOI: 10.5152/ADDICTA.2022.22029

of transcription factors. Heat shock proteins are also described as essential proteins that play a crucial role in cell survival (Lianos et al., 2015).

High or low levels of HSPs have been associated with numerous diseases such as cancer, atherosclerosis, diabetes, asthma, transplant rejection, Alzheimer's disease, arthritis, multiple sclerosis, neurodegeneration, ischemia, immunity to infectious agents, and dehydration-induced nephropathy (Ataş, 2021; Cui et al., 2015; Sarman et al., 2020). The existence of a significant relationship between HSPs and cigarette exposure may lead to new study areas in terms of the possibility of HSP-induced pathophysiology between smoking and the diseases it causes. Intracellular HSPs were used in the majority of studies on the relationship between smoking and HSPs. This study aims to evaluate the effects of smoking on plasma HSP90, HSP70, and HSP40 levels.

Methods

Patient Recruitment

This cross-sectional case – control study was conducted on patients who applied to outpatient clinics for smoking cessation and routine control in our faculty hospital between November 2015 and February 2016. Seventy-five smoking men between the ages of 30 and 65 years and 82 nonsmoking men were included in the study. The study protocols were in accordance with the ethical standards of the Declaration of Helsinki and were approved by the Ethic Committee of our University numbered by 2015/229. Written informed consent was obtained from all participants who participated in this study. The study has an effect size of 0.5 and a power of 93%.

People with a history of cerebrovascular events, neurological impairment, cardiovascular disease, infection, renal or hepatic insufficiency, malignancy, and receiving anticonvulsant and/or nephrotoxic medication were excluded from the study.

Smoking Characteristics

Those who had never smoked or smoked less than 100 cigarettes in their life were included in the nonsmoker group (WHO (World Health Organization), 1998). Active smokers were included in the smoking group. Carbon monoxide (CO) tests in expired breath with the piCO™ Smokerlyzer Breath (Bedfont Scientific, Harrietsham, England) instrument were used to verify the smoking status of the cases. The piCO™ Smokerlyzer Breath device can measure CO levels in breath expired between 0 and 150 ppm (*Smokerlyzer® Range For Use with PiCO™, PiCO^{baby}™ and Micro^{+}™ User Manual*, 2020). Those with CO levels of 6 ppm or less were evaluated as non-smokers (*Smokerlyzer® Range For Use with PiCO™, PiCO^{baby}™ and Micro^{+}™ User Manual*, 2020). Fagerström test for nicotine dependence questions was used in the scoring and classification of addiction (Fagerstrom et al., 1990). Face-to-face interview techniques were applied to all participants. We used the following formula to calculate the total amount of cigarettes: cigarettes (package/year) = number of packages smoked daily × number of active smoking years.

Blood Collection and Laboratory Analysis

Venous blood samples were taken into evacuated tubes anticoagulated with ethylenediaminetetraacetic acid after 8 – 12 hours of fasting and centrifuged for 10 minutes (900 *g*, 4°C). Plasma samples were separated and stored at −80°C until analysis. Heat shock protein levels in plasma samples were determined using ELX 800 plate reader (Biotek, Mainz, Germany) and Human HSP ELISA kit (MyBioSource, Southern California, San Diego [USA]). Detection limits for testing were 0.03, 0.09, and 0.06 ng/mL for HSP90, HSP70, and HSP40, and in-study and interstudy precision was below 15%. (Marion et al., 2020; Zimmerman et al., 2014). Hemogram and biochemistry samples were not stored at −80°C. Hemogram and biochemistry samples were studied routinely in Selcuk University Faculty of Medicine Biochemistry Laboratory.

Statistical Analysis

The data were evaluated in terms of normality. The Student's *t*-test was used to compare measurements of a specific variable of two separate groups, and the Mann – Whitney *U* test was used for normally distributed groups and abnormally distributed groups. Chi-square tests were used to evaluate the relationship between the smoking and nonsmoking groups in terms of categorical variables. Spearman test was used to determine the relationship between numerical variables for normally distributed groups and abnormally distributed groups. Correlation coefficient (*r*) values between .000 and .249 were accepted as a weak relationship; between .250 and .499 an average relationship; between .500 and .749 a strong relationship; and between .750 and 1.000 a very strong relationship. A *p*-value <.05 was considered statistically significant with a 95% confidence level. Statistical Package for Social Sciences statistical software was used for Windows 21.0 for the analysis of all data.

Results

There is no statistically significant difference between the age, body mass index, and sociocultural levels of individuals in the smoking and nonsmoking groups.

Smoking characteristics of the smoking group are shown in Table 1. Plasma HSP levels were found to be significantly higher in smokers. Plasma HSP90, HSP70, and HSP40 levels of the smokers were 34.45 ± 17.26 pg/mL, 0.93 (0.08 – 3.78) pg/mL, and 7.85 ± 1.41 pg/mL, respectively. Plasma HSP90, HSP70, and HSP40 levels of the nonsmoker groups were 20.99 ± 8.57pg/mL, 0.73 (0.08 – 2.78) pg/mL, and 6.96 ± 1.23 pg/mL, respectively ($p < .001$, $p = .009$, $p < .001$) (Figures 1 and 2).

Table 1.
Smoking Characteristics of the Smoker Group

	Mean ± SD	Median	Minimum – Maximum
First smoking age (years)	15.74 ± 3.83	16	9 – 26
Number of cigarettes daily			
Duration of smoking (years)	25.60 ± 10.88	22	10 – 54
Package/year	28.21 ± 18.68	26	7 – 100
Fagerström score	5.74 ± 2.32	6	1 – 10

SD = standard deviation.
Package/year: the number of cigarettes daily × duration of smoking (years).

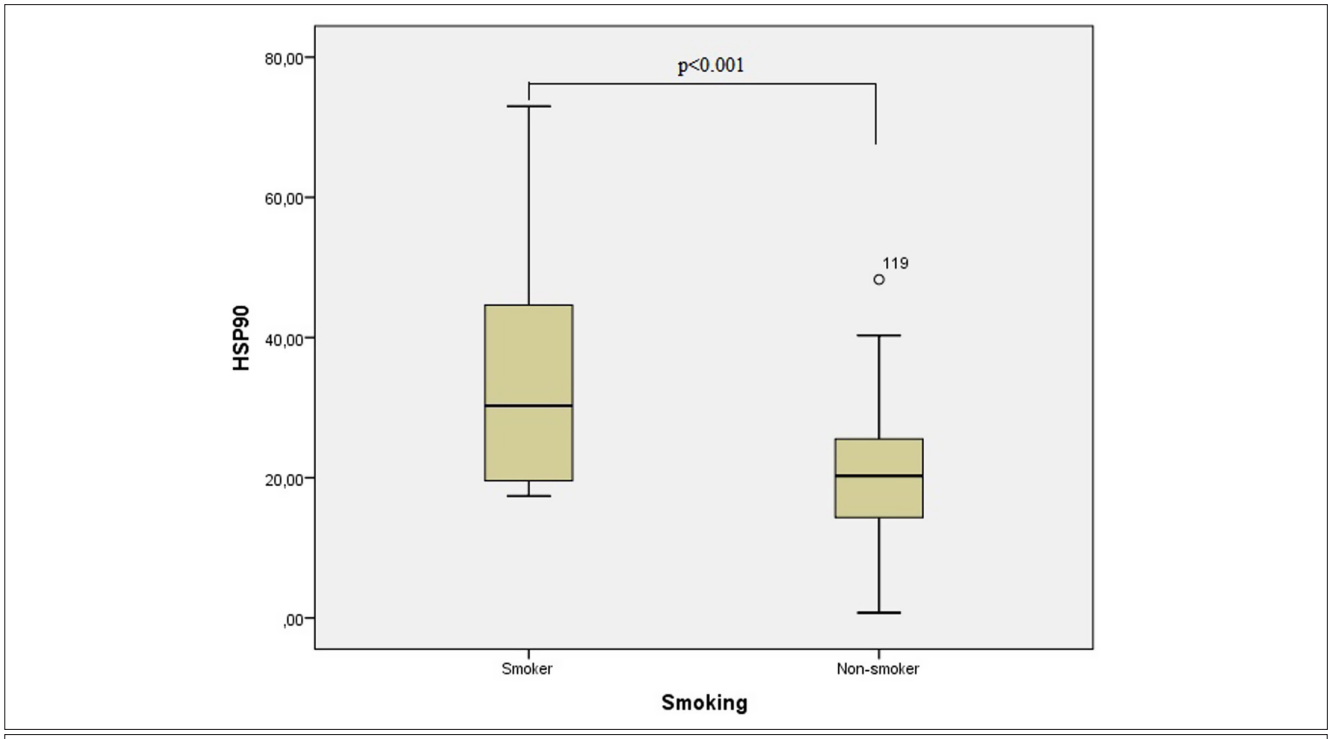


Figure 1. Plasma HSP90 Levels. HSP = Heat Shock Proteins.

Blood values of both groups are summarized in Table 2. Thyroid-stimulating hormone (TSH), high-density lipoprotein (HDL), and hemoglobin were significantly lower, and white blood cell (WBC) was significantly higher in smokers ($p < .005$).

Carbon monoxide was moderately correlated with HSP90 ($r = .385$, $p < .001$) and weakly correlated with HSP70 and HSP40 ($r = .201$, $p = .012$; $r = .230$, $p = .004$) (Table 3). There were no significant correlations between HSP90, HSP70, and HSP40 levels ($p > .050$). All three HSPs were not correlated with age, the number of cigarettes smoked daily, the number of cigarettes smoked so far (package/year), body mass index, TSH, low-density lipoprotein, HDL, total cholesterol, triglyceride, urea, creatinine, fasting blood sugar, WBC, hemoglobin, platelet, alanine aminotransferase, and aspartate aminotransferase ($p > .050$).

There was no significant correlation between systolic and diastolic blood pressure and HSPs ($p > .05$).

Discussion

We aimed to determine how smoking affects plasma HSP levels and found that smoking significantly increases plasma HSP90, HSP70, and HSP40 levels. There was no significant correlation between plasma HSPs and the number of cigarettes smoked daily, the number of cigarettes smoked so far (package/year), duration,

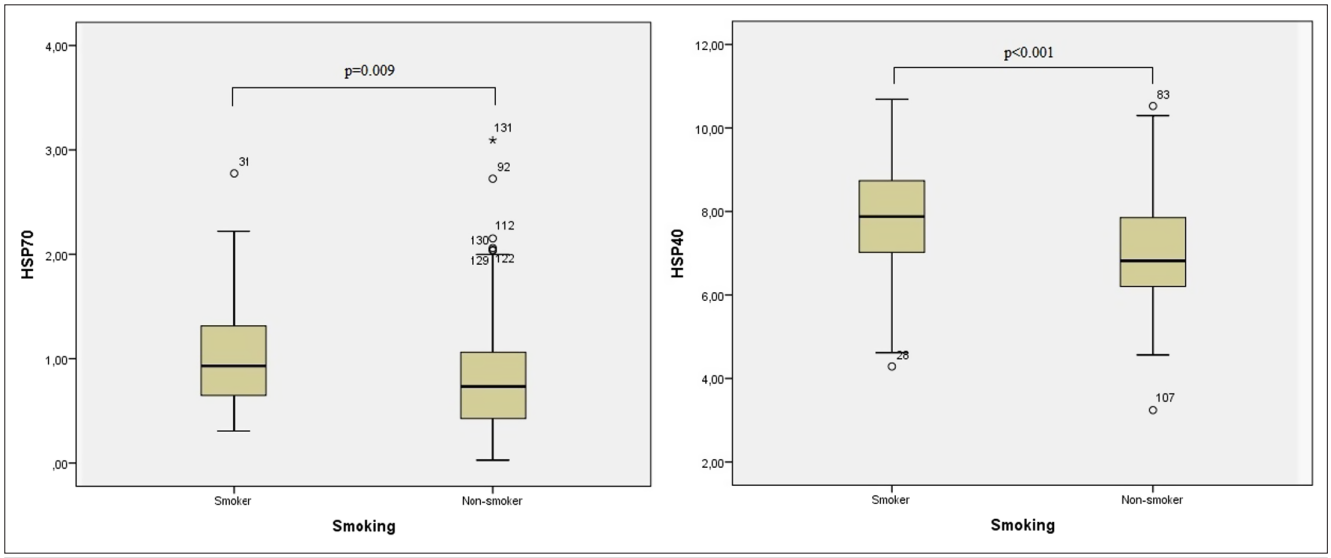


Figure 2. Plasma HSP70 and HSP40 Levels. HSP = Heat Shock Proteins

Table 2.
Comparison of Blood Parameters Between Smokers and Nonsmokers

	Nonsmokers (*n* = 82) (Mean ± SD) Median (Min-Max)	**Smokers (*n* = 75) (Mean ± SD) Median (Min-Max)**	***Z/t***	***p***
HSP90	20.99 ± 8.57	34.45 ± 17.26	6.268	***<.001*****
HSP70	0.73 (0.08 – 2.78)	0.93 (0.08 – 3.78)	-2.629	***.009****
HSP40	6.96 ± 1.23	7.85 ± 1.41	4.198	***<.001*****
TSH	2.06 ± 0.85	1.76 ± 0.74	-2.451	***.015****
LDL	130.61 ± 32.99	136.13 ± 47.03	.857	.393**
HDL	41.25 ± 7.67	37.28 ± 6.15	-3.558	***<.001*****
Total cholesterol	202.86 ± 41.62	205.00 ± 38.69	.351	.726**
Triglyceride	180.79 ± 69.70	176.58 ± 21.99	-.192	.848**
Urea	28.34 ± 4.70	26.82 ± 5.38	-1.894	.060**
Creatinine	0.84 ± 0.10	0.83 ± 0.09	-.627	.531**
FPG	97.68 ± 10.16	96.64 ± 13.74	-.184	.854**
WBC	7.18 ± 1.37	8.62 ± 1.74	5.757	***<.001*****
Hemoglobin	15.05 ± 0.97	15.54 ± 0.72	3.524	***.001*****
Platelet	239.12 ± 61.68	234.70 ± 45.01	-.508	.612**
ALT	27.60 ± 12.71	27.54 ± 15.33	-.028	.978**
AST	22.98 ± 8.14	22.06 ± 12.46	-.553	.581**

HSP = heat shock protein; TSH = thyroid-stimulating hormone; LDL = low-density lipoprotein; HDL = high-density lipoprotein; FPG = free plasma glucose; WBC = white blood cells; ALT = alanine aminotransferase; AST = aspartate aminotransferase.
*Mann – Whitney *U* test; **Student's *t*-test. The bold and italic values mean $p < 0.05$.

and the number of cigarettes smoked even though cigarettes have been found to significantly increase plasma HSP90, HSP70, and HSP40 levels.

Extracellular HSP70 (eHSP70) can activate the inflammatory response and lead to proinflammatory effects while intracellular HSP70 exerts protective and anti-inflammatory effects (Krause et al., 2015; Mathur et al., 2011). Several studies have confirmed the proinflammatory effects of eHSP70 in different cells, including human bronchial epithelial cells (Hulina et al., 2018; Mathur et al., 2011). A recent study showed that higher levels of plasma HSP70 might be associated with an increased risk of COPD among coal workers (Cui et al., 2015). It is known that smoking is a significant risk factor for COPD. It can be inferred that HSPs can have a part in the relationship between COPD and smoking.

Wu et al. exposed human airway smooth muscle cells to cigarette smoke in vitro. Hypo-concentration cigarette smoke (1.25% and 2.5%) increased the intracellular HSP70 expression according to their results. In contrast, the expression of intracellular HSP70 decreased significantly at high cigarette smoke concentrations (5.0%, 10.0%, and 20.0%). It was stated in the study that 1.25% daily corresponds to 1.25% smoking and 20% daily corresponds to approximately 20 cigarettes (Wu et al., 2013). These data are consistent with the current study indirectly. In the current study, daily smoking ranged from 6 to 50 cigarettes per day, so the exposure level of individuals in our study was similar to the group exposed to high cigarette smoke concentration. Krause et al. suggested that chronically elevated eHSP70 may cause an inflammatory response and intracellular HSP70 reduction (Krause et al., 2015). Therefore, increased plasma HSP levels in chronic smokers were likely to trigger proinflammatory processes. This condition

Table 3.
Age, the Number of Cigarettes Daily, Package/Year, CO, and BMI Correlation with HSPs

		Age	**The Number of Cigarettes Daily**	**Package/Year**	**CO**	**BMI**	**HSP90**	**HSP70**	**HSP40**
HSP90	*r*	–.112	.040	.030	.385	–.155	1.000	.085	.116
	p	.164	.731	.801	***<.001***	.062	–	.287	.146
HSP70	*r*	–.179	.086	.111	.201	.001	.085	1.000	.089
	p	.058	.465	.344	***.012***	.997	.287	–	.270
HSP40	*r*	–.149	.089	.089	.230	.027	.116	.089	1.000
	p	.062	.446	.449	***.004***	.733	.146	.270	–

may have decreased the amount of intracellular HSP according to the reports of Krause et al., which may negatively affect the protection of cells against oxidative stress.

HSP90 is an antiapoptotic protein and is associated with many signaling pathways, and heat shock is the best-studied member of the protein family and works in conjunction with HSP90, HSP70, and HSP40 (Parimi & Tsang, 2014). All three HSP groups were significantly higher in smokers, but there was no significant correlation between HSP90, HSP70, and HSP40 in our study. In the current study, overexpression of HSPs was demonstrated in healthy men who smoke. How smoking affects HSP levels in the abovementioned conditions that cause overexpression of HSPs and the clinical effects of this situation are candidates for new studies.

The function and role of HSP40 are not fully understood. The HSP40 family reportedly regulated the HSP70 function. Parallel high expression of HSP40, HSP70, and HSP90 in brain tumors and high expression of HSP40 in lung cancer tissues have been reported. In addition, tumor growth has been associated with HSP40 (Qiu et al., 2006). Furthermore, a study concluded that the progression of colorectal cancer is associated with overexpression of HSP40 (Kurzik-Dumke et al., 2008). The relationship between smoking and HSPs was shown in our study. It comes to mind that HSPs may have a role in the pathophysiology of the smoking – cancer relationship in cancers associated with smoking and HSPs. However, we cannot reach this conclusion with our study. It only suggests a hypothesis.

No significant relationship was found between HSP70 and hypertension in our study, although there are reports of overproduction of HSP70 in hypertension (Dhingra et al., 2006).

Viruses do not have heat shock proteins and rely on host HSPs for viral protein folding. Viruses require host HSPs for survival and can cause overexpression of HSPs in the infected cell (Goulhen et al., 2003; Lee et al., 2010). Not surprisingly, all HSPs (HSP90s, HSP70s, HSP60s, HSP40s, and HSP27) participated in coronavirus infection (Cao et al., 2012; Wan et al., 2020), proposing a good target for anti-COVID-19 (coronavirus disease-19) drug development. Early evidence suggests that the risk of adverse health outcomes (ventilator attachment and death) for patients with a history of smoking is dramatically increased compared to nonsmokers and is associated with higher admission rates to intensive care units (Harapan et al., 2020; Vardavas & Nikitara, 2020). The World Health Organization (WHO) confirmed that smokers may experience serious complications of COVID-19 compared to nonsmokers based on the recent literature (*WHO Statement: Tobacco Use and COVID-19*, 2020). HSPs may also play a role in the complex relationship between COVID-19 and cigarettes when HSPs are interpreted with the relationship between COVID-19 and HSP in the current literature according to the results of our study.

There was a significant positive correlation between CO and HSP levels. Carbon monoxide can have a direct impact on HSPs as well as an indirect impact. We did not find any studies explaining the relationship between CO and HSP in our literature review. This topic is apparently an area where new studies are needed.

Preclinical trials have proved that overexpression of the HSPs increases tumor growth, metastatic potential, and resistance to chemotherapy in rodent models (Ciocca et al., 2013; Pavan et al., 2014). Many clinical trials have also shown its association with promoting drug resistance, aggressive cancers, metastasis, and poor patient outcomes (Ciocca et al., 2013; Pavan et al., 2014). The inhibition of HSPs is thus emerging as a novel strategy for cancer therapy. In the present study, we showed that plasma HSP levels increase with smoking. Data from several clinical studies suggest that continuation of smoking during therapy for tobacco-related cancers is associated with lower response rates to chemotherapy and/or radiotherapy, and even with decreased survival (Ciocca et al., 2013; Pavan et al., 2014). The present study and these results in the literature are not sufficient to claim that HSPs also play a role in the pathophysiology of the effects of smoking in cancer patients. However, we think it is an interesting hypothesis for new studies. The most effective way to avoid the harmful effects of smoking is not to smoke. A better understanding of the pathophysiology between smoking and related diseases will show new targets for the prevention and treatment of these diseases. We believe that proving its harms with scientific evidence will strengthen physicians' belief in the fight against smoking and increase the deterrence of smoking even though it is known worldwide that smoking is harmful.

Limitations, and Directions/Suggestions for Future Research

This is a cross-sectional study. A prospective study may provide more comprehensive details. In the present study, we demonstrated that HSPs levels increase. But we do not know how it affects smoking-related diseases. This condition is a new subject for further studies.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Selçuk University (approval no.: 2015/251).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.K., K.M.; Design – M.K., K.M.; Supervision – K.M; Materials – M.K., K.M., B.S., H.V.; Data Collection and/or Processing – M.K., K.M., B.S., H.V.; Analysis and/or Interpretation – M.K., K.M.; Literature Review – M.K., K.M., B.S., H.V.; Writing – M.K., K.M.; Critical Review – M.K., K.M.

Acknowledgments: The authors thank all patients for being included in the study and Selçuk University Scientific Research Projects Coordinator.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: This study was supported by Selçuk University, Scientific Research Projects (project number: 15401179).

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Genişletilmiş Özet

Sigara İçenlerde Plazma Isı Şoku Protein Düzeylerinin Değerlendirilmesi

Giriş

Sigara dumanı Kronik Obstrüktif Akciğer Hastalığı (KOAH), karsinogenez ve kardiyovasküler hastalık gibi hastalıklarda en önemli risk faktörlerinden biri olarak kabul edilmektedir. Sigara tüm kanser ölümlerinin yaklaşık %30' unu oluşturmaktadır. Tütün ürünleri, başlıca önlenebilir ölüm nedenidir.

Heat Shock Proteinleri (HSP) polipeptid proteinlerdir ve çoğu organizmanın temel işlevlerini yerine getirirler. HSP'ler, hipoksi, hiperoksi, ultraviyole ışığına ve kimyasallara maruz kalma, viral ajanlar, cerrahi stres, beslenme yetersizlikleri gibi stres koşullarına yanıt olarak hücre tarafından sentezlenen yüksek oranda korunmuş moleküler şaperonlardır. HSP'ler boyutlarına göre HSP90, HSP70, HSP60, HSP40 ve HSP27 dahil küçük HSP'ler olarak sınıflandırılmıştır.

Yüksek veya düşük HSP seviyeleri, kanser, transplantasyon reddi, ateroskleroz, Alzheimer hastalığı, diyabet, artrit, multipl skleroz, astım, nörodejenerasyon (Huntington koreası ve Parkinson hastalığı), serebral ve miyokardiyal iskemi, dehidrasyon kaynaklı nefropati ve enfeksiyöz ajanlara karşı bağışıklık gibi çok sayıda hastalık ve hastalık dışı durumla ilişkili bulunmuştur. HSP'ler ile sigara arasında anlamlı ilişki bulunması sigara ve sebep olduğu hastalıklar arasında HSP kaynaklı bir patofizyoloji olabilirliği açısından yeni çalışma alanları doğurabilir. Sigara ile HSP ilişkisi arasında yapılan çalışmalar daha çok hücre içi HSP'ler ile değerlendirilmiştir. Bu çalışmada, sigaranın plazma HSP 90, HSP 70 ve HSP 40 düzeyleri üzerindeki etkilerini değerlendirmeyi amaçladık.

Yöntem

Mevcut çalışma kesitsel vaka-kontrol çalışmasıdır. 30-65 yaş arası 75 sigara içen erkek ile 82 sigara içmeyen erkek dahil edildi. Serebrovasküler olay, nörolojik bozukluk, kardiyovasküler hastalık, enfeksiyon, böbrek veya karaciğer yetmezliği, malignite öyküsü olan ve antikonvülsan ve / veya nefrotoksik ilaç tedavisi gören kişiler çalışma dışı bırakıldı. Çalışma protokolleri Helsinki Bildirgesi'nin etik standartlarına uygun olup, Üniversitemiz Etik Kurulu tarafından 2015/229 sayılı etik kurul kararı ile onaylanmıştır. Çalışmanın etki büyüklüğü 0,5 ve gücü %93'tür.

Hayatında hiç sigara içmeyenler veya hayatında 100'den az sigara içenler sigara içmeyenler grubuna dahil edildi. Sigara içen gruba aktif sigara içenler dahil edildi. Olguların sigara içme durumunun doğrulanması piCO Smokerlyzer Breath Bedfont Scientific cihazı ile ekspire edilen nefeste karbonmonoksit (CO) ölçümleri ile yapıldı. Bağımlılığın puanlanması ve sınıflandırılmasında Fagerstrom Nikotin Bağımlılık Testi kullanılmıştır. Anketler yüz yüze görüşme teknikleri ile tüm katılımcılara uygulanmıştır.

Plazma örneklerindeki HSP seviyeleri ELX 800 plaka okuyucu (Biotek, Almanya) ile İnsan HSP ELISA kiti (MyBioSource, ABD) kullanılarak belirlendi. Test için tespit limitleri HSP90, HSP70 ve HSP40 için 0,03ng / ml, 0,09 ng / ml ve 0,06 ng / ml idi ve çalışma içi ve çalışma arası kesinlik % 15'in altındaydı. Hemogram ve biyokimya numuneleri aynı gün Üniversitemiz Biyokimya Laboratuvarında rutin olarak çalışıldı.

Tüm verilerin analizinde Windows 21.0 için SPSS (Statistical Package for Social Sciences) istatistik programı kullanıldı.

Bulgular

Sigara içen ve içmeyen grublar arasında yaş, vücut kitle indeksi ve sosyo-kültürel düzeyleri arasında istatistiksel olarak anlamlı bir fark yoktu.

Sigara içenlerde plazma HSP düzeyleri anlamlı olarak daha yüksek bulundu. Plazma HSP90, HSP70 ve HSP40 seviyeleri sigara içen grupta sırasıyla34,45$\pm$17,26 pg/mL, 0,93 (0,08-3,78) pg/mL ve 7,85$\pm$1,41 pg/mLidi. Plazma HSP90, HSP70 ve HSP40 seviyeleri sigara içmeyen grupta sırasıyla2099$\pm$8,57pg/mL, 0,73(0,08-2,78) pg/mL ve 6,96$\pm$1,23 pg/mL idi (Sırasıyla $p<0,001$, $p=0,009$, $p<0,001$).

Sigara içenlerde Tiroid Sitimulan Hormon (TSH), Yüksek DansiteliLipoprotein (HDL) ve hemoglobin anlamlı derecede düşüktü. Beyaz Kan Hücresi(WBC) anlamlı derecede yüksekti ($p<0,005$). CO ile HSP90 arasında pozitif ve orta düzey ilşkili bir korelasyon ($r = 0,385$ $p = <0,001$) ve CO ile HSP70 ve HSP40 arasında pozitif yönlü ve zayıf düzey ilşkili bir korelasyon vardı ($r = 0,201$ $p = 0,012$, $r = 0,230$ $p = 0,004$) (Tablo 3) HSP90, HSP70 ve HSP40 seviyeleri arasında anlamlı bir korelasyon yoktu ($p> 0,050$). Her üç grup HSP ile yaş, günlük içilen sigara sayısı, şimdiye kadar içilen sigara miktarı (paket / yıl), Vücut Kitle İndeksi(VKİ), TSH, Düşük DansiteliLipoprotein (LDL), HDL, total kolesterol, trigliserid, üre, kreatinin, açlık kan şekeri, WBC, hemoglobin, platalet, Alanin Aminotransferaz (ALT) ve Aspartat Aminotransferaz (AST) arasında anlamlı bir ilişki yoktu ($p> 0,050$).

Sistolik ve diyostalik tansiyonlar ile HSP'ler arasında anlamlı bir korelasyon yoktu ($p>0.05$).

Tartışma

Bu çalışmada sigaranın plazma HSP düzeyleri üzerine nasıl etki ettiğini belirlemeye çalıştık ve sigara içmenin plazma HSP 90, HSP 70 ve HSP 40 düzeylerini önemli ölçüde artırdığını bulduk. Sigara içmenin plazma HSP 90, HSP 70 ve HSP 40 düzeylerini önemli ölçüde artırdığı bulunmasına rağmen, plazma HSP' leri ile günlük içilen sigara sayısı, şimdiye kadar içilen sigara sayısı (paket / yıl), sigara içme süresi ve miktarıarasında anlamlı bir korelasyon yoktu.

Hücre içi HSP 70, koruyucu ve anti-enflamatuar etkiler uygularken, hücre dışı HSP 70 (eHSP 70), inflamatuar yanıtı aktive ederek proinflamatuar etkiye yol açabilir. Kömür işçilerinde yapılan yakın tarihli bir çalışmada, yüksek plazma Hsp70 düzeylerinin artan KOAH riski ile ilişkili olabileceğini göstermiştir. Sigaranın KOAH için önemli bir risk faktörü olduğu bilinmektedir. KOAH ve sigara arasındaki ilişkide HSP'lerin rol oynayabileceği akla gelmektedir.

İnsan hava yolu düz kas hücrelerini in vitro olarak sigara dumanına maruz bırakıldığı bir çalışmada, düşük konsantrasyonlu sigara dumanı (%1,25 ve %2,5) hücre içi HSP70 ekspresyonunu arttırdı. Buna karşılık, yüksek sigara dumanı konsantrasyonlarında (%5.0, %10.0 ve %20.0) hücre içi HSP70'in ekspresyonu önemli ölçüde azaldı. Çalışmada %1,25 sigara dumanının günde bir sigara içmeye karşılık geldiği, günlük %20'nin ise yaklaşık 20 sigaraya tekabül ettiği belirtilmiştir. Bu veriler bizim çalışmamızı dolaylı olarak desteklemektedir. Çalışmamızda günlük sigara içimi günde 6 ile 50 adet arasında değişiyordu, bu nedenle çalışmamızdaki bireylerin sigaraya maruz kalma düzeyi yüksek sigara dumanı konsantrasyonuna maruz kalan grup ile benzerdir. Krause ve ark. kronik olarak yükselmiş eHSP70'in inflamatuar bir yanıta ve hücre içi HSP70 azalmasına neden olabileceğini bildirdi. Dolayısıyla çalışmamızda kronik olarak sigara içenlerde plazma HSP düzeyleri artarak proinflamatuar süreçleri başlatmada etkili olması muhtemelken, Krause ve ark.'larının bildirimlerine göre hücre içi HSP miktarlarını azaltmış olabilirler ve bu da hücrelerin oksidatif sitrese karşı kornmasını olumsuz yönde etkileyebilecektir.

Çalışmamızda, her üç HSP grubu da sigara içenlerde anlamlı olarak daha yüksekti ancak HSP 90, HSP 70 ve HSP 40 arasında anlamlı bir korelasyon yoktu.

Yakın zamanda yapılan bir çalışma, beyin tümörlerinde HSP 40, HSP 70 ve HSP 90'ın paralel yüksek ekspresyonunu ortaya çıkardı. Ayrıca, akciğer kanseri dokuları üzerinde yapılan bir araştırma, HSP 40'ın yüksek oranda eksprese edildiğini gösterdi. Bazı raporlar, tümör büyümesinin modülasyonunda bazı HSP 40 ailesi üyelerinin katılımını açıklamaktadır. Ayrıca, yakın tarihli bir çalışma, kolorektal kanserin ilerlemesinin HSP 40'ın aşırı ekspresyonu ile ilişkili olduğu sonucuna varmıştır. Sigara içen sağlıklı erkeklerde HSP'lerin aşırı ekspresyonu olduğu çalışmamızda gösterildi. HSP'lerin aşırı ekspresyonu olan yukardaki durumlarda sigara içmeye devam etmenin HSP düzeylerini daha da fazla artırıp artırmadığı ve bu durumun klinik etkileri yeni çalışmalara konu olmaya adaydır.

Sigara ve HSP'lerle ilişkili kanserlerde sigara-kanser ilişkisinin patofizyolojisinde HSP'lerin rolü olabileceği akla gelmektedir. Ancak çalışmamızla bu sonuca varamayız.

Sigaranın zararlı olduğu dünya çapında bilinmesine rağmen, zararlarını bilimsel kanıtlarla kanıtlamanın doktorların sigarayla mücadeleye olan inancını güçlendireceğine ve sigaranın caydırıcılığını artıracağına inanıyoruz.