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DETERMİNATION OF SOME ANTIOXIDANT ENZYME LEVELS IN HAIRDRESSER EMPLOYESS IN THE CENTRAL DISTRICTS OF VAN PROVİNCE

Van İlinin Merkez İlçelerinde Kuaför Çalışanlarında Bazı Antioksidan Enzim Düzeylerinin Belirlenmesi

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ABSTRACT

Although hairdressing is not a dangerous profession, it is known that hairdressers are exposed to chemical combinations known as allergens, carcinogens or organic solvents. In this study, it was aimed to determine the levels of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH), which are known as some important antioxidants, and malondialdehyde (MDA), the end product of lipid peroxidation and an indicator of oxidative stress, in hairdresser workers in Van. 33 female hairdresser employees working in the central districts of Van (Edremit, Tusba and Ipekyolu) were included in the scope of the study as subjects, and voluntary participants using hairdresser services were included as the control group. Venous blood was taken from those who accepted the study, examined in the laboratory, and the results were statistically analyzed. The difference between group means for MDA, CAT, SOD and GSH was statistically significant ($p<0.05$). As a result, it can be said that hairdressers, whose professional lives will last for many years, are in the potential risk group in the formation of diseases such as eczema, asthma, cardiovascular diseases, cancer, in which reactive oxygen derivatives also play a role, since they are constantly exposed to physical and chemical factors.

Keywords: Catalase, Glutathione, Hairdresser workers, Malondialdehyde, Superoxide dismutase.

ÖZ

Kuaförlük tehlikeli bir meslek olmasa da kuaförlerin alerjenler, kanserojenler veya organik solventler olarak bilinen kimyasal kombinasyonlara maruz kaldıkları bilinmektedir. Bu çalışmada, Van'daki kuaför çalışanlarında bazı önemli antioksidanlar olarak bilinen süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon (GSH) ile lipid peroksidasyonunun son ürünü ve oksidatif stres düzeyinin göstergesi olan malondialdehit (MDA) düzeylerinin belirlenmesi amaçlanmıştır. Araştırma kapsamına Van ili merkez ilçelerinde (Edremit, Tusba ve İpekyolu) çalışan 33 kadın kuaför çalışanı denek olarak, kuaför hizmetlerinden faydalanan gönüllü katılımcılar kontrol grubu olarak dahil edildi. Çalışmayı kabul edenlerden venöz kan alındı, laboratuvarda incelendi ve sonuçları istatistiksel olarak analiz edildi. MDA, CAT, SOD ve GSH için grup ortalamaları arasındaki fark istatistiksel olarak anlamlıydı ($p<0.05$). Sonuç olarak mesleki yaşamları uzun yıllar sürecek olan kuaförlerin, sürekli fiziksel ve kimyasal etkenlere maruz kaldıkları için egzama, astım, kalp-damar hastalıkları, kanser gibi reaktif oksijen türevlerinin de bulunduğu hastalıkların oluşumunda potansiyel risk grubunda olduğu söylenebilir

Anahtar kelimeler: Glutatyon, Katalaz, Kuaför çalışanları, Malondialdehit, Süperoksit dismutaz.

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INTRODUCTION

Although hairdressing is not a dangerous profession, it is known that hairdressers are exposed to combinations of chemicals known as allergens, carcinogens or organic solvents. These chemicals, which are found in nail and skin care products as well as hair products, can cause allergic dermatitis, asthma, rhinitis and even cancer when taken by skin or respiratory tract. Cancers associated with hairdressing include lung, breast, ovarian, cervical, lymphoma, bladder, pancreatic, and salivary gland cancers (Pignatti et al., 2013; Preston, 2008). A previous study has shown an increased risk of breast cancer among hair dye users (Heikkinen, 2015). Similarly, it is thought that exposure to chemicals in the aromatic amine structure found in hair dyes play a role in increasing the risk of bladder cancer in hairdressers (Şüküroğlu & Burgaz, 2018).

These chemicals act as free radicals in the body that damage compounds such as protein and DNA. When it comes to continuous exposure to chemicals, free radicals increase in humans and damage healthy cells over time. Normally, tissue damage caused by free radicals in the body is controlled by antioxidant defense systems. The increase in free radicals in the body slow down the antioxidant defense systems and cause oxidative stress. In this case, dysfunction in body cells and tissues and various related diseases may occur. At the same time, hairdressers are exposed to electromagnetic fields as they constantly use blow dryers/hair dryers, which adversely affect health (Eloff et al., 2013; Heikkinen, 2015; Preston, 2008). As free radicals are highly reactive and short-lived, they are difficult to measure directly (Altınışık, 2000). The measurement of MDA is widely used to determine the damage caused by oxidative stress in cells. SOD, glutathione peroxidase (GPX), glutathione reductase (GR), and CAT are among the most important enzymatic antioxidants that prevent the accumulation of free radicals and the initiation of lipid peroxidation. These enzymes inside the cell destroy superoxide and peroxides before they react with metal catalysts to form more reactive species. Measurement of antioxidant enzyme activities such as SOD and CAT are indirect ways to understand the antioxidant defense status of the organism (E. Çetin, N. Çetin & Küçük, 2011; Karabulut & Gülay, 2016).

In line with this information, we aimed to determine the levels of MDA (an indicator of oxidative stress level and the end product of lipid peroxidation), and some important antioxidants such as SOD, CAT, GSH in hairdresser workers in some regions of Van province.

MATERIAL AND METHOD

33 female hairdressers working in the central districts of Van (Edremit, Tusba and İpekyolu) were included in the study. People working in manicure/pedicure, haircut, hair dyeing and styling, skin care in the hairdresser's salon were determined as the subjects and voluntary participants using hairdresser services were determined as the control group. After going to the workplaces and giving information about the study, written consent was obtained and blood samples were collected from those who accepted the study. Blood samples taken from 30 volunteers in the control group were brought to Van Yuzuncu Yil University (YYU) Biochemistry Laboratory in the Faculty of Science, Department of Chemistry, at the end of each day and centrifuged. After separating the serums, they were kept at -80°C until the study day. In the blood serum samples taken, the level of MDA, which was the end product of lipid peroxidation and SOD, CAT, and GSH, which had an important place in protecting cells against oxidative stress, were determined by spectrophotometric method. After examining the same parameters in serum samples obtained from blood samples taken from healthy control groups, the results obtained from both groups were statistically analyzed and interpreted.

Determination of Biochemical Parameters

Determination of SOD Activity

SOD activity was determined by using the proposed method of (Popov et al., 2003). SOD accelerates the dismutation of hydrogen peroxide and molecular oxygen of superoxide radicals (O_2 •−) formed during the oxidative energy production. This method is based on the reading of topic density resulted from using of xanthine and xanthine oxidase in which superoxide radicals that generated from the blue colored formazan dye of the nitro blue tetrazolium (NBT) in the optical density wavelength of 560 nm. The SOD that exists in the sample serum inhibits the formazan reaction by excluding superoxide radicals from the environment. Under the experimental conditions, 1unit of SOD is the %50 inhibition of NBT reduction rate.

%Inhibition = [(Blank OD – Sample OD) / Blank OD] × 100

Determination of CAT Activity

Serum (0.1 mL) was added to a quartz cuvette containing 1.4 mL of 30 mM H_2O_2 prepared in potassium phosphate buffer (50 mM, pH 7.4). The change in absorbance was monitored by psectrophotometer at 240 nm for 30 seconds (Aebi, 1974).

Activity = $(2.3/\Delta x) \times [(\log A1/\log A2)]$.

Activity: calculated in U/L

Δx = 30 seconds

2.3 = 1 μmol optical density of H_2O_2 in 1 cm light path

Determination of GSH Level

The reduced GSH was measured as the final product of the reaction was achieved, that was the formation of the yellow color, of obtained clear liquid of sulfhydryl groups and DTNB (5,5'-(dithiobis 2-nitrobenzoic acid). Measurement of the GSH level in the serum blood was done in 412 nm wavelength in the spectrophotometer (Beutler et al., 1963).

Activity (mg/dl) = [(OD2-OD1)/13600 ×E1 1.25] ×1000

OD1: First absorbance before addition of DTNB at 412 nm.

OD2: Second absorbance after addition of DTNB at 412nm.

E1: 1 in the calculations

13600 is the molar extinction coefficient of the yellow color that formed during the interaction of GSH and DTNB.

Determination of MDA Level

The reaction of fatty acids with free radicals result in MDA, which was the final product of lipid peroxidation, was measured with thibarbituric acid that gives a colored form (Gutteridge, 1995). 200 mL from the blood was taken and put into 1 tube. 800 mL phosphate buffer, 25 mL BHT solution, and 500 mL of %30 TCA were added. The tubes were stirred with vortex and kept on ice for 2 hours. Then centrifuged at 2000 rpm for 15 minutes, 1 mL from supernatant was taken and transferred to other tubes. Then 75 mL of EDTA and 250 mL of TBA were added. Tubes were mixed in the vortex and kept in a hot water bath for 15 minutes. Then, they were brought to room temperature and their absorbance was read at UV/V spectrophotometer at 532 nm.

Calculation of MDA level:

C=F* 6.41* A

C: Concentration

F: Dilution factor

A: Absorbance

Statistical Analysis

Descriptive statistics were expressed as Mean, standard Deviation. T-test was used in cases with normal distribution condition. Mann-Whitney U test was used in cases where normal distribution condition was not provided. The statistical significance level was taken as $p<0.05$

and the SPSS statistical software version 19.0 (SPSS Inc, Chicago, III, USA) pack was used for analyses.

Ethical Declaration

For this study, permission was obtained from the Ethics Committee of Yuzuncu Yil University, Faculty of Medicine, Interventional Clinical Research, with the letter dated 04/03/2020 and numbered 11, and the Helsinki Declaration criteria were taken into consideration.

RESULTS

Descriptive statistics and comparison results of the groups were summarized. When Table 1 was examined, the difference between group averages for MDA, CAT, SOD, and GSH were statistically significant (p <0.05). According to these results, the mean of MDA in the hairdresser staff group was found to be high compared to the average in the control group, while it was found to be low in the CAT, SOD, and GSH hairdresser staff group compared to the control group (**Figure1**, and **Table 1**).

Table 1. Descriptive Statistics and Comparison Results in The Hairdresser Staff and Control Groups

0	Group	n	Mean±Std. Deviation	*p*
MDA (µmol/mL)	Hairdresser Staff	33	0.66±0.05	0.001
	Control	30	0.27±0.06	
CAT (U/mL)	Hairdresser Staff	33	0.13±0.014	0.001
	Control	30	0.25±0.018	
SOD (U/mL)	Hairdresser Staff	33	41.73±18.94	0.001
	Control	30	77.69±9.87	
GSH (mg/dL)	Hairdresser Staff	33	0.001±0.0006	0.001
	Control	30	0.03±0.012	

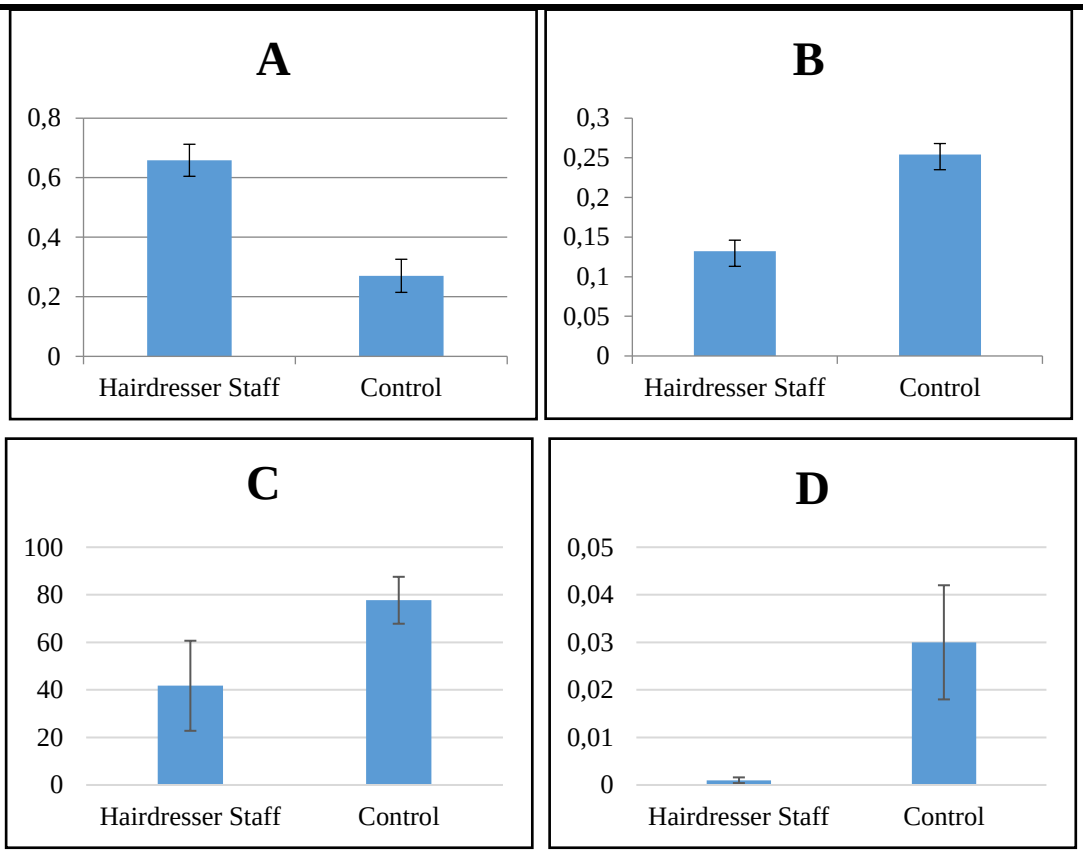

Figure 1. Levels of MDA (A), CAT (B), SOD (C), and GSH (D) in Groups

DISCUSSION

We actually need free radicals for proper immune function. Reactive molecules that occur naturally during the conversion of nutrients into energy using oxygen are used by the liver for detoxification and destroying damaged cells, bacteria, and viruses (Akkuş, 1995). UV rays, drugs, immunological reactions, radiation, stress, smoking, exposure to alcohol and chemicals are environmental factors that contribute extra to the formation of free radicals. The abundance of free radicals formed as a result of continuous exposure disrupts the balance of antioxidants and free radicals in the body. Since the increase in reactive products damages cell components such as lipid, protein and DNA, they contribute to the formation of conditions such as neurodegenerative diseases, cardiovascular diseases, diabetes, acute renal failure, laryngitis, asthma, bronchitis, dermatitis, and cancer (Çömelekoğlu, Mazmancı & Arpacı, 2000; Eloff, Preston, Pretorius, Du Plessis & Laubscher 2013; Leino, 1999; Ma, Lin, Chen, Huang & Li, 2010; Sulaiman, Demir, Soyoral & Demir, 2021). Since the working conditions of hairdressers and the standards to be complied with are not well-adjusted, these workers are frequently exposed to many risk factors all over the world, including occupational diseases such as asthma and other respiratory problems, contact dermatitis, poor posture, and cancer (Dryson, ’t

Mannetje, Walls, McLean & McKenzie, 2007; Ferrari, Moscato & Imbriani 2005; Moscato & Galdi, 2006). Studies in the literature on the adverse systemic effects of hairdressing have revealed the relationship between hair dyes and human malignancies. It has been stated by the International Agency for Research on Cancer (IARC, 1993) that the risk of urinary bladder cancer, lung cancer, and non-Hodgkin lymphoma is high in hairdressers. The profession has therefore been deemed 'possibly carcinogenic' (IARC, 1993). In a study conducted in 24 states of the USA, it was confirmed that hairdressers were exposed to a significant risk, primarily lung, stomach, pharyngeal, all lymphatic, and hematopoietic cancers (Lamba, Ward, Weeks & Letal, 2001). A 39-year cohort study of the incidence of neoplastic diseases in hairdressers found that male hairdressers showed an extreme risk of upper respiratory-digestive system, lung, and colon cancer. Again in the same group, an increased risk of pancreatic, lung, and cervical cancer was observed in female hairdressers (Czene, Tiikkaja & Hemminki. 2003). In addition, epidemiological studies have investigated the risk of bladder cancer among hairdressers and hair dye consumers in relation to harmful components such as aromatic amines contained in hair dyes (Golka et al., 2004). It has been shown that 0.1% to 0.5% of permanent (oxidative) hair dyes containing aromatic amines such as p-phenylenediamines and p-aminophenols used in beauty salons are absorbed through the skin in humans (Bolt & Golka, 2007). Some of the chemicals used in hair dye products has been reported to be carcinogenic in animals (Bjarte, 2005; Harling, Schablon, Schedlbauer, Dulon & Nienhaus, 2010).

In this study, there is a continuous exposure to chemicals and non-ionizing radiation, as those who have been in the hairdressing profession for 10 years or more are included in the study. P-phenylenediamine is the main aromatic amine in hair dye and is one of the common causes of contact sensitivity in an exposed person, possibly related to the formation of oxygen radicals (Picardo, Zompetta & Marchese, 1992). Studies have shown that topically pre-treated antioxidants such as alpha-tocopherol and SOD effectively protect skin cells from oxidative damage caused by ultraviolet light (Hamanaka, Miyachi & Imamura, 1990). In this study, MDA value was found to be significantly higher in hairdresser employees ($p<0.05$). In another study, a statistically high MDA concentration was found in hairdressers (Menicagli, Marotta & Menicagli, 2018).

In the current study, most of the hairdressers who do hair dyeing, hair cutting, washing, hair styling, and epilation were using gloves and masks while dyeing hair, but were not using the counted protection equipments while preparing the dye, washing, and cutting the hair. Lind, Johnsson, Lidén, Meding & Boman (2015) found measurable amounts of resorcinol in both hands of hairdressers after haircuts (Lind, Johnsson, Lidén, Meding & Boman, 2015).

Resorcinol is a chemical found in most oxidative hair dye products, especially disrupt thyroid function. Another study has reported that nitrile rubber gloves provide better protection against hair dye exposure than polyvinylchloride (PVC) and natural rubber latex (NRL). The same study has suggested that rubber allergen-free PVC gloves are the best choice to minimize the risk of contact allergy from hair dye (Lind, Johnsson, Lidén, Meding & Boman, 2017). Since NRL gloves were more economical and provide ease of use, hairdressers generally used NRL and disposable plastic gloves in our study. All the halls had natural ventilation instead of extraction ventilation. It has been reported that problems related to eyes, nose, throat, lungs, and skin are common among hairdressers and their clients due to poor indoor air quality in hairdressing salons (Leino, 1999). In another study, volatile organic compounds (VOC) in sprays were accepted as dangerous air pollutants and it was suggested that they could cause pulmonary and cardiovascular symptoms (Ma et al., 2010). Hairdressers' reliance on natural ventilation and insufficient natural ventilation, especially in winter, increases their exposure potential.

Oxidative stress is defined as any irregularity between pro-oxidants and antioxidants. Studies have shown that antioxidants reduce the effects of free radicals and prevent cell damage (Sulaiman et al., 2021). An increase in lipid peroxidation levels in healthy tissues is an indicator of tissue damage. Picardo et al. (1996) reported that hair dye application causes oxidative stress due to the formation of free radicals due to the chemical components in the dye (Picardo, M., Zompetta, Grandinetti, Ameglio, Santucci, Faggioni & Passi, 1996). In addition, they observed a decrease in antioxidant enzyme activities such as SOD, CAT, and GSH after exposure to hair dye, and an increase in membrane lipid peroxidation level depending on the exposure time. In our study, SOD and CAT values in hairdresser employees were lower than healthy people ($p<0.05$) (Figure1, and Table 1). SOD is an endogenous antioxidant enzyme that catalyzes the conversion of superoxide radical (O_2^-) into less harmful hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). CAT is the endogenous enzyme that breaks down hydrogen peroxide (H_2O_2) into water and oxygen. A low SOD causes an increase in superoxide radicals in the body. Superoxide radical can turn into toxic products as a result of various reactions. Similarly, low CAT causes accumulation of hydrogen peroxide in the organism. Hydrogen peroxide is not a free radical, but forms a hydroxyl radical (OH). GSH is a very important endogenous antioxidant, it reacts with free radicals and peroxides, protecting cells against oxidative damage. In this study, the GSH value was lower in hairdresser workers than in healthy individuals ($p<0.05$). Low GSH causes an increase in oxidative stress (Karabulut & Gülay, 2016). Again, it can be thought that the decrease in the amount of enzymes reacts with lipid peroxidation, thus

reducing the oxidative stress that may occur against the chemicals that employees are exposed to at work. P-phenylenediamine is the main aromatic amine in hair dye and is one of the common causes of contact sensitivity in an exposed person, possibly related to the formation of oxygen radicals (Picardo et al., 1992). Studies have shown that topically pre-treated antioxidants such as alpha-tocopherol and SOD effectively protect skin cells from oxidative damage caused by ultraviolet light (Hamanaka et al., 1990). Our study results were consistent with previous similar studies (Çömelekoğlu, Mazmancı & Arpacı, 2000; Gündüz & Demir, 2020; Sulaiman, Demir, Soyoral & Demir, 2021). In a study investigating lipid peroxide levels, antioxidant enzyme activities such as GSH, SOD, (GSHPx), CAT in erythrocytes, and DNA damage in lymphocytes in young women exposed to hair dyeing, modification of antioxidant enzyme activities and lymphocyte DNA damage were shown (Sim et al., 2005). The ingredients of hair dyes, which are frequently made in hairdressing salons, have medium to low acute toxicity. In recent years, the use of hair dyes has increased, especially in developed countries, and in parallel, sensitivity to hair dyes has become widespread in general populations. In our study we observed that exposure to hair dyeing caused modifications in antioxidant enzyme activities. Therefore, it is clearly seen that more studies are needed to investigate the relationship between the harmful effects of hair coloring and the antioxidant status.

CONCLUSION

As a result, it can be said that hairdressers, whose professional lives will last for many years, are in the potential risk group in the formation of diseases such as eczema, asthma, cardiovascular diseases, cancer, since they are constantly exposed to physical and chemical factors. Hairdresser employees and persons in this sector should be encouraged to inform about the toxic effects of chemicals and to reduce exposure. For example, local exhaust ventilation in places where hair dyes are mixed, closing the lids of the products when not in use, applying the dye after the hair is cut, and well ventilated salon reduce the possible risks. In addition, hairdressers should be informed about the necessity and correct use of personal protective equipment such as gloves and masks. It should be emphasized that people who constantly work with electrical devices that generate electromagnetic fields should keep the blow dryer away from them in order to reduce the dangers of these machines. It is also recommended that they give importance to a balanced diet as much as possible. There is also a need for new studies in which the risks are investigated more in order to prevent occupational diseases in hairdresser employees.

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