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Araştırma Makalesi

The Effects of Long-Term Exposure To Textile Dyes On Serum Cytokine And Antioxidant Enzyme Activities

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Abstract

The dyehouse works can be used in almost all areas of the industry. Nowadays, the paints used in industry are very diverse with their different chemical properties and different application methods. Oxidative stress and cytokine levels were measured to determine the occupational exposure status of 40 workers in dyehouses of textile industry and 40 employees working in other office work. The oxidant/antioxidant parameters superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx) were found to be statistically significantly lower in the dye-exposed group compared to the control group ($p<0.05$). The parameters of inflammation biomarkers tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin 1 beta (IL-1β) were statistically significant higher in the dye-exposed group, compared to the control group ($p<0.05$). As a result, it is understood that the oxidant-antioxidant and anti-inflammatory cytokine balance status of workers in the dyehouse of a large-scale factory will deteriorate over time.

Keywords: Anti-inflammatory cytokines, antioxidant, dyehouse worker, worker health

INTRODUCTION

Painting is a coating method applied to color or protect any object. Paint types with different properties that can be applied to almost all materials are produced in industry. The painting job can be used in almost all areas of the industry. Today, the paints used in industry are very diverse with their different chemical properties and different application methods. Many products such as textiles, food, industrial parts, vehicles, furniture are subjected to dyeing at the final stages of their production. Paint; is a chemical mixture that consisting of binders, solvents, pigments and additives. Dyehouse or paint workers are exposed to some harmful solvents and paint removers that containing aromatic hydrocarbons. Solvent and paint removers are containing some of aromatic hydrocarbons that have clastogenic activity. Aromatic hydrocarbons form a mixture containing genotoxic substances (such as benzene, xylene and toluene). Occupational chronic exposure to these substances is considered genotoxic. There is little information about genotoxic damage (chromosomal and DNA damage) in workers exposed to the dye, and detection of the damage depends on a good understanding of the carcinogenic effects of these substances (Villalba-Campos., et al. 2016). In addition, genotoxic agent markers formed as a result of occupational exposure are antioxidant and anti-inflammatory cytokines. Free radicals, which are constantly formed during normal metabolic reactions in living things and by external factors such as radiation, toxic chemicals, drugs, are neutralized by antioxidant defense mechanisms. There is a balance between antioxidant mechanisms and free radical formation. In case of disruption of the mentioned balance, the amount of free radicals increases and oxidative damage occurs in molecules such as lipid, carbohydrate, protein and DNA. During the disease process, oxidative stress occurs in molecule, tissue and cell damage (Ercan et. al., 2012, Gutteridge et. al., 1993). Oxidative stress creates a cellular redox imbalance seen in a variety of cancer cells. For this reason, it can be said that oncogenic stimulation may be associated with redox imbalance. The resulting reactive oxygen species (ROS) can cause genetic changes in active genes and cause DNA damage. DNA mutation has a critical importance in carcinogenesis. High oxidative DNA lesions (8-OH-G) in various tumors attract attention and strongly influence this damage in the etiology that causes cancer (Ercan et. al., 2012). There are many defense mechanisms to destroy free radicals and reduce the damage they cause. These are known as “antioxidant defense mechanisms” (Altan et. al., 2006). Antioxidants neutralize the damage caused by oxidant molecules with both intracellular and extracellular defense (Altan et. al., 2006). This system is endogenous and that divided into two as enzymatic and non-enzymatic (Çaylak et. al., 2011). Some of the enzymatic ones are antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) (Pekcan et. al., 2011). Acute phase response is stimulated by cytokines released from monocytes and macrophages at the site of inflammatory lesions or infections. Cytokines are cellular regulatory proteins. These proteins are secreted by specific cells against different stimuli and thus the behavior of target cells is affected (Yarım et. al., 2006). Cytokines; they are soluble peptides that affect cell growth, maturation or functions. Cytokines are primarily involved in host defense against infections and diseases (Ergonul

et. al., 2009). Major cytokines held responsible in the pathogenesis of inflammation; It is IL-1, IL-6 and TNF-α. IL-1β; It can be produced by many cell types, including monocyte, B cells, keratinocytes, mesangial cells, and endothelium (Ergonul et. al., 2009). It consists of a wide variety of hazardous substances such as dyes, organic solvents and heavy metals. Biomonitoring is an important tool for assessing occupational health risk. Therefore, in this study, we analyzed exposure levels and changes in oxidative stress biomarkers and cytokines in the paint industry.

MATERIALS and METHODS

Study design

With the approval of Fırat University Non-Invasive Research Ethics Committee, dated 28 March 2019, decision number 05/13, the participants were informed about ethical rules. Study participants signed informed consent forms in accordance with the Declaration of Helsinki. Oxidative stress and cytokine levels were measured to determine the occupational exposure status of employees in Denizli textile industry dyehouses and other office work. Socio-demographic data the history of smoking and occupational protective equipment were interviewed with all participating workers and a personal questionnaire about work and lifestyle was answered by all individuals and recorded.

Exposure status

Control group: 40 workers (employees not exposed to paint who in administrative divisions and other office department). Exposed group: 40 workers (working at least 1 year who directly exposed to raw material weighing, solvent and paint in the dyehouse)

Laboratory analysis

Biochemical examinations were performed at Fırat University Medical Faculty Medical Biochemistry Laboratory. Blood samples (5 mL) were taken from 80 participants when their an empty stomach. Blood serums were separated by centrifugation at 4000 g for 5 - 10 minutes. It was kept at -20 ˚C until the day of the study. After thawing blood samples taken from the study groups and with separated serums; SOD, CAT, GSH, GPx, liver function tests (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) and inflammation marker (IL-6, TNF-α) levels were measured in serum. Oxidative stress/antioxidant parameters SOD, CAT, GSH, GPx levels were analyzed by ELISA in 96-well microplates using appropriate kits (Cayman, Michagen, USA). The washing procedure was repeated five times, then the detection antibody was added, followed by the addition of substrate and the stop solution, and the absorbance ELISA (BIOTEK ELx800) was read in the reader. Commercial kit measuring glutathione reductase enzyme was used to determine GSH concentration. SOD concentration was expressed as U/mL, GSH concentration as μM, CAT concentration as U/L, GSH concentration as μM, GPx concentration as U/L. Inflammation biomarkers TNF-α, IL-6, cytokine quantification according to human-specific kit protocols were performed in 96-well microplates following the instructions with a commercial kit (Bioscience, Vienna, Austria). The washing procedure was repeated five times and the detection antibody was added. The absorbance was read in an ELISA (BIOTEK ELx800) reader at 450 nm by adding 50 μL of stop (phosphoric or sulfuric acid) solution after incubation in the dark for 30 minutes at room temperature (23 ^{0}C) after substrate addition. Using the absorbance data, the

concentration of each cytokinin was obtained in picograms per mL (pg/mL).

Statistical analysis

The data were evaluated with SPSS statistical package program (IBM SPSS Version 22.0) (Armonk NY. 2013). Parametric tests were used by controlling the data using Shapiro-Wilk test and variance homogeneity "Levene" test. Independent Samples T (Student T) test was used. Statistical significance was accepted at the level of $p<0.05$. Data were presented as mean ± standard deviation (SD) values.

RESULTS

Laboratory analysis

Serum oxidant/antioxidant parameters (SOD, CAT, GSH, GPx), liver function tests (AST, ALT), and inflammation biomarkers (IL-6, TNF-α) were determined by analysis (Table 1, 2). It was observed that oxidant/antioxidant parameters (SOD, CAT, GSH, GPx) in the exposure group were statistically significantly lower than the control group ($p<0.05$). Inflammation biomarkers TNF-α, IL-6 and IL-1β parameters were found to be statistically significantly higher in the exposed group than in the control ($p<0.05$). SOD levels were 116.0 ± 9.2 U/mL in the control group and 92.1 ± 8.1 U/mL in the exposed group. CAT levels were 33.7 ± 3.4 U/L in control group and 25.2 ± 2.8 U/L in exposed group. The average GSH levels were 2.5 ± 0.1 μM and 2.18 ± 0.22 μM in the control group, and exposed group, respectivly. The mean GPx level was 1.35 ± 0.22 U/L in the control group and 1.07 ± 0.12 U/L in the exposed group. TNF-α values were 41.51 ± 3.11 pg/mL in control group and 51.14 ± 4.31 pg/mL in exposed group. The average IL - 6 levels were 65.59 ± 3.21 pg/mL and 82.12 ± 4.58 pg/mL in the control group, and exposed group, respectively (Table 1).

Table 1 Serum antioxidant and inflammation biomarkers (SOD, CAT, GSH, GPx, TNF-α, IL-6, IL-1β).

Laboratory tests	Control group (n = 40)	Exposed group (n = 40)	p
SOD (U/mL)	116.00 ± 9.20	92.10 ± 8.10	p<0.05
CAT (U/L)	33.70 ± 3.40	25.20 ± 2.80	p<0.05
GSH (μM)	2.50 ± 0.10	2.18 ± 0.22	p<0.05
GPx (U/L)	1.35 ± 0.22	1.07 ± 0.12	p<0.05
TNF-α (pg/mL)	41.51 ± 3.11	51.14 ± 4.31	p<0.05
IL-6 (pg/mL)	65.59 ± 3.21	82.12 ± 4.58	p<0.05
IL-1β (pg/mL)	101.46 ± 5.26	122.47 ± 6.24	p<0.05

Data are presented as mean ± standard deviation. Control group: not exposed to dye. Exposed group: direct exposed to dye. Significant difference at $p<0.05$ (A statistically significant difference was accepted).

Table 2 Liver function biomarkers

Serum biochemistry parameters	Control group (n = 40)	Exposed group (n = 40)	p
AST (U/L)	17.2 ± 3.40	19.40 ± 4.10	NS
ALT (U/L)	15.5 ± 2.50	18.8 ± 3.20	NS

Data are presented as mean ± standard deviation. Control group: not exposed to dye, Exposed group: direct exposed to dye, NS: Not significant

Although AST and ALT enzyme activities were higher in the exposed group than in the control group, this value was not statistically significant.

Life situations of participants

Family history, nutritional status, body mass index information, as well as smoking and alcohol use, frequency of computer, telephone and hair dryer use were recorded from the participants in the study.

DISCUSSION

Free radicals occur during the functioning of normal metabolic events in the organism or under the influence of various environmental factors such as environmental agents (pesticides, aromatic hydrocarbons, toxins, solvents, etc.), stress, radiation. Free radicals can also be formed during normal metabolic activities of our body (eg, after feeding). However, industrial wastes, sun rays, cosmic rays, ozone, especially the gases from vehicle exhausts, heavy metals, viruses, cigarette, alcohol, stress, residual products formed as a result of fat metabolism in the body, various chemicals, water and air are environmental factors that create free radicals. The paint industry is a heavy business in which its employees are negatively affected by paints and solvents and often suffer from occupational lung diseases. Inhalation of dyes and solvents in business life can negatively trigger oxidative stress and inflammation mechanisms as well as lung cancer and various lung diseases. The main source of occupational exposure is the use of dyes and organic solvents in industrial production (Martínez-Alfaro M. et al. 2006). In the studies conducted; It is said that the properties of the chemical solvent in subjects determine the degree of distribution in the environment and the toxicological profile of the exposed persons (Campagna D. et al. 2001). Prolonged inhalation of the dye solvent toluene adversely affects many organs in humans and rats (Ciarroca M. et al. 2012, Martínez-Alfaro M. et al. 2011). Chronic exposure to solvents leads to loss of multi-organ function and the development of various pathological conditions (Baydas G. et al. 2005). The immune system is of great importance in maintaining the health of living, and it is also essential for vital tissues such as the lungs. The immune system plays an important role in maintaining health, especially in the lungs. It shows that it can be the target of toxic effects caused by various chemicals, including paint residues and paint removal products. It is known that the levels of pro-inflammatory cytokines (IL-4 and IL-6) increase significantly in the serum of dyers (Karagözler AA. et al. 2002). IL-6 acts as both a pro-inflammatory and anti-inflammatory cytokine. The antagonist to regulatory T-lymphocytes, IL-6 helps the growth of B-lymphocytes (Pestka S. et al. 2004). In our study, serum antioxidant parameters (SOD, CAT, GSH, GPx) and liver function tests (AST, ALT) and inflammation biomarkers (IL-6, TNF-α, IL-1β) were determined as a result of the analysis (Table 1, 2). Antioxidant parameters (SOD, CAT, GSH, GPx) in the control group were found to be significantly higher than the exposed group to dye ($p<0.05$). The parameters of inflammation biomarkers TNF-α, IL-6 and IL-1β were found to be statistically significantly higher in the exposed group compared to the control group ($p<0.05$). There was no statistically significant change in liver function tests (AST and ALT). Considering the findings we obtained after the study, it is understood that oxidative stress increases with dye exposure and therefore reduces antioxidant enzyme levels negatively. In

addition, an expected increase in anti-inflammatory cytokine levels was observed after oxidative damage and changing balance in favor of oxidants. We found that when compared with the literature, we got similar results. Blood mediated inflammatory response can injured critical organs (Gotts JE. et al. 2016). TNF-α, IL-6 and IL-1β are classified as proinflammatory cytokines (Dinarello CA. 2000). High levels of TNF-α, IL-6, which are among the proinflammatory cytokines, have been associated with chronic obstructive pulmonary disease (COPD) and asthma (Şakar A. et al. 2004, Stankiwicz W. et al. 2002). According to the data we obtained from our study, the high values of this cytokines suggest that diseases such as asthma and COPD may occur.

CONCLUSION

As a result, it is understood that the after dye exposure, oxidant-antioxidant and anti-inflammatory cytokine balance status of workers in the dyehouse of a large-scale factory will deteriorate over time. It is necessary to focus more on occupational health and safety issues that are included in the field of medicine and health today. Precautions should be increased by providing in-service training on the necessity of using necessary precautions and protective equipment for workers. In addition, support should be obtained from preventive physicians and public health experts in terms of working hours and occupational diseases. It may be recommended to perform blood tests at regular intervals in a year in order to keep the anti-inflammatory cytokines in balance in return for the decrease in antioxidants. These recommendations may also require new and comprehensive studies. Reduced antioxidant enzymes, supplemented with various nutritional habits and food supplements, can also reduce oxidative stress by increasing the level of rescue of employees and providing a stress-free work environment. Considering all these suggestions and recommendations, keeping in mind that occupational health and safety is a society problem will ease the burden on the health sector.

LIMITATIONS OF THE RESEARCH

A more comprehensive study in Turkey could have led to better suggestions. In the study, using one factory province is the most important constraint for this study. In this study; Since the small sample is used, new studies with increased number of participants are needed.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

FINANCIAL DISCLOSURE

All authors declare no financial support.

ETHICAL COMMITTEE APPROVAL

Before the study, permissions were obtained from local ethical committee. Fırat University, Non-Invasive Research Ethics Committee, 05/13 - 28 March 2019. Some of data of this study were presented as an oral presentation at the 2Nd International Medical Congress of İzmir Democracy University (IMCIDU 2020) on December 17-19, 2020.

REFERENCES

Altan, O., Sahan, U., Ipek, A., Aydın, C. 2006. Effects of oxygen supplementation on embryonic survival, haematological parameters and plasma glucose level of broiler chicks. Archıv Fur Geflugelkunde, 70: 64-68.

Baydas, G., Ozveren, F., Akdemir, I., Tuzcu, M., Yasar, A. 2005. Learning and memory deficits in rats induced by chronic thinner exposure are reversed by melatonin. J Pineal Res, 39: 50–56.

Campagna, D., Stengel, B., Mergler, D., Limasset, JC., Diebold, F. et al. 2001. Color vision and occupational toluene exposure. Neurotoxicol Teratol, 23: 473–480.

Ciarroca, M., Tomei, G., Fiaschetti, M., Caciari, T., Cetica, C., et al. 2012. Assessment of occupational exposure to benzene, toluene and xylenes in urban and rural female workers. Chemosphere, 87: 813–819.

Çaylak, E. 2011. Hayvan ve bitkilerde oksidatif stres ile antioksidanlar. Tıp Araştırmaları Derg, 9(1): 73-83.

Dinarello, CA. 2000. Impact of basic research on tomorrow's medicine. Chest, 118(2): 503-508.

Ercan, N., Fidancı, UR., 2012. Piyodermalı köpeklerde idrarda 8-hidroksi-2'-deoksiguanozin (8-OHdG) düzeyleri. Ankara Üniv Vet Fak Derg, 59: 163-168.

Ergonul S., Askar T. 2009. Anaplasmosisli Sığırlarda Isı Şok Protein (HSP), Malondialdehit (MDA), Nitrik Oksit (NO) ve İnterlökin (IL-6, IL-10) Düzeylerinin Araştırılması. Kafkas Univ Vet Fak Derg, 15(4): 575-579.

Gotts, JE., Matthay, MA. 2016. Sepsis: pathophysiology and clinical management. BMJ, 353: i1585.

Gutteridge, JM. 1993. Free radicals in disease processes: A compilation of cause and consequence. Free Radic Res Commun, 19(3): 141-158.

IBM SPSS, IBM Corp. 2013. Released. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: USA.

Karagözler, AA., Mehmet, N., Batçioglu, K. 2002. Effects of long-term solvent exposure on blood cytokine levels and antioxidant enzyme activities in house painters. J Toxicol Environ Health, 65: 1237–1246.

Martínez-Alfaro, M., Contreras-Alcaraz, Y., Carabez-Treio, A., Leo-Amador, GE. 2011. Oxidative stress effects of thinner inhalation. Indian J Occup Environ Med, 15(3): 87–92.

Martínez-Alfaro, M., Palma-Tirado, L., Sandoval-Zapata, F., Carabez-Trejo, A. 2006. Correlation between formamidopyrimidine DNA glycosy- lase (Fpg)-sensitive sites determined by a comet assay, increased MDA, and decreased glutathione during long exposure to thinner inhalation. Toxicol Lett, 163: 198–205.

Pekcan, Z., Çınar, M., Gürkan, M., Kumandas, A. 2011. Ankara Keçilerinde Propofol ve İzofluran Anestezisinin Oksidatif Stres Üzerine Etkileri. Atatürk Üniversitesi Vet Bil Derg, 6(3): 217-222.

Pestka, S., Krause, CD., Sarkar, D., Walter, MR., Shi, Y., et al. 2004. Interleukin-10 and related cytokines and receptors. Annu Rev Immunol, 22: 929–979.

Stankiwicz, W., Dabrowski, MP., Chcialowski, A., Plusa, T. 2002. Cellular and cytokine immunregulation in patients with COPD and bronchial asthma. Mediators Inflammation, 11(5): 307-12.

Şakar, A., Var, A., Onur, E., Güvenç, Y., Yorgancıoğlu, A. 2004. Astım olgularında IL-1β, IL-6 ve TNFα düzeyleri. Türk Klinik Biyokimya Derg, 2(1): 017-021.

Villalba-Campos, M., Chuaire-Noack, L., Sánchez-Corredor, MC., Rondón-Lagos, M. 2016. High chromosomal instability in workers occupationally exposed to solvents and paint removers. Mol Cytogenet, 20: 9: 46.

Yarım, GF., Nisbet, C., Ocal, N., Ciftci, G., Coskuner, A. 2006. Şap hastalıklı danalarda plazma monosit kemoatraktan protein-1 ve İnterlökin-1α düzeylerinin ve bu düzeylerle kan lenfosit ve monosit sayıları arasındaki ilişkilerin incelenmesi. Ankara Üniv Vet Fak Derg, 53: 91-95.