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MONITORING THE OXIDATIVE STRESS IN HAIRDRESSERS' IN EXPOSURE TO THE VAPORS FROM THE DYES OF THE HAIR , BY ANALYSIS OF SALIVARY MALONDIALDIALDEYDE WITH THE USE OF DIPSTICKS

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ABSTRACT

OBJECTIVES: The objective of this study is determinate with a semi quantitative method (dipsticks), the oxidative stress, produced by exposure to dyes of hairdressers, measuring the concentration of salivary malondialdehyde (MDA).
MATERIALS AND METHODS: Saliva samples are provided by the hairdressers and a control group. The values of MDA are determinate using dipsticks, in a range ≤ 3 and ≥ 7 nM / ml, and For 14 random samples of saliva, the MDA, was also determined spectrophotometrically. The date is statistically examined by Univariate (UVA) and Multivariate (MVA) analysis method
RESULTS: The values of MDA determined with dipsticks are statistically correlated with those obtained spectrophoto metrically, p≤0. 05. For the control group, at UVA) no variable is significant, (p≥0.05), and at MVA, age and smoking significantly increase the levels of MDA (p≤0.05). For hairdressers group at UVA, the age and the place of work increase the MDA. (p≤0.05) and at MVA only the place of work remains significant. (≤0.05). At MVA, besides the type of work, also the age and smoking significantly increase the level of MDA.
DISCUSSION: The data shows in hairdressers a significant increase in salivary MDA as a function of age, and smoke, then control sample the main increase is for hairdressers regardless of their occupational work. For the direct exposure to vapors arising from hair dyes by a proportional intake of free radicals and MDA, that are also generated with increasing age. and smoking
CONCLUSIONS: The saliva test with dipsticks is reliable to determine the degree of oxidative stress, especially for the oral cavity, in the professional exposure of hairdressers

INTRODUCTION

Hairdressing work is classified as probable carcinogenic, based on an excess risk for bladder cancer. However, it is highly controversial if current work still entails exposure to carcinogens, or if this has been eliminated by restricting the use of several aromatic amines in hair dyes. The large individual variation in the products used, biological half-lives have challenged the classic occupational hygiene assessment of hairdressers' exposure to aromatic amines and with several other intermediates that serve due hair coloring. This process can be divided into three phases: primary, intermediate dye (the p-phenylediamine and p-aminophenol), attaches to the hair, then reacts with a compound called coupler (1-naphthols, m-diamines, resorcinol, m-amino-phenole, pyrazolones), to form the azocompound or chromophore system Chemicals as p-phenylenediamine and aminophenyl have been suggested as possible carcinogens or mutagens in experimental studies. (Occup Environ Med, 2015; Johansson, ?). The current evidence provides limited evidences on the association between personal hair dye use and human cancer risk, except for the possibility of hematopoietic cancers and to a lesser extent, bladder cancer. Several methodological issues should also be considered in future studies, including completed hair dye use information such as on the timing, duration, frequency and type of hair dye product use.

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It also must be considered as well as other concomitant risk factors that may contribute to that linked the exposure of hairdressers to hair dyes or cigarette smoking and age of those exposed. In fact, cigarette smoking is that resulting from the formation during combustion of a strong oxidative stress "chemical (Bjartved, 2005)" that involves practically all compounds present in tobacco, and that leads to the formation of free radicals that are chemical compounds that have an unpaired electron in the orbital more external, which makes them a very reactive species, in the sense that the electronic balance, is re-established very quickly, with the attack of them to a another atom also having unpaired electron. The end result is what is called a "chain reaction", the faster and quantitatively important depending on the species involved and their concentration In the smoke the combustion temperatures reach very high values and in these conditions was calculated, whereas a puff of smoke standards is equivalent to about 35 cm3 and that a cigarette is consumed in about 10 aspirates, one has that the amount of radicals introduced in the body is estimated in a range of $10^{13} \div 10^{18}$ radicals / cigarette smoked (Zaga, 2002). The other question is the age of those exposed that as has been demonstrated in studies of smokers and non-smokers, consumes more oxidative stress probably in relation to the mitochondrial dysfunction. This phenomenon has long been considered a major cause of aging and age-related diseases (Acta Biomed, 2016). Mitochondrial free radical theory of aging postulate that somatic mitochondrial DNA mutations that accumulate on the life cause excessive production of reactive oxygen species that damage macromolecules and affect the function of cells and tissues. In fact, studies have shown that the oxidative capacity, maximum decreases with age, while increases reactive oxygen species production. As previously stated in this context is very difficult the assessment of mutagenic and/or carcinogenic damage due to exposure to hair dye, especially with routine blood tests. The aim of this work is to study oxidative stress possibly induced by exposure of hairdressers to hair dye, using dipsticks, semiquantitative, quickly and effectively while method. This assessment will take account of two parameters that we consider very important and that is smoking and age, which in this particular season of work generally coincides with the time of exposure, through the analysis of malondialdehyde in saliva using dipsticks

MATERIALS AND METHODS

For semi-quantitative determination of salivary MDA was used test strips of DFI (Dream Future Innovation), and for reading the results was used the FRC 505 Analyzer in the same DFI. The value expressed in nM /ml in salivary MDA is expressed in four different ranges: ≤ 3 mM NORMAL; CAUTION 3-5; HIGH 5-7; ≥ 7 VERY HIGH. Saliva samples were collected from groups of volunteers, 27 and 21 control population, in the morning and after two hours of taking food, and reviewed instantly, for immersion in the fluid dipstick and read them with the Scanner FRC 505. In fourteen samples "random", between smokers seven, and seven for the control group, the MDA were measured in compliance with the most common tests thiobarbituric acid (TBA), and the results compared with the corresponding values obtained with dipsticks, through statistical processing with the Fisher Exact Test From the study excluded people with a history of diseases that can be a source of generation of excess free radicals, such as diabetes and cardiovascular disease. We excluded those who have anamnesis reported using alcohol in a non-moderate, those taking antioxidants, such as vitamins or supplements containing curcumin and quercetin polyphenols, and finally even those people who perform strenuous anaerobic exercise.

RESULTS

In this study, saliva samples from volunteers has been made after a careful history of the oral cavity to rule out possible diseases that could affect the characteristics of a normal salivary composition excluding that arising from a regular cell metabolism. The first result, estimated in the table one is obtained by comparing the values obtained with the MDA dipsticks and with the type of analysis with spectrophotometric assay at TBA, performed on the same sample of saliva for each of fourteen volunteers, seven hairdressers and seven for control group The values in Table 1 indicate that there is a statistically significant difference: $p \leq 0.05$ by Fisher exact test., according with a precedent study executed by the same authors (Acta Biomed, 2016).

Table 1. Comparison of mda values (nm / ml) with dipstickst and tba assay

H = hairdressers c= control

TEST DIPSTICKS		TEST TBA	
SAMPLE *	MDA	SAMPLE *	MDA
1 H	≥7	1H	7.2
2H	5-7	2H	6.9
3H	5-7	3H	6.0
4H	5-7	4H	6.2
5H	3-5	5H	5
6H	3-5	6H	4.1
7H	≤3	7H	2.8
1C	3-5	1C	4.2
2C	3-5	2C	4.1
3C	3-5	3C	4.8
4C	≤3	4C	2.0
5C	≤3	5C	2.2
6C	≤3	6C	3.0
7C	≤3	7C	1.5

After this test, with which we ascertained the sovapponibilità and therefore the reliability of the results obtained with the dipstick, as on the other hand, we had conducted in a recent study, (), MDA values were determined in the control group and in the hairdressers (see Table 2). Salivary MDA concentration values obtained were statistically analyzed and the results are given in tables 3, 4.5

For control samples at Univariate analysis, no variable is significant,but at multivariate analysis, age and smoking significantly increase the levels of MDA. (see Table 3). For a hairdressers sample in Univariate analysis, the age and the place of work increase the levels of MDA and at Univariate analysis, the age and the place of work increase the levels of MDA.,(see Table 4). Only the type of work increases the level of MDA. Different and fundamental is the result of the last statistical processing carried out by examining both the samples At Univariate analysis, besides the type of work, also the age and smoking significantly increase the level of MDA; moreover, the type of work has the highest impact.

Table 2 . Total values of salivariy mda concentration (nm/ml) in hairdressers and control groups

Legend: W = Women; M = Man; Np = Private Workplace; Cc = Shopping Center

Hairdressers					control		
Sex	age	working	smoking	mda	age	smoking	mda place
W	35	NP	not	≥3 ≤ 5	23	syes	≤3
W	56	NP	not	≥3≤5	34	si	≥ 5≥ 7
W	64	NP	not	≥5≤7	33	not	≤3
W	35	NP	YES	≥3≤5	40	yes	≥3≤ 5
W	42	NP	YES	≥3≤5	33	not	≤3
W	30	CC	YES	≤3	34	yes	≤3
W	28	CC	not	≥ 3≤ 5	50	yes	≥5≤ 7
W	31	CC	not	≥3≤ 5	44	yes	≤3
W	25	CC	not	≤3	60	not	≥3≤ 5
W	44	NP	YES	≥ 5 ≤7	22	yes	≤3
W	39	CC	YES	≥3 ≤5	31	yes	≥3≤ 5
W	35	NP	YES	≥≤ 5 7	45	yes	≥3≤ 5
W	39	CC	not	≥3 ≤5	39	not	≤3
W	42	NP	YES	≥3≤ 5	27	yes	≤3
W	36	NP	YES	≥ 5≤ 7	35	not	≤3
W	34	NP	not	≥5≤ 7	28	not	≤3
M	55	NP	not	≥ 3≤ 5	23	not	≤3
W	26	CC	not	≤3	29	not	≤3
W	33	NP	not	≥ 3 ≤5	19	yes	≤3
M	27	CC	no	≥ 3≤ 5			
W	44	NP	YES	≥5≤ 7			
W	34	CC	not	≥ 5 ≤7			
W	31	CC	YES	≥ 3≤ 5			
W	30	CC	not	≤3			
W	29	CC	not	≥ 3≤ 5			
W	25	cc	not	≥3≤5			
W	19	CC	YES	≤3			
W	19	CC	YES	≤3			

Table 3. Analysis of control sample

Univariate Analysis			Multivariate Analysis		
VARIABLE	COEFFICIENT	p-VALUE	VARIABLE	COEFFICIENT	p-VALUE
AGE	0.0909	0.0909	AGE	0.0866	0.0079
SMOKING	2,3832	0.0567	SMOKING	1.2974	0.0404
SEX	1,5628	0.3350	0/1	3.2176	0.0144
			0/2	5.4184	0.0003

Table 4. Analysis of Only Hairdresser Sample

Univariate Analysis

VARIABLE	COEFFICIENT	p-VALUE
WORKING NOT SHOPPING CENTER	2.7669	0.0173
AGE	0.0967	0.0460
SMOKING	0.9303	0.2566
SEX	-0.3982	0.7577

At Multivariate Analysis Only the Place of Work Increase the Levels of Mda

Table 5 .Analysis of all Samples

Univariate Analysis			Multivariate Analysis		
VARIABLE	COEFFICIENT	p-VALUE	VARIABLE	COEFFICIENT	p-VALUE
AGE		0.0170	AGE	0.01106	0.0031
SMOKING		0.09804	SMOKING	1.8338	0.0117
WORK AS HAIRDRESSER		2.0253	WORK AS HAIRDRESSER	2.6512	0.0007
SEX		0.4477	0/1	5.6721	0.0006
			0/2	8.6033	≤0.0001

sesso	età	luogo	MDA	fumo	ETA'	SESSO	FUMO	MDA
F	48	NP	≥3 ≤ 5	no	44	F	no	≤3
F	56	NP	≥3≤5	no	46	F	no	≤3
F	64	NP	≥5≤7	no	23	F	si	≤3
F	35	NP	≥3≤5	si	34	M	si	≥ 5≥ 7
F	42	NP	≥3≤5	si	33	F	no	≤3
F	30	CC	≤3	si	40	F	si	≥3≤ 5
F	28	CC	≥ 3≤ 5	no	33	M	no	≤3
F	31	CC	≥3≤ 5	no	34	F	si	≤3
F	25	CC	≤3	no	50	F	si	≥5≤ 7
F	44	NP	≥ 5 ≤7	si	44	F	no	≤3
F	39	CC	≥3 ≤5	si	60	F	no	≥3≤ 5
F	35	NP	≥≤ 5 7	si	22	F	si	≤3
F	39	CC	≥3 ≤5	no	31	F	si	≥3≤ 5
F	42	NP	≥3≤ 5	si	45	F	si	≥3≤ 5
F	36	NP	≥ 5≤ 7	si	39	F	no	≤3
F	34	NP	≥5≤ 7	no	27	F	si	≥3
M	55	NP	≥ 3≤ 5	no	35	F	no	≤3
F	26	CC	≤3	no	28	F	no	≤3
F	33	NP	≥ 3 ≤5	no	23	F	no	≤3
M	27	CC	≥ 3≤ 5	no	29	F	no	≤3
F	44	NP	≥5≤ 7	si				
F	34	CC	≥ 5 ≤7	no				
F	31	CC	≥ 3≤ 5	si				
F	30	CC	≤3	no				
F	29	CC	≥ 3≤ 5	no				
F	25	cc	≥3≤5	si				
F	29	NP	≥3≥5	no				

In the multivariate analysis of all samples, the interaction term between being hairdresser and the place of work does not remain significant (see Table 5).

DISCUSSION

Before analyzing the data obtained by monitoring of salivary MDSA, you should check if the hypothesis that forms the basis of this study, which is a possible correlation between the lipid peroxidation and hair dyes, you will find a correlation not only theoretical, but also practical with work featured in literature. In a recent work (Environ Mol Mutagen, 2016) has been measured Telomere length in peripheral blood leukocytes. Telomeres are DNA-protein structures located at the ends of eukaryotic chromosomes composed of hundred Grand of tandem repeats TTAGGG. The main function of Telomeres is maintaining the integrity of chromosomes, preventing their erosion and the phenomenon known as end-fusion, and thus adjusting cell lifespan [Blasco, 2005 (7) . The hairdressers had shorter Telomeres than non-hairdressers. Some factors are known to accelerate the erosion of Telomeres, including oxidative stress (Trends Biochem Sci. 2002), and then this study stands in line with other experiments, aims to verify whether oxidative stress induced by hair dyes.. Salivary MDA concentration data (see table 2) detected in the hairdressers show an initial analysis, though not strictly statistics, a high incidence of occupational exposure.

In twenty-seven hairdressers, twenty (74%) are under forty years of age, and all in the absence of particular pathologies which alone can justify an increased oxidative stress. Well, eleven of them (55%), non-smokers, are salivary MDA concentration high, unlike the control group, which had this phenomenon in only four employees out of fourteen (28%)- This study tested essentially that this correlation exists and is a predominant parameter than the other two risk factors considered, the smoke and the age ; this is clear not only from the Univariate analysis and Multivariate statistical analysis of the two samples at taken together (see table 5) but also in the analysis of only hairdresser samples (see table 4):.in both cases the p-value related to occupational exposure is much lower than those related to smoking and age, both however statistically significant. As a final note, the lack of influence of the workplace (see table 5), most likely because direct exposure of hairdressers suffer little in positive terms, any more air changes, that there are usually in stores are in malls, rather than in small shops

Conclusion

The use of dipsticks for the assay of MDA salivary in hairdressers and in the control group is a method of semi-quantitative rapid and valid for the conditions for taking the samples, less subject to alteration phenomena of compounds in the steps of analyzing. the results of this study, it can be said that exposure to hair dye causes a significant increase of oxidative stress in the hairdressers.

This factor involves a complex of metabolic disorders that produce free radicals, which are added to those induced by the consumption of cigarettes and those arising from the typical processes of aging. These data show a potential carcinogenic risk, we think possible in many anatomical sites, well-known, vascular damage, and produced by an excess of free radicals

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