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MONITORING OF OXIDATIVE STRESS INDUCED BY CIGARETTE SMOKE BY ANALYSIS SALIVARY MALONDIALDEHYDE WITH THE USE OF DIPSTICKS

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ABSTRACT

Objectives: Determinate with a semi quantitative method, the oxidative stress produced by tobacco smoke, in function of the number of cigarettes and the age of the smoking, measuring the concentration of salivary malondialdehyde (MDA), using the dipsticks,

Materials and Methods: Saliva samples are provided by a smoking group, divided into three subgroups for consumption of cigarettes / day (≤ 7 ; $7 < 20$; ≥ 20), and by the control group. The values of MDA are determinate using dipsticks, in a range ≤ 3 and ≥ 7 nM / ml, and in two subgroups: (≤ 45 and / or > 45 years old), for 14 random samples of saliva, the MDA, was also determined spectrophotometrically. The data were analyzed statistically with "Logistic Regression Method"

Results: The values of MDA determined with dipsticks are statistically correlated with those obtained spectrophotometrically, $p \leq 0.05$. For consumption of cigarettes (≥ 7 / day), the MDA concentration have an increase statistically significant compared to the control: $p \leq 0.05$, and proportional to the number of them. Cigarettes consumption ≤ 7 / day not involve any increase in MDA indicative vs. control: $p = 0.075$. The age ≥ 45 years, in smokers as in non-smokers by cigarette consumption, significantly increase the MDA, $p \leq 0.029$

Discussion: Improvement of the cigarettes results in a proportional intake of free radicals and MDA, resulting in lipid peroxidation, which is also generated with increasing age.

Conclusions: The saliva test with dipsticks is reliable to determine the degree of oxidative stress, especially for the oral cavity, in cigarettes smoking

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INTRODUCTION

Epidemiological studies have made it clear that tobacco smoke is one of the most important environmental risk factors such as morbidity and mortality, and 'estimated (Vineis *et al.*, 2004) that smoking tobacco is now responsible for about 30% of all cancer deaths in major industrialized countries. In addition smoking is due to a large number of deaths from cardiovascular, pulmonary, and is responsible for many degenerative diseases. In 2000, 4.8 was attributed to the fumes 4.8 million deaths worldwide, of which about half in most industrialized countries, (Peto and Lopez, 2001), and projection in 2030 indicates a number of 30 million. According to recent studies, also smoke a little, or use different types of cigarettes considered less harmful, is not a reasonable solution.

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Smoking also only 5 cigarettes / day involves risks, in particular for the onset of the infarct, despite this significantly lowers the risk of developing lung cancer. These data, are highlighted in a recent study conducted in France, presented at the European Conference 2015 "in the Lung Cancer (ELCC) in Geneva, Switzerland (Greiller, 2005) and the same results are expressed in the review, published in the journal Tobacco Control (Bjartved, 2005), which it analyzed three studies carried out in Sweden, USA, Denmark.. In the Swedish study, in both sexes, smoking 1-4 cigarettes per day was associated with a significantly higher risk of dying from ischemic heart disease, and women also increased the relative lung cancer. The research was conducted on 7495 people aged 47-55 and for which certain parameters for the primary prevention for cardiovascular diseases, were screened in 1970-1973 and followed for 12 years. All the survivors were invited to a second screening in the period 1973-1977 and followed for 7 years. In men, smoking 1-4 cigarettes per day at the first

screening, they had an odds ratio for the occurrence of myocardial infarction of 2.8, and for other types of death of 2.0. In men with stable habits of smoking, the corresponding odds ratio adjusted for both projections were 4.6 for heart attack and 3.4 for other coronary heart disease. The risk of dying from cancer for smokers light 'was not significantly increased. In the USA was carried out by questionnaire on smoking habits, a study at follow-up (1976-1988) out of a total of 117,006 women, with a questionnaire, updated every two years and 'was determined that. The adjusted relative risk for women who smoke 1-4 cigarettes a day was 1.94 for a myocardial infarction, and the baseline increases with the consumption of tobacco over a minimum of two cigarettes a day. Basically the same conclusions are those listed in the Danish study conducted on 6505 women and 5644 men aged 30, in 1976-7 he followed for nearly 22 years. The hazard of the tobacco is mainly due to inhalation of an aerosol containing in addition to nicotine, around 1010 particles / ml, consisting of about 1000 particles of polymeric material, having a substrate of porous carbon, on which are absorbed heavy metals, polycyclic aromatic hydrocarbons as nitrosaminic and azarenic compounds. (Hecht, 1999) The particulate phase that is inhaled with the smoke of tobacco, contain about 3500 compounds, most of which are in proportion to the weight ratio, is of type mutagenic or carcinogenic, such as benzene, the 2 -naftilamine , 210Po, 226Ra, 228Ra, nickel, cadmium, benzo [a] pyrene. In practice, according to a recent study by IARC (Hoffman, 1997), in cigarette smoke are at least 55 compounds for which there is an almost established evidence in the initiation of the process of cancer genesis...Another big risk related to cigarette smoking is that resulting from the formation during combustion of a strong oxidative stress "chemical" that involves practically all compounds present in tobacco, and that leads to the formation of free radicals. In very simple terms the free radicals, 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. The production of free radicals is very high. at temperatures around 37 ° C and most of the radicals is greatly reactive so that their concentration becomes very low, estimated at $10^{-9} \div 10^{-4}$ M in solution, corresponding to $10^{10} \div 10^{13}$ radicals per cm³ of the gas phase 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 radical thus formed may be "long life" in the corpuscular phase (phase tar), and short-lived, in the gaseous phase (gas phase). The primary radical of the "tar phase" is constituted by the complex quinone-hydroquinone, a redox system very active and capable of reducing molecular oxygen to superoxide radical and thus to hydrogen peroxide and hydroxyl radical. The "gas phase" of the cigarette smoke contains rather small alkyl and alkoxy groups with a significantly higher reactivity to the radicals of the phase corpuscular. The life of free radicals is

very short and takes place in the immediate vicinity of the production site. However, if they are not immediately neutralized by a physiological acceptor, the radicals attack the different constituents intracellular within a radius of action vary according to the type of radical itself. Free radicals show their toxic activity only when they are produced with a rate or in an amount such that it cannot be inactivated by the defense systems of the cell. In this case they are able to react with all the constituents of the cell and the cell matrix, resulting in a condition called "oxidative stress" and all classes of biological molecules are potential "targets" for the attack of free radicals, should, however, assert that free radicals are natural products of our cellular metabolism, which uses them in the defense from bacterial and viral infections of various kinds, and their production as opposed to a regular physiological balance, increases with age. Many studies have shown that excessive endogenous production of free radicals, and / or their indirect intake, can cause in addition to the known phenomena of aging, degenerative diseases also much more important as cancer, stroke, Alzheimer's, Parkinson's, arthritis and others. The most important degenerative process in the load of free radicals, however, remains that which arises from lipid peroxidation, which leads to the formation of compounds potentially very harmful to the DNA, and among these the malondialdehyde (MDA), which among other things has the ability to diffuse rapidly in the various tissues, (Mc Mel, 2007). It is very important to dose the concentration of free radicals in the human body, mainly in conditions of oxidative stress. There are many ways to check the activity of free radicals in the body: between these the test on serum and urine for metabolites such as MDA, and / or, always in the same biological fluids the concentration of the antioxidant compounds which decrease can be a indirect index of oxidative stress going on in the body. These tests that determine whether the blood from an individual is affected by activities of free radicals, are, invasive, and do not usually have a good reproducibility for the possible degradation during the extraction phase of the metabolites of lipid peroxidation. The urine test with which it dispenses the MDA is more accurate (50x) blood test, and the results are often much faster...The range of concentrations usually accepted in Italy (8) for a normal value of MDA is for the blood and in the urine 3. 0 / nM / L. In any case, the diagnostic methods, used to determine the concentration of MDA in biological fluids such as HPLC, the colorimetric test with thiobarbituric acid (TBA), they are expensive and require much time. At the present time few works have studied the influence of cigarette smoking on the values of MDA in human saliva (Demirtas, 2014), in the absence of other pathologies, and to analyze these concentrations with a rapid and low cost test These result therefore interesting, and one of the objectives of this work, analyzing the concentration of MDA salivary function of the number of cigarettes smoked per day, taking into account also the age smokers, with a dipsticks for a rapid of semi-quantitative, non-invasive, reliable, reproducible and at low cost test.

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 / m l in salivary MDA is expressed in four different ranges:

≤ 3 mM / L NORMAL; CAUTION 3-5; HIGH 5-7; ≥ 7 VERY HIGH

Saliva samples were collected from groups of volunteers, 25 smokers and 25 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), (8), 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 exercise, anaerobic .

The group of smokers has been divided into three subgroups according to the quantity of cigarettes / day smoke Group 1 ≥ 20 cigarettes / day Group 2 7 / day Group 3 ≤ 7 cigarettes / day:

"ligh smokers"

All fifty volunteers, and not smoking, were then divided into two groups based on age:

Group 1 ≤ 45 years

Group 2 ≥ 45 years.

The study results were statistically processed by the method:

"logist ordinal regression"

All of the study volunteers ii purposes only knowledge was sought their occupation, and a group of smokers, as with other non-smokers, was asked whether more or less routinely felt a sensation of dry mouth. These data were statistically analyzed

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 smokers and seven non-smokings. The values in Table 1 indicate that there is a statistically significant difference: $p \leq 0.05$ by Fisher exact test.

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

| TEST DIPSTICKS | | TEST TBA | |
|----------------|----------|----------|-----|
| SAMPLE * | MDA | SAMPLE * | MDA |
| 1 F | ≥ 7 | 1F | 7.0 |
| 2F | 5-7 | 2F | 6.6 |
| 3F | 5-7 | 3F | 5.8 |
| 4F | 5-7 | 4F | 6.5 |
| 5F | 3-5 | 5F | 5 |
| 6F | 3-5 | 6F | 4.6 |
| 7F | ≤ 3 | 7F | 2.8 |
| 1A | 3-5 | 1A | 4.2 |
| 2A | 3-5 | 2A | 4.7 |
| 3A | 3-5 | 3A | 4.8 |
| 4A | ≤ 3 | 4A | 2.0 |
| 5A | ≤ 3 | 5A | 2.2 |
| 6A | ≤ 3 | 6A | 3.0 |
| 7A | ≤ 3 | 7A | 1.5 |

Table 2. Salivary mda levels in smokers and control population

| Smoker e- age - Sigarettes* | Mda nm/ml | Not Smoker -Age | Mda nm/ml |
|--------------------------------|--------------|-----------------|--------------|
| 1 71 2 | 5-7 | 1 54 | 3-5 |
| 2 73 3 | 5-7 | 2 78 | ≤ 3 |
| 3 72 1 | 3-5 | 3 77 | 3-5 |
| 4 70 2 | 3-5 | 4 55 | 3-5 |
| 5 66 1 | 3-5 | 5 55 | ≤ 3 |
| 6 65 3 | 5-7 | 6 52 | ≤ 3 |
| 7 66 1 | ≤ 3 | 7 70 | ≤ 3 |
| 8 55 3 | ≥ 7 | 8 53 | 3-5 |
| 9 54 3 | 3-5 | 9 59 | ≤ 3 |
| 10 49 1 | ≤ 3 | 10 46 | 3-5 |
| 11 47 2 | 3-5 | 11 55 | ≤ 3 |
| 12 47 1 | ≤ 3 | 12 50 | ≤ 3 |
| 13 25 2 | 3-5 | 13 44 | 3-5 |
| 14 44 3 | 3-5 | 14 27 | ≤ 3 |
| 15 32 1 | ≤ 3 | 15 32 | 3-5 |
| 16 30 2 | 3-5 | 16 24 | ≤ 3 |
| 17 27 2 | ≤ 3 | 17 33 | ≤ 3 |
| 18 43 3 | 5-7 | 18 39 | ≤ 3 |
| 19 32 3 | 3-5 | 19 38 | ≤ 3 |
| 20 34 2 | ≤ 3 | 20 34 | ≤ 3 |
| 21 36 1 | ≤ 3 | 21 27 | ≤ 3 |
| 22 38 2 | 5-7 | 22 20 | ≤ 3 |
| 23 38 1 | 3-5 | 23 18 | ≤ 3 |
| 24 37 1 | ≤ 3 | 24 39 | ≤ 3 |
| 25 20 2 | 3-5 | 25 20 | ≤ 3 |

Legenda : ^ Cigarettes 1 ≤ 7 Cigarettes 2 $\geq 7 \leq 20$ - Cigarettes 3 ≥ 20 /die

The results of the concentrations of MDA salivary, obtained using only the dipsticks for both groups, smokers and non-smokers, are indicated in the table 2 where it is also is also indicated, age, and for the smokers, the number of cigarettes consumed per day This last parameter analyzed, namely the number of cigarettes smoked per day, resulted in a classification smokers into three subgroups, and the results are given in Table 3. All data on the various tests carried out and illustrated in the above Tables, 2 and 3, were statistically analyzed using "ordinal logistic regression method", see table 4.

In accordance with this statistical analysis it may be concluded that:

- Be smoking level 2 or 3, implies a significantly increased risk
- Be smoking Level 1, it does not involve a significant increase in MDA compared with the control.

- Older age ≥ 45 years, it has a significant effect on increasing the MDA, both in smokers, and in the control.

Table 3. Value mda (nm / l) according to the number of cigarettes

Smoking (a) ≤ 6.7 cigarette consumption / die
 Smoking (b) consumer $\geq 7 \leq 20$ cigarettes / die
 Smoking (c) ≥ 20 cigarettes consumption / die

| Smokers a | Md a | Smokers b | Mda nm/ml | smokers c | mda nm/ml |
|-----------|----------|-----------|-----------|-----------|-----------|
| 1 | 3-5 | 1 | 5-7 | 1 | 5-7 |
| 2 | ≤ 3 | 2 | 3-5 | 2 | 5-7 |
| 3 | ≤ 3 | 3 | ≤ 3 | 3 | ≥ 7 |
| 4 | ≤ 3 | 4 | 3-5 | 4 | 3-5 |
| 5 | ≤ 3 | 5 | 3-5 | 5 | 3-5 |
| 6 | ≤ 3 | 6 | ≤ 3 | 6 | 5-7 |
| 7 | 3-5 | 7 | ≤ 3 | 7 | 3-5 |
| 8 | ≤ 3 | 8 | 5-7 | 8 | 5-7 |

higher in smokers compared to not smokers. For the study of these degenerative phenomena described in the literature are essentially two types of methods, both for the blood is for urine. The first, the most commonly used is based on the assay of MDA by colorimetric method with the test of thiobarbituric acid, TBA, the second much more demanding and expensive is carried out in HPLC. The appropriate methods are used on serum and urine primarily because the plasma MDA, stored after the step of alkaline hydrolysis is not very stable, even if it can increase the stability, with its extraction in butanol (Grotta, 2007). The MDA urinary appears to be much more stable (12), but the values of the concentrations determined in this biological fluid directly affected by the possible development of a possible oxidative stress which can occur for multiple and sometimes trivial causes in the district urinary, and not perceived and reported, by patients at the time of the survey

Table 4.

| Parameter | B | Hypothesis test | | | Exp (B) | 95%Wald Confidence Interval for Exp(B) | | |
|----------------|-------------------|-----------------|--------|-----|---------|--|--------|-----------|
| | | Wald Chi Square | df | Sig | | Lower | Upper | |
| Intercept | Mda $\leq 3/ 3-5$ | 2.274 | 8.088 | 1 | 0.04 | 26.425 | 2.767 | 252.384 |
| | Mda 3-5/ ≥ 5 | 5.287 | 17.043 | 1 | 0.00 | 537.426 | 27.169 | 10630.714 |
| Age | | 0.45 | 4.744 | 1 | 0.029 | 1.004 | 1.004 | 1.089 |
| Smokers 3 vs 0 | | 4.986 | 17.448 | 1 | 0.00 | 146.290 | 14.101 | 1517.633 |
| Smokers2vs 0 | | 2.411 | 6.496 | 1 | 0.011 | 11.144 | 1.745 | 71.154 |
| Smokers1vs 0 | | 0.251 | 0.82 | 1 | 0.775 | 1.298 | 0.230 | 7.186 |

Table 5. Detection of feeling "dry mouth" in samples of smokers and non-smokers

| Smokers | Dry mouth | Not smokers | dry mouth |
|---------|-----------|-------------|-----------|
| 1)A ● § | + | 1)● § | + |
| 2)A ● § | + | 2)● | |
| 3)A ● § | + | 3)● § | + |
| 4)B ● § | + | 4)● | |
| 5)C ● § | | 5)● | + |
| 6)C | + | 6) | |
| 7)C § | | 7) | |
| 8)C | | 8) | |
| 9)C | + | 9) | |
| 10)C | | 10) | |

In Table 4, are some data regarding the replies, quite subjective to some volunteers smoking, these divided by the number of cigarettes / day and non-smokers, relatively to the presence or not of a continuous sensation of "dry mouth."For this data has not been performed statistical processing, but it is clear that the feeling of dry mouth is practically absent in the younger population also including lighth smokers.

DISCUSSION

Cigarette smoke inhalation with normal breathing can affect the body balance or homeostasis between second oxidative and reducing and cause the generation of free radicals The direct result of cigarette smoking or stimulated by it, causes oxidative stress, and can initiate the breakdown of lipids in the cell membrane. The MDA, such as the end product of lipid peroxidation, is a molecule chemically very active and can have harmful effects on protein structure especially at the intracellular level, but also tissue for its diffusive properties. These data are reported in the literature for active and passive smoking (Demirtas, 2014), and are confirmed with studies and experiments on animals (Mc Mel, 2007). In all studies, the MDA levels measured in samples of blood and / or saliva were

Ability to use saliva to monitor the health and disease of a patient is a highly desirable objective for the promotion of health and health research. The saliva has long been proposed and used as a diagnostic tool (Kaufman, 2002) because it is easily accessible, not using invasive means of one of its collection, short times for the same harvest, inexpensive and can be used for purposes of mass screening (Samaranayake, 2007). As a drawback for the use of saliva as a diagnostic fluid is the knowledge that the markers that are sought generally in it, are generally present in amounts lower than in serum (Miller, 1994). However, with the use of new laboratory equipment or even with alternative methods, in recent years we have achieved remarkable clinical results. In this study it has been suggested and realized the use of a rapid test for the analysis of the salivary MDA, through the use of dipsticks The use of this device, it allows the monitoring in direct contact with saliva without invasive stages of levies, storages, operations of centrifugation, etc. Moreover, this procedure allows collection and doses the MDA from an anatomical site, the oral cavity, directly in contact with the source of formation of free radicals. In this study, Table I, has confirmed the attainment of this first goal: it is clear from the statistical analysis performed by the method of "logistic regression" that

the values of the concentration of salivary MDA determined using the dipsticks, are similar to those obtained by spectrophotometric with TBA, and the correlation is statistically indicative: $p \leq 0.05$. Final data of the experiment summarized in Table 2, indicate the values of salivary MDA in smokers than in control subjects, while in Table 3, these concentrations are relative to the number of cigarettes smoked for day. It is clearly seen from Table 2 the values of salivary MDA, statistically processed, Table 3, for a consumption of cigarettes a day or more than seven, increase, significantly and in proportion to the number of them: smokers Group 3 $p = 0.00$, $p = 2$ smokers group 0.011. Ricordando that the conditions for the examination of the salivary MDA were made strictly with the same procedures, mainly, respecting a minimum interval of two hours of taking food and drinks, this trend can be explained primarily as the result of an oxidative stress proportional to the amount of free radicals inhaled with the smoke and with persistence, (chronicity) of such effects. Similar experimental data are also reported in a recent work (9), while other studies (Solak, 2005), (Isik, 2007), draw the same conclusion, however, by analyzing the variations that smoking involves not on consultation of MDA, but on other parameters indicators of stress oxidative caused by cigarette smoke, such as reduced glutathione.

The second result of this study regards the values of salivary concentration of MDA in function of the number of cigarettes smoked per day. The analysis indicates that there is a statistically indicative between so-called "light smokers" and non-smokers and that the pose problem if that data can be scientifically acceptable and it is obvious that the answer is no. It's impossible, in fact, scientifically accept the possibility that there is no "threshold limit values" (doses "safe") for carcinogens underneath which is established not the process of carcinogenesis, and then even one cigarette can in this sense be dangerous. As regards the other damage caused by smoking of cigarettes instead we can state that they are higher or lower as a consequence of the number of cigarettes smoked, though, the body is not able to dispose of all the substances consumed in a short time, and then smoking a few cigarettes a day, some damage however it suffers.

Perhaps the real problem posed by the result of the concentration of salivary MDA "light smokers" is that when we talk about the harm of smoking on the one hand you have to consider the various target organs separately, on the other hand take into account that some diseases depend not only radical free, but also other compounds such as nicotine, which act on the cardiovascular system, depending also on the state of health of the smoker. It is, in fact, obvious that five cigarettes smoked by a person with, for example, by an ischemic heart disease that are more dangerous for a person with a healthy heart. A recent study (Pieri, 2014) found that in smokers to have an effective reduction of 27% in the risk of developing lung cancer should be reduced by at least 50% the number of Mr. / day; while for other smoking-related diseases (heart attack, stroke, chronic obstructive pulmonary bronchus), while halving the Mr. / day, is not observed any reduction indicative of risk. Smoking also only 5 cigarettes / day involves risks, in particular for the onset of the infarct, despite this significantly lowers the risk of developing lung cancer.

The result obtained in this study, relatively to the low concentration of salivary MDA found at light- smokers, may be to consider that saliva on which the test is performed most affected by the biochemical conditions of the oral cavity, rather than the whole organism in the saliva contains mucins, glycosylated proteins, that if not altered and / or decreased ensure perfect protection of the oral cavity (Duca, 2015). Smoking is one of the elements that may deteriorate their structure (Taniguchi, 2012), but in the case of "light smokers" intake of it is diluted, over many hours and it can be assumed that the amount of salivary mucins is little altered so that the physiological turnover of them quickly re-establish the conditions for a 'good lubrication oral. The light smokers, and also more generally younger people (see Table 5) were unlikely to have the sensation of dry mouth, which means that the cells of the oral mucosa are sufficiently protected and not subject to phenomena of lipid peroxidation. There is no doubt that in the production of exogenous free radicals over exposure to the sun (ultraviolet radiation in general), to stress, the high consumption of alcohol. exposure to polluted environments, a 'intense physical activity, a diet too rich in protein and fat animals, treatments based on drugs (especially steroids that cause an abuse of glucose into cells which are no longer able to dispose of it), the smoke is deemed to be the most dangerous still remember that with a single suction cigarette in our organism enter concentrations of free radicals in the order of 10¹³-15, and this excess produces an oxidative stress is not controllable even at a young age.

In case of oxidative stress can be observed early: reduced memory and concentration, sleep disturbances, decreased sex drive, white hair, baldness, appearance of wrinkles and spots on the skin, reduced muscle mass and increased body fat deficiency of the immune system, increased cardiovascular risk, decreased energy, stress and anxiety and depression. This mechanism of exogenous production of free radicals in cigarette smoke and the resulting increased salivary MDA, does not justify, see Table IV, the result indicates that as independent of smoking, there is a statistically significant correlation between increasing age, and increase in MDA. The explanation for this may be admitting the theory of Harman (21) or mitochondrial hypothesis expressed already in 1956. The mitochondrial dysfunction has long been considered a major cause of aging and age-related diseases. 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.

The hypothesis of Harman has been seriously challenged by recent studies showing that reactive oxygen species evoke metabolic health and longevity, perhaps through mechanisms that include autophagy. The purpose of this review is to scan the growing literature on the mitochondria from the point of view of research on aging and try to identify the priority issues that should be addressed in future research. This theory has been challenged, modified and extended by many, but remain two key arguments in support of it: first, there is evidence that an imbalance between antioxidant / oxidant occurs with age

and this results in an accumulation of oxidized macromolecules and damaged and second, the accumulation of oxidative damage causes by involving the formation of a phenotype aged and degenerated. This second point is one that is most questioned recently challenged by several recent studies (22). The mitochondrial hypothesis justifies the data obtained in this study that correlate statistically indicative increased salivary MDA in older people regardless be smoking or non-smoking. This, taking into accounts the parameters shown in Table V and related to the subjective perception of dry mouth expressed by two samples of the population, and smoking control, leads to three other important considerations. The process of lipid peroxidation in smokers older, even in the absence of disease, is the result of two distinct and important sources of training with a final result on double-degenerative effects throughout the body. The majority age in smokers also entails that all components of the smoke, such as carcinogenic substances, and of course the free radicals, exert their effects on a cable in a chronic oral deprived of its main natural defense, or the protein layer contained in the saliva. The feeling of dry mouth, or xerostomia is in fact one of the main symptoms of the smoker, and must be a spy of the effects of smoking. The smoke, which in any case is to be considered truly dangerous for the possible onset of disorders of the oral cavity and the larynx, it is even more for those people suffering from autoimmune diseases. 19), which then they suffer from a chronic xerostomia.

Conclusions

- The use of dipsticks for the assay of MDA salivary in smokers 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.
- 2 Smoking produces in almost all smokers, even those with lower age and lower consumption of tobacco to the number of cigarettes, an oxidative stress proportional.
- 3 The consumption of a few cigarettes the day, it seems not to produce very relevant effects, with values of MDA salivary comparable to the control group.
- 4 The increasing age, obviously referring to smokers, involves a probable accentuation of the risk, because with his increase, the process of production and physiological regularization of the protective layer of the oral cavity, decreases, and further can be so damage the first digestive tract and larynx.
- 5 The use of dipsticks for the analysis of the salivary MDA is therefore a valuable complement for the monitoring the health status of oral cavity and larynx

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