The Exposure to Pollutants of the Auto Repair Workers: Monitoring their Oxidative Stress

Roberto Menicagli, O. Marotta¹, L. Menicagli²

Department of Biochemistry, Romabiomed Research Lab, Mediglia, ¹Department of ENT, University Federico II, Naples, ²Department of ENT, IRSCC Policlinico San Donato, University of Milan, Milan, Italy

Abstract

Background and Aim: Auto repair workers are exposed to multiple pollutants, each of them potentially risks, dangerous for several target organs. The aim of this study is to identify their possible overall effect, by monitoring the concentration of salivary malondialdehyde, index of oxidative stress. Materials and Methods: Malondialdehyde of 25 male workers, smokers and non-smokers, further divided into two subgroups relatively to the amplitude of their working place, was monitored, in the saliva, with the Thiobarbituric acid method. The control group consists of 12 and 13 male smokers, and 13 non-smokers. Univariate (UVA) and Multivariate (MVA) analysis methods were used to analyze the results. Results: No variable is significant ($P \geq 0.05$) for the control group using UVA, while age and smoking significantly increase the levels of MDA ($P \leq 0.05$) using MVA. For workers group, the age and the place of work increase the MDA ($P \leq 0.05$) using UVA analysis, while only the place of work remains significant ($\leq 0.05$) using MVA analysis. MVA analysis reveals that, besides the type of work, also the age and smoking significantly increase the level of MDA, as a result of a higher exposure to pollutants. Conclusions: You can check the cumulative effect of pollutants on auto repair workers, by monitoring the salivary malondialdehyde.

Keywords: Auto repair, free radicals, malondiadehyde, mechanics

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to produce energy, the remaining 5% gives rise to free radicals, in non-harmful quantities, as it serves to the immune defense system and to eliminate germs and other pathogens. There are other conditions that contribute to the formation of free radicals, and they may occur both in normal health conditions and in case of pathologic situations. When the amount of free radicals exceeds a certain levels and the body fails to eliminate their excess, they accumulate in the tissues damaging the cells. The damage over time can evolve by creating local or systemic problems (affecting the entire body), thus generating a clinical picture called “oxidative stress.” This stress results in a deterioration of the cells and the tissues that originate with an evident loss of efficiency. It should be highlighted that unlike other diseases, usually identified with medical examination and specific investigations, oxidative stress is hard to identify because it does not generate well-defined symptoms. This condition becomes even more difficult to evaluate and monitor in the various working environments that could be large and well ventilated, rather than small workshops. The workers’ habits and lifestyles are different as well, mainly due to the smoking habit, and to different ages. The aim of this study is to identify their possible overall effect, with the dosage of the concentration of salivary malondialdehyde, oxidative stress index.

**Materials and Methods**

In this study, saliva samples from volunteers have been obtained after a careful anamnesis regarding diseases of the oral cavity that could affect the characteristics of a normal salivary composition other than arising from a regular cell metabolism. We excluded those who have had reported alcohol abuse, workers under antioxidants therapies such as vitamins or supplements containing curcumin and quercetin polyphenols, and finally workers that performed strenuous anaerobic exercise.

Saliva samples were collected from groups of volunteers, 25 workers in auto repair, and 25 control populations, in the morning and after 2 h of food intake. Volunteers rinsed their mouth with water prior to the collection of saliva samples, and samples were placed in test tubes, where they were held for up to 5 min. Collected samples were kept at \(-20^\circ{}\text{C}\) until the laboratory analysis for the measurements of MDA level, with the thiobarbituric acid (TBA) colorimetric test we run.

Samples taken to measure MDA values in saliva were processed with TBA and boiled for 30 min, and then spectrophotometrically scanned at 532 nm; this method is in fact from dual boiling, where during the initial heating, proteins were precipitated from bound MDA; during second heating, total MDA entered into a reaction with TBA to create a colored complex in a hot and acidic environment. The concentration of MDA is calculated as indirect proportion to the absorbance delivered by the color complex at 532 nm generated by MDA. In this analysis, 10% trichloroacetic solution (TCA; Merck), and 0.675% TBA solution (Merck) were used.

The data of salivary MDA were statically analyzed, with Univariate (UVA) and Multivariate method (MVA). In our study, the variables that may affect the results are three: age, smoking, and workplace, intended as size of the locals. For this purpose, we have chosen the UVA and MVA method.

With the UVA method you assume that the response variable is influenced only by one factor, while with the MVA you assume that response variable is influenced by multiple factors (and even combinations of factors). In our study, houses, age, smoking, and work places MDAs are statically analyzed, with UVA, and MVA method.

**Results**

Salivary MDA concentration values obtained in the two groups are presented in Table 2 with evident difference in auto mechanics. Furthermore, these data are statistically analyzed with UVA and MVA method, and their interpretation is given in Tables 3–5. For control samples at UVA analysis, no variable is significant, while at MVA analysis, age and smoking significantly increase the levels of MDA [Table 3]. For the auto mechanics group in UVA analysis, age and the place of work increase the levels of MDA, while at UVA analysis, the age and the place of work increase the levels of MDA [Table 4]. Only the type of work and not the working place increases the level of MDA. Different and fundamental is the result of the last statistical processing carried out by examining both the samples. At UVA 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. In the MVA analysis of all samples, the interaction between being workers and the place of work does not remain significant [Table 5].

**Discussion**

Salivary MDA concentration data [Table 1] detected in the auto mechanics show at an initial analysis, though not strictly statistic, a high incidence of occupational exposure. In 27 workers, 27 (74%) are under 40 years of age, and all without particular pathologies which could justify an increased oxidative stress. Eleven of them (55%), non-smokers, have high salivary MDA concentrations, unlike the control group, in which it was observed only in four employees out of 14 (28%)-This study proved that this correlation exists and

### Table 1: Principal pollutants for every risk factors

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|----|
| Gas exposure to fumes | Exposure to welding fumes | UV and IR local radiations | Exposure to welding fumes | UV and IR local radiations | Exposure to welding fumes | UV and IR local radiations | Exposure to welding fumes | UV and IR local radiations | Exposure to welding fumes | UV and IR local radiations |

1. Gas exposure to fumes
2. Exposure to welding fumes
3. UV and IR local radiations
4. Exposure to welding fumes
5. UV and IR local radiations
6. Exposure to welding fumes
7. UV and IR local radiations
8. Exposure to welding fumes
9. UV and IR local radiations
10. UV and IR local radiations
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Table 2: Values of salivary malondialdehyde concentration (nm/ml) in the groups

| Age | Workers group | Control group |
|-----|---------------|---------------|
|     | WP | Smoking | MDA | Age | Smoking | MDA |
| 35  | S  | Not    | 3.8 | 23  | Yes     | 2.3 |
| 56  | S  | Not    | 3.9 | 34  | Yes     | 5.1 |
| 64  | S  | Not    | 6.0 | 33  | Not     | 1.8 |
| 35  | S  | Yes    | 4.1 | 40  | Yes     | 3.9 |
| 42  | S  | Yes    | 4.3 | 33  | Not     | 1.7 |
| 30  | G  | Yes    | 2.0 | 34  | Yes     | 2.8 |
| 28  | G  | Not    | 4   | 50  | Yes     | 5.1 |
| 31  | G  | Not    | 4   | 44  | Yes     | 2.6 |
| 25  | G  | Not    | 1.9 | 60  | Not     | 3.5 |
| 44  | S  | Yes    | 5.7 | 22  | Yes     | 2.3 |
| 39  | G  | Yes    | 4.2 | 31  | Yes     | 3.5 |
| 35  | S  | Yes    | 6.0 | 45  | Yes     | 3.5 |
| 39  | S  | Not    | 3.8 | 39  | Not     | 1.9 |
| 42  | S  | Yes    | 4.2 | 27  | Yes     | 1.8 |
| 36  | S  | Yes    | 6.2 | 35  | Not     | 1.6 |
| 34  | S  | Not    | 5.3 | 28  | Not     | 2.0 |
| 55  | S  | Not    | 4.1 | 23  | Not     | 1.4 |
| 26  | G  | Not    | 2   | 29  | Not     | 1.2 |
| 33  | G  | Not    | 3.7 | 19  | Yes     | 1.9 |
| 27  | G  | Not    | 3.6 | 33  | Not     | 1.6 |
| 44  | S  | Yes    | 6.1 | 41  | Yes     | 3.9 |
| 34  | G  | Not    | 5.5 | 43  | Not     | 2.0 |
| 31  | G  | Yes    | 4.4 | 57  | Yes     | 3.3 |
| 30  | G  | Not    | 2   | 26  | Not     | 1.4 |
| 29  | G  | Not    | 3.8 | 33  | Yes     | 2.9 |

WP: Working place. S: Smart auto-repair working place, G: Great auto-repair working place, MDA concentration: nM/ml, age in years.

MDA: Malondialdehyde

Table 3: Analysis of control group

Univariate analysis | Multivariate analysis
| Variable | Coefficient | P | Variable | Coefficient | P |
|-----------|-------------|---|----------|------------|---|
| Age       | 0.0909      | 0.0909 | Age      | 0.0866     | 0.0079 |
| Smoking   | 2.3832      | 0.0567 | Smoking  | 1.2974     | 0.0404 |

Table 4: Analysis of only workers group

Univariate analysis

| Variable | Coefficient | P |
|----------|-------------|---|
| Working small auto-repair | 2.7669 | 0.0173 |
| Age      | 0.0967      | 0.0460 |
| Smoking  | 0.9303      | 0.2566 |

Table 5: Analysis of all groups: Workers and control

Univariate analysis | Multivariate analysis
| Variable | Coefficient | P | Variable | Coefficient | P |
|----------|-------------|---|----------|------------|---|
| Age      | 0.0748      | 0.0170 | Age      | 0.01106     | 0.0031 |
| Smoking  | 0.9804      | 0.09804 | Smoking  | 1.8338      | 0.0117 |
| Workers  | 2.0253      | 2.0253 | Workers  | 2.6512      | 0.0007 |

is a predominant parameter than the other two risk factors considered (smoke and age). This observation is clear not only from the UVA and MVA statistical analysis of the two samples [Table 4] but also in the analysis of the workers group alone, [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 [Table 5] is most likely because direct exposure of the workers suffer little in positive terms, because of the air changes, that are usually more frequent in spacious auto repair places, rather than in small working places. It also must be considered that as other concomitant factors may contribute to improve the risks set out above. For the auto repair workers, they are principally the pollutants exposure, the cigarette smoking, and the increasing age.

The main pollutants, or risk factors, that involved the workers in the car repair section are essentially fine dust, lead, polycyclic aromatic containing gases, the CO, and mineral oils. For each pollutant, the biological damage and the ways for monitoring are reported in literature, and in some cases also an estimation of the oxidative stress induced individually.[10-13]. In our study, it cannot be affirmed which one specifically has raised in a statistically significant way the values of salivary MDA among workers compared to the control group; this consideration may be even more important, if considered the synergistic contribution that certainly can have smoking and age, with the other risk factors. The cigarette smoking,[14,15] in fact, induces a strong oxidative phase: during combustion, there is a “chemical stress” that involves practically all compounds present in tobacco, and that leads to the formation of free radicals with others in the “chain reaction,” the faster and quantitatively important depending on the types involved and their concentration. The amount of radicals introduced in the body is estimated in a range of 1013 ÷ 1018 radicals/cigarette smoked.[16] The other question is the increasing age of those exposed to lead, exhaust gas with the polycyclic aromatic hydrocarbons, CO, and other pollutants related to the mitochondrial dysfunction. This phenomenon has long been considered a major cause of aging and age-related diseases, and those effects add up to a longer time of exposure to environmental pollutants, for older workers. In this study, we investigated the oxidative stress possibly induced by exposure to pollutants at the auto repair workers, the monitoring of salivary malondialdehyde may results the better system. This assessment will consider two parameters that are very important and that is smoking and age, which in this particular kind of work generally coincide with the time of exposure, through the analysis of malondialdehyde in the saliva using dipsticks.

The other issue is the age of those exposed; as has been demonstrated in studies of smokers and non-smokers that oxidative stress increases in relation to the mitochondrial dysfunction and according to the age of the person.
This phenomenon has long been considered a major cause of aging and age-related diseases.\textsuperscript{[17,18]} mitochondrial free radical theory of aging postulated that somatic mitochondrial DNA mutations accumulated during lifetime cause excessive production of reactive oxygen species that damage macromolecules and affect the function of cells and tissues. In fact, studies have shown that maximum oxidative capacity decreases with age while reactive oxygen species production increases.

**Conclusions**

In our country, auto repair workers are subject to regular and effective controls for the occupational exposures, by the competent authorities. Our study with biological monitoring has not found anything that can suggest the effect of a pollutant on their body, but in any case the kind of their work brings to a series of small exposures, undetectable except as cumulative. This effect, which can be defined oxidative stress, is evident from the values of MDA, encountered on those employees, compared to the control group.

To that, biological effect is added the effect induced by smoking and age, which is evident also in the control group as well. Our study highlights how important is to quantify this oxidative stress, especially for the head and neck district, in the professional exposure.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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