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Comparative analysis of methemoglobin, oxygen saturation and hematological parameters in smokers and non-smokers: An observational analytical cross-sectional study

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Abstract

Introduction: The formation of methemoglobin (MetHb) occurs through the oxidation of iron in hemoglobin, impairing its capacity for oxygen association and deoxygenation. On exposure to oxidizing agents, such as those present in cigarettes, this process may be more frequent, causing an increase in serum MetHb. Objectives: To evaluate and compare methemoglobin, oxygen saturation and hematological parameters between smokers and non-smokers. Materials and methods: Observational case-control study with participants classified as smokers and non-smokers, in equal number and gender. Smokers classified as moderate to very high dependence degree by the Fagerström tolerance questionnaire were included. In all subjects, oxygen saturation was assessed using portable pulse oximetry, methemoglobin levels by spectrophotometric method and hematological parameters by an automated analyzer. Parametric (Student's T-test) and non-parametric (Mann-Whitney U) tests were performed for comparison of mean values between groups. Results: There were no changes in methemoglobin rates and hematological parameters, both in relation to clinical reference values and in the statistical difference between groups. The oxygen saturation values were significantly higher in the smoking group, 96.4% versus 94.8% (p = 0.04). Conclusions: Despite the potential deleterious effects of cigarettes, in this study it was found that smoking was not a determinant of changes in methemoglobin rates and hematological parameters, when compared with non-smokers. Further studies are suggested with a robust sampling population, complementary analysis of hematological and physiological factors and verification of comorbidities, in order to elucidate a greater relationship between the presented parameters.

Keywords: Hematology; Tobacco Use Disorder; Blood Physiological Phenomena.

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Introduction

Smoking is recognized as a chronic and epidemic disease by the World Health Organization (WHO) and as one of the biggest causes of preventable early deaths in the world.[1] In Brazil, it is one of the main risk factors for death, disability or decreased productivity in people with chronic diseases, generating high costs for public health and reducing life expectancy by about six years compared to non-smokers.[1] Its relevance is due to the presence of nicotine, a psychoactive substance that generates physiological, psychological and behavioral dependence on the consumer. Burning tobacco exposes the smoker to more than 4,000 substances with different chemical properties, of which approximately 60% with carcinogenic activities and 40% recognized as toxic substances to the human body.[2,3]

The harmful effects of chronic exposure to cigarette substances are important for the onset and aggravation of pulmonary and cardiovascular diseases. The incidence of respiratory tract diseases, in general, is directly associated with smoking, such as chronic obstructive pulmonary disease (COPD) and lung cancer. While for others, the influence on the frequency, injuries on the onset, course and outcomes of the diseases is reported.[4] In smokers, the molecular, structural and functional changes in the respiratory and vascular tract are reported, such as decreased defense and cell repair functions, increased inflammatory response and decreased concentration and activity of ascorbic acid and total antioxidants.[2,5] Cell death - both by apoptosis and necrosis - induced by exposure to cigarette smoke can be explained by an increase in oxidative stress in the alveolar tissue, with its effects extending systemically through the bloodstream, affecting the blood and vascular tissues itself.[5]

Hemoglobin (Hb) is a globular protein contained within erythrocytes that is responsible for transporting blood gases, such as oxygen (O_2) and carbon dioxide (CO_2).[6,7] During this association, an electron from the iron present in the heme group can be transferred to oxygen, changing it from a ferrous (Fe^{2+}) to a ferric

(Fe^{3+}) state. In the deoxygenation process, the electron can return to the iron in heme molecule, reducing it to the ferrous state again. This iron auto-oxidation occurs naturally in healthy individuals, and the conversion of methemoglobin is carried out by the body through protective mechanisms that attempt to reverse the oxidative stress situation by reducing it to Fe^{2}+. These mechanisms occur due to physiological and enzymatic systems, such as the action of ascorbic acid, glutathione reductase (GSH), through NADH-dependent cytochrome-b5 reductase and NADPH-dependent methemoglobin reductase.[7,8]

However, about 0.5 to 3.0% of Hemoglobins do not perform this conversion, remaining in the ferric state. This prevents the formation of new reversible bonds with molecular oxygen, thus forming methemoglobin (MetHb). Therefore, the formation of MetHb is due to excessive Hb oxidation (increased production) or decreased activity of reducing enzymes. These factors can cause a decrease in circulating oxygen, and, in this situation, the hemoglobin saturation curve presents a dissociation deviation, distorting its normally sigmoid shape. In addition, MetHb decreases the cooperative release capacity of the oxygen molecule, impairing tissue oxygenation.[9,10]

Methemoglobinemia is considered as a 1.5% increase in the MetHb concentration in relation to total hemoglobin, impairing oxygen distribution. It is understood that methemoglobinemia is a possible cause of anemic hypoxia, that is, a product of the decrease in functional hemoglobin, being presented as a reduction in the oxygen transport capacity when the partial pressure of O_2 and cardiac output are regular or high. The low saturation reading of pulse oximeter (SpO_2) can support the diagnosis of methemoglobinemia in patients with central cyanosis, but the co-oximeter is a gold standard method equipment for the determination of MetHb.[9]

Methemoglobinemia can have an acute onset or can occur due to chronic exposure to methemoglobinic agents, such as drugs, pesticides, herbicides, fertilizers, industrial chemicals, nitrites and nitrates present in water and food, as well as exposure to smoke.[9,10] There is a wide variation in which such substances are able to cause methemoglobinemia, avoiding a quantitative determination in relation to exposure and the clinical condition presented. Hence, the reduction in the number of functional erythrocytes or changes in hemoglobin by chemical induction, as mentioned above, may lead to methemoglobinemia.[9,10]

Thus, the present study aimed to evaluate changes in methemoglobin, oxygen saturation and hematological clinical parameters in smoking volunteers compared to non-smoking counterparts.

Materials and methods

The present study, characterized as an analytical observational cross-sectional case-control study, was approved by the Research Ethics Committee of Universidade Paulista, Number 3.186.586/CAAE 03249518.1.0000.5512. It respected all the ethical principles described in resolution 466/12 of the Brazilian National Health Council, especially in which participation is free and spontaneous and with a confidential identity to avoid exposure. The Informed Consent Form was signed by the participants after explanation about the research objectives, being the primary and indispensable inclusion criterion in the sample.

The individuals participating in this study were recruited and invited from March to May 2019. The smoking volunteers had the inclusion criteria to be over 18 years old and to have nicotine dependence assessed through the Fagerström Tolerance Questionnaire (FTQ) translated into the Portuguese.[11] The questionnaire consists of six items, each having 2 to 4 alternatives scored to each answer. At the end, the result is obtained by the sum score, which varies from 0 to 10, indicating the level of dependence of the smoker. The reference score rank used in the questionnaire are: 0 to 2 very low; 3 to 4 low; 5 medium; 6 to 7 high; and 8 to 10 very high dependence.[11] Smoking volunteers who obtained medium to very high levels of dependence as a result were included. For the group of non-smokers, individuals over 18 years-old who reported never having smoked before were included. It was considered as an exclusion criterion, in both groups, the self-reported presence of hematological, acute or chronic respiratory diseases or other conditions that impact the blood count and methemoglobin levels, such as cancer, anemia, surgeries less than a week and chronic exposure or acute to other methemoglobinizing agents, such as those mentioned above.[9,10]

As suggested by the resolution 466/12 of the Brazilian National Health Council, as a research benefit to encourage changes to healthy attitudes, all volunteers were instructed about the harmful effects of cigarettes and the existence of the National Tobacco Control Program to stop smoking, offered at no cost by the Brazilian Ministry of Health through the National Cancer Institute.

Venous blood were collected in partnership with a private diagnostic medicine laboratory, in the facility with best access for each volunteer, with no prior preparation in relation to fasting or previously defined hour.

The laboratory has certifications that aim to guarantee the necessary quality for the analysis of this study (College of American Pathologists – CAP, Accreditation Program for Clinical Laboratories – PALC and ISO 9001).

Determination of methemoglobin assays were performed using the spectrophotometric method using a co-oximeter device, Cobas b221® (Roche Diagnostics, USA), according to the manufacturer's protocol. The technique consists of dissolving the blood in a lysing reactant and aggregating it to a high vibrational frequency hemolyser. Then the sample was loaded to an optical cuvette to measure the absorbance at seven predefined wavelengths, which allow the differentiation of the hemoglobin fractions in distinctive absorptions. With the absorbance data, the equipment itself uses matrix equations to calculate the concentrations of each fraction.

The hematological parameters observed in the red series were: Erythrocyte, Hemoglobin, Hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW). The assays were performed using the automated Sysmex ® analyzer device, according to the manufacturer's protocol. For RBC, Ht and RDW, the flow impedance with hydrodynamic focus methodology was used by the equipment. For Hemoglobin, colorimetry was used by the device, where all forms of hemoglobin are converted to a stable form and measured at 550nm in spectrophotometry. Finally, the MCV, MCH, MCHC parameters were obtained through calculations from the values presented above by the device itself.

The determination of oxygen saturation (SpO_2) was performed by a portable pulse oximetry device, the Nonin GO2 Achieve (Nonin Medical Inc, MN, USA), with an oxygen saturation accuracy range between 70% to 100% SpO_2.[12] The device was placed on the volunteer's index finger to measure the SpO_2 transported in the blood as a percentage of its total transport capacity, ideally above 89% SpO_2.

For data analysis, Microsoft Excel® was used for data arrangement and descriptive analysis. To verify the data distribution, the Shapiro Wilk test was used, available on the Sdittami online platform. A parametric test (Student's t test) was chosen for the comparison between two groups that had data with normal distribution, available online on the Evanmiller platform. Data in which at least one group had non-normal distribution were analyzed using the Mann-Whitney U test, available on the Socscistatistics platform. Difference of means were considered with association probabilities lower than 5% error ($p<0.05$).

Results

A total of twenty-four volunteers residing in the Federal District participated by signing the consent form. They were allocated into 2 groups according to the smoking characteristic, i.e. active smokers or non-smokers, each containing the same number of people and gender equivalence, that is, 6 men and 6 women in each smokers and non-smokers groups. For all parameters analyzed, there was no significant difference between men and women, so all comparisons were made only between the smoking characteristic. The Fagerström test for the inclusion of the twelve smoking volunteers showed a result of 58.3% for moderately dependent smoking volunteers, 25% with high dependence and 16.7% with very high dependence to nicotine.

The oxygen saturation presented in both groups was within the reference value recommended for a healthy adult individual by the Brazilian Society of Pulmonology and Phthisiology (SBPT), i.e., greater than 89%. Data distribution was non-normal and the Mann-Whitney U test was used. The mean and standard deviation of the values for the smoker group was 96.4 ± 1.1% (with a minimum value of 95% and a maximum of 98%), and for non-smokers 94.8 ± 1.8% (with a minimum value of 93% and maximum of 98%). The means between the groups of smokers and non-smokers had a significant difference ($p = 0.04$).

In the assessment of methemoglobin saturation, all values were below clinical reference, that is, less than 1.5%. From the statistical analysis it can be inferred that the mean in the smoker group was 0.72 ± 0.09% (with a minimum value of 0.50 and a maximum of 0.80%) and in the non-smoker group it was 0.72 ± 0.08% (with minimum values of 0.60 and maximum of 0.80%), with no significant difference ($p = 0.95$). Table 1 describes the oxygen saturation and methemoglobin parameters for individual and group means.

The hematological parameters under study did not show any discrepancy in relation to the clinical reference values, either individually or in the means of each group. In the statistical analysis, the parameters had a normal distribution and the Student's t-test was used to calculate the differences between the means of the groups. The exception was the MCV, which obtained free distribution and the Mann-Whitney U test was used. The results presented by the groups were compared and no significant difference was shown within the p value for each parameter (Table 2).

Table 1. Determination of oxygen saturation (SpO_2) and methemoglobin (MetHb) in a group of smoking volunteers according to the levels of dependence using the Fagerström tolerance questionnaire and non-smokers, described individually and in groups by mean ± standard deviation. Statistical significance considered for $p < 0.05$

Smokers	Smokers Dependence score	Smokers SpO2	Smokers MetHb	Non-Smokers	Non-Smokers SpO2	Non-Smokers MetHb
S-1	8	98%	0,7%	NS-1	94%	0,8%
S-2	5	97%	0,8%	NS-2	94%	0,8%
S-3	5	96%	0,5%	NS-3	96%	0,8%
S-4	5	98%	0,8%	NS-4	96%	0,6%
S-5	6	96%	0,7%	NS-5	98%	0,6%
S-6	5	96%	0,7%	NS-6	95%	0,8%
S-7	6	98%	0,7%	NS-7	95%	0,7%
S-8	5	96%	0,8%	NS-8	93%	0,7%
S-9	7	97%	0,8%	NS-9	96%	0,8%
S-10	5	95%	0,8%	NS-10	92%	0,6%
S-11	5	95%	0,8%	NS-11	92%	0,7%
S-12	8	95%	0,6%	NS-12	97%	0,8%
Mean ± standard deviation	**5,83 ± 1,19**	**96,3% ± 1,2%[a]**	**0,727 ± 0,1%[b]**		**94,8% ± 1,9%[a]**	**0,725 ± 0,09%[b]**

Legend: S = Smokers Volunteer; NS = Non-Smokers Volunteers; SpO_2 = Oxygen saturation; MetHb = methemoglobin.
Clinical reference value for $SpO_2 > 89\%$.
Clinical Reference Value for MetHb: Unexposed = 0.04 to 1.52%; Toxic level = above 15%; Lethal level = above 70%.
P-value a = 0.04; P-value b = 0.99.
Authorship: The authors (2021).

Table 2. Evaluation of the hematological parameters of the group of smokers and non-smokers, described as mean ± standard deviation. Statistical significance considered for $p < 0.05$

Hematological Parameters	Smokers (n = 12)	Non Smokers (n = 12)	p-value	Reference Values	
				Adult Men	Adult Women
Erythrocyte (/mm^3)	5,04 ± 0,2	4,90 ± 0,4	0,56	4,5 – 6,0	4,0 – 5,4
Hemoglobin (g/dL)	15,2 ± 0,8	14,4 ± 1,0	0,15	13,0 – 16,5	12,0 – 15,8
Hematocrit (%)	44,2 ± 2,2	42,8 ± 2,9	0,38	36,0 – 54,0	33,0 – 47,8
MCV (fl)	87,6 ± 3,3	87,6 ± 3,3	0,90	80,0 – 98,0	80,0 – 98,0
MCH (pg)	30,3 ± 1,3	29,4 ± 0,8	0,24	26,8 – 32,9	26,2 – 32,6
MCHC (g/dL)	34,4 ± 0,7	33,6 ± 0,4	0,05	30,0 – 36,5	30,0 – 36,5
RDW (%)	13,1 ± 0,5	13,3 ± 0,4	0,72	11,0 – 16,0	11,0 – 16,0

Legend: MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; RDW = Red Cell Distribution Width.
Authorship: The authors (2021).

Discussion

In the present study, it was noted that even with medium to very high levels of dependence and frequent exposure to harmful agents present in cigarettes, the group of smokers did not show potential changes in methemoglobin or hemogram parameters. It was assumed that the amount of agents to which smokers are exposed is not sufficient to cause increased MetHb formation or to alter hematological parameters in the present sample.[10] For the group of non-smokers, all variables evaluated were within the clinical limits of the reference values.

Although it was not observed in the present study, others have shown that smoking caused an increase in carbon monoxide levels, being able to modify some of the hematological parameters, such as hemoglobin, leukocytes, MCV, MCH and RDW.[13,14] The increase in carbon monoxide in smokers was observed in another study in 5% of carboxyhemoglobin (COHb), based on the comparison of results obtained in smoking and non-smoking volunteers. However, no differences and changes in methemoglobin levels were observed between the groups in those studies.[13,15,16]

In this research, a statistical significance was observed in the evaluation of oxygen saturation, showing that the mean saturation in the control group, even within the reference value, was lower than the mean presented by smokers. It was considered, as observed in another study,[15] that the high saturation presented by smokers is due to the presence of carboxyhemoglobin and the limitation of the oximeter in distinguishing between oxyhemoglobin, carboxyhemoglobin, methemoglobin and other fractions of this molecule. Therefore, methemoglobin could not be considered here as interfering in the results of O_2 saturation, as it was within the reference values in both groups.[15]

The difference observed in oxygen saturation may occur in cases that different hemoglobins are not distinctable.[9] The pulse oximeter uses only two light wavelengths for hemoglobin and oxyhemoglobin, not being able to distinguish carbon monoxide from O_2, due to the amount of light absorbed being similar and falsely raising the saturation.[9] A similar case also occurs with methemoglobin, when elevated, even in small amounts, it provides erroneous estimates of oxygen saturation, delivering a higher saturation value than the real one.[17]

The difficulty in determining the direct quantitative relationship between occupational exposure to a potentially harmful agent and the likely presentation of physiological and hematological changes has been described in toxicology.[9] Although MetHb is considered an indicator of the exposure effect, it is important to note that there is a diversity of potentially methemoglobinizing agents from the tobacco combustion, which in turn also have variations related to toxicokinetics and toxicodynamics, indicating acute or chronic changes in various tissues in the human body.[2-4]

Other authors have shown different MetHb results. Despite using the same methodology for determination, the sample number used in other was higher than in this study (856 volunteers, 377 smokers and 479 non-smokers). The authors found MetHb levels in smokers was significantly higher compared to non-smokers, respectively MetHb = 0.63% and 0.56%, however, these values are still considered clinically normal.[18]

Another study aiming to verify the increase in MetHb in smokers observed the mean methemoglobin saturation of the smokers group (n=15) clinically and statistically higher compared to the non-smokers group (n=15).[19] However, the authors used manual methods to perform the determination, and in the present study the automated methodology was the one of choice, also including quality control for assurance and safety of the results.

No changes were observed in the hematological parameters evaluated in the present study. Due to exposure to carbon monoxide, hemoglobin levels could be altered, impairing oxygen bounding and an increase in the number of red blood cells as compensation.[10,20] However, the present findings corroborate other studies that obtained the absence of hematological alterations as results.[15,16,21]

The low number of individuals in each group can be pointed out as a limitation of the study, which did not allow the calculation of the statistical sampling power to be determined in a robust way. By using a larger sample, homogeneity could be assessed in relation to other factors associated with smoking, such as age, clinical, dietary, anthropometric and demographic variables.[22,23] The present study did not assess in detail the health conditions of the individuals present in the sample. This bias could change the results, however it may have been minimized by the proposed exclusion criteria. Still, comorbid conditions that impair oxygen transport, such as anemia, heart disease and lung disease, are associated with cases of low-grade methemoglobinemia,[24] and, therefore, should be considered in future studies.

Still as a recommendation for future studies, arterial blood gases can be used for a more accurate measurement of oxygen saturation and also of the partial pressure of carbon dioxide. In addition, it is necessary to compare MetHb levels with complementary tests of inflammatory markers, such as C-reactive protein, xanthine oxidase and/or lactate dehydrogenase, and also the determination of total plasma antioxidant activity, or specific antioxidants, such as vitamin C or vitamin E, as cigarette consumption can decrease the concentration and activity of these markers and increase their systemic inflammation effects.[25] It is suggested to perform the laboratory dosage of these parameters and relating to the methemoglobin values, once that, to date, there are no statistical analysis studies that performed the comparison of reduced blood values of these antioxidant and inflammatory markers in smokers influencing in the methemoglobin values.

Conclusion

In the present study, the evaluation between groups of smoking and non-smoking volunteers did not detect alterations in methemoglobin saturation and hematological parameters, both in relation to clinical reference values and in the statistical analysis among groups. In the oxygen saturation, a higher mean was observed in smokers compared to the control group, being extremely important for future perspectives in research discussions focusing on the evaluation of detailed hemoglobin parameters presented in smokers. Further research is needed, as the topic lacks recent work with automated technologies. In addition, new studies with a larger sample group and the performance of additional determinations are also suggested, such as, for example, the relation methemoglobin with carboxyhemoglobin, or even the dynamic acute effect of cigarette smoke inhalation on hematological and methemoglobin parameters.

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