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RESEARCH ARTICLE

Tobacco influence in heavy metals levels in head and neck cancer cases

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Abstract
Heavy metals intoxication is known to be risk factors for various diseases, including cancer. These metals may be presented in food and soil as well as in leaf and tobacco smoke. The aim of this study was to correlate the exposure to heavy metals stemming from tobacco and head and neck squamous cell carcinoma carcinogenesis. Analysis of lead, copper, manganese, arsenic, chromium, and cadmium by atomic absorption spectrophotometry was performed in whole blood samples from 91 patients: 68 smokers with oral cavity, pharynx, or laryngeal cancer; 8 non-smokers with oral or larynx cancer; and 15 non-cancer smokers with tobacco-related diseases (control group). No differences were found in metals quantifications, except a significant difference was observed ($p = 0.0223$) with higher mean in copper levels for non-smokers with cancer. The present study concluded, for the groups evaluated, it was not possible to prove the relationship between the studied metals in the development of the neoplasm. On the other hand, the results of copper demonstrated a correlation with smokers with cancer and lower levels of circulating copper.

Keywords Tobacco · Carcinoma, squamous cell · Head and neck neoplasms · Metals, heavy

Introduction

Smoking is the leading cause of illness and accounts for more than 7 million deaths per year. Tobacco use in its different forms is related to several types of cancer, such as lung, stomach, bladder, and head and neck region cancer (WHO 2017). Squamous cell carcinoma (SCC) accounts for 90% of malignant neoplasms of the mouth, pharynx, and larynx and has smoking and alcoholism as main known risk factors (WHO-Cancer-prevention 2017). However, diet, environmental, occupational, and genetic factors have also been pointed out as aspects of interest for the study of this type of neoplasm (Abnet et al. 2015; Curado and Hashibe 2009; Stewart and Wild 2014).

To the present time, more than 7000 chemical substances have been identified in cigarette smoke, including aromatic hydrocarbons, aldehydes, ketones, and heavy metals (Condoluci et al. 2016; Stewart and Wild 2014). Although some metals are considered essential for biological functions, balance, high exposure to lead (Pb), cadmium (Cd), arsenic (As), nickel (Ni), and chromium (Cr), which are present in soil, contaminating food, and tobacco smoke, are also considered risk factors for cancer (Condoluci et al. 2016; Galazyn-Sidorczuk et al. 2008; Koedrith et al. 2013; Maret 2016; Tsai et al. 2017; Viana et al. 2011). From the contamination of soil, tobacco leaves can be exposed to the metals: lead (Pb), cadmium (Cd), arsenic (As), nickel (Ni), and chromium (Cr), copper (Cu), and zinc (Zn), but its concentrations are dependent on plant growth and soil pH (the lower soil pH, the higher the metal contamination capacity) (Golia et al. 2009). All the agriculture area studies demonstrate the necessity to regulate the tobacco leaves from countries that proved to have a high amount of heavy metals on tobacco cigarettes (Ajab et al. 2014; Pourkhabbaz and Pourkhabbaz 2012). Some important studies demonstrated that the tobacco leaves'

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contamination is in different concentration from raw tobacco, ash tobacco, and processed tobacco (Ajab et al. 2014; Eneji et al. 2013; Regassa and Chandravanshi 2016).

Some carcinogens as physical, chemical, or viral agents are well known, but heavy metal's relation to malignant neoplasms and the mechanism by which it induces carcinogenesis remain unclear (Condoluci et al. 2016; Galazyn-Sidorczuk et al. 2008; Koedrith et al. 2013; Maret 2016; Tsai et al. 2017; Viana et al. 2011; Wise et al. 2017). Metals such as cadmium, arsenic, nickel, and chromium are already classified as carcinogenic to humans (Groups 1 and 2) by the International Agency for Research on Cancer (WHO-IARC. 2014).

Currently, toxicogenomic researchers study several mechanisms, such as DNA damage and repair, as well as the oxidative stress present in multiple processes, such as autophagy, angiogenesis, inflammation, epigenetic alterations, metabolic reprogramming, and genomic instability (Koedrith et al. 2013; Wise et al. 2017; Wu et al. 2016). Furthermore, low concentration of some heavy metals can have an important influence on the double strand breaks (DSBs) at the DNA level and can thereby cause mutagenic changes to the cell repair process (Morales et al. 2016).

Recently, heavy metal's contribution to metastasis processes has also been discussed, as well as its use in new generations of anticancer drugs and anti-metastatic agents (Fouani et al. 2017).

Chronic exposures to low concentrations of arsenic were associated with diseases such as hepatic fibrosis, cirrhosis, hepatocellular carcinoma, melanosis, hyperkeratosis, diabetes mellitus, immunological disorders, pulmonary infections, hypertension, and cardiovascular and neuromuscular diseases (Ahmed et al. 2013). Similarly, exposure to high environmental nickel levels was related to an increased risk of oral carcinoma and premalignant lesion development (Tsai et al. 2017).

Copper and iron analysis and their relationship with immune complexes in premalignant lesions and mouth SCC have been considered as possible disease biomarkers (Tiwari et al. 2016).

The aim of this study was to correlate the exposure to heavy metals stemming from tobacco and head and neck SCC carcinogenesis. The hypothesis of the present study was that the contamination of tobacco leaves on crops or during the industrial production leads to the posterior contamination of the smokers which led to head and neck SCC carcinogenesis. The levels of heavy metals evaluated in blood samples from patients with a diagnosis of oral, pharynx, and larynx cancer were lead, copper, manganese, arsenic, chromium, and cadmium.

Material and methods

Ninety-one individuals were selected with a diagnosis of oral, pharynx, or larynx SCC, which was confirmed by hematoxylin-eosin histopathological exam, in the Laboratory of Pathology from Santa Casa de Misericordia de São Paulo Hospital (Sao Paulo, SP, Brazil). All the samples were diagnosed as squamous cell carcinoma. The groups were composed of 68 smokers or ex-smokers and 8 individuals who had never smoked. The study also included 15 smokers with chronic vascular diseases and no history of malignant neoplasms. The groups were divided in Group 1—smokers with cancer ($n = 68$), Group 2—smokers without cancer ($n = 15$), and Group 3—non-smokers with cancer ($n = 8$). The patients' demographics, smoking profiles, and cancer stages are described in Table 1.

Table 1 Demographic characteristics of the 91 study participants

	Group 1	Group 2	Group 3
N	68	15	8
Gender			
Male[a]	56 (61.54%)	9 (9.9%)	2 (2.2%)
Female[a]	12 (13.19%)	6 (6.6%)	6 (6.6%)
Age, years[b]	61.7 ± 8.03 (47–82)	70.47 ± 5.28 (62–77)	72.13 ± 15.55 (45–92)
Age of smoking initiation, years[b]	15.66 ± 3.76 (6–30)	18.27 ± 5.23 (13–30)	
Duration of smoking, years[b]	40.63 ± 10.46 (10–67)	40.6 ± 12.46 (15–58)	
Smoking history pack/years[b]	60.91 ± 29.35 (5–167.5)	52.17 ± 26.85 (4.5–110)	
	Smokers $n = 83$		
FTND, score[b]	5.72 ± 2.33	4.93 ± 2.69	
Low to moderate[a]	24 (28.92%)	7 (8.43%)	
High or very high[a]	44 (53%)	8 (9.64%)	
Disease site[a]			
Mouth	10 (13.15%)		7 (9.21%)
Pharynx	21 (27.63%)		
Larynx	38 (50%)		1 (1.32%)
Disease stage[a]			
I	15 (19.74%)		4 (5.26%)
II	5 (6.58%)		2 (2.63%)
III	16 (21.05%)		0
IV	32 (42.10%)		2 (2.63%)

FTND, Fagerström Test for Nicotine Dependence; ND, nicotine dependence. [a] Values are expressed as *n* (%), except where otherwise indicated. [b] Values are expressed as mean ± SD

In this study, blood samples (5 mL) were collected from patients with a diagnosis of oral, pharynx, and larynx SCC. Regarding smoking, the study included active smokers, former smokers, and non-smokers who were all regularly followed at the Department of Head and Neck Surgery, Medical Sciences College, Irmandade da Santa Casa de Misericórdia de São Paulo (ISCMSP), São Paulo, SP, Brazil.

The present work was approved by the ethics committee of ISCMSP (Protocol number 147/10) and complies with the international guidelines of human use on sciences. All the participants agreed to participate in the research and signed an Informed Consent Term (ICT).

Clinical and oncological data were extracted from medical records. The criteria for classification as active smokers, former smokers, and non-smokers were based on the study carried out at the Brazilian Thoracic Association (Reichert et al. 2008). Individuals who had smoked at least one hundred cigarettes in their lifetime and who smoked daily or occasionally were considered to be active smokers, and individuals who had stopped smoking for more than one year before the interview were considered to be former smokers. Non-smokers were those individuals who had never smoked or had consumed fewer than 100 cigarettes during their lifetime and smokers patients answered a questionnaire about their smoking profile, which consisted of questions regarding their current use of cigarettes and their derivatives, the type of tobacco and the age they began the use, the total amount of their consumption per day, and the period of tobacco use. Nicotine addiction was assessed using the Fagerström Test for Nicotine Dependence (FTND) (Fagerstrom 2012; Reichert et al. 2008).

Smoking patients answered a questionnaire about their smoking profile, which consisted of questions regarding their current use of cigarettes and their derivatives, the type of tobacco, the age they began to use, the total amount they consumed per day, and the period of tobacco use. Nicotine addiction was assessed using the Fagerström Test for Cigarette Dependence (FTCD). The FTCD is only used for smokers of industrialized cigarettes. Regarding the onset of smoking, the mean age of the cancer patients was 15.66 years old, and the non-cancer group had a mean age of 18.27 years old (Fagerstrom 2012).

The final score obtained was classified into five levels of nicotine dependence: very low (0 to 2 points), low (3 to 4 points), moderate (5 points), high (6 to 7 points), and very high (8 to 10 points). Smoking history was quantified by pack-years of cigarettes smoked (Reichert et al. 2008).

For metal level evaluation, 5.0 mL of blood was collected on EDTA collect tube (Laborimport, Osasco, Sao Paulo, Brazil) and kept on − 20 °C freezer, until the analysis. Atomic absorption spectroscopy technique was used to determine the lead, copper, manganese, arsenic, chromium, and cadmium levels at the Center of Toxicological Assistance (CEATOX), Sao Paulo State University (Unesp), Institute of Biosciences of Botucatu (Botucatu, Sao Paulo, Brazil). For the measurements, the certification was granted by the ISO 17025 certification from Adolfo Lutz institute with INMETRO (National Institute of Metrology, Quality and Technology, Rio de Janeiro, RJ, Brazil) which is the equivalent of ANAB institute of the USA (National Accreditation Board, Milwaukee, WI, USA).

In order to prepare the samples, a microwave oven (Provecto®–DGT–100 plus) in the proportion 3:1 with nitric acid (HNO_3 65%) using a specific program (Number 18) was supplied by the microwave manufacturer (P. S 2001; Vogel 1981). The analysis conditions were optimized with hollow cathode lamps (Photron PTY, Narre Warren, Victoria, Australia) specific to each chemical element analyzed. The atomic absorption spectrometer AA 932 model (GBC Scientific Equipment, Dandenong, Australia) was optimized to read blood samples (Athanasopoulos 1994).

The results were expressed in $\mu g\ dL^{-1}$, in which the "<" sign represented values below the technique limit detection. For this study, the following limits of detection (LoD) and quantification (LoQ) were obtained: for lead, LoD 0.05 $\mu g\ mL^{-1}$ and LoQ 0.5 $\mu g\ mL^{-1}$; cadmium, LoD 0.01 $\mu g\ mL^{-1}$ and LoQ 0.1 $\mu g\ mL^{-1}$; for copper, LoD 0.02 $\mu g\ mL^{-1}$ and LoQ 0.2 $\mu g\ mL^{-1}$; chromium, LoD 0.01 $\mu g\ mL^{-1}$ and LoQ 0.1 $\mu g\ mL^{-1}$; manganese, LoD 0.01 $\mu g\ mL^{-1}$ and LoQ 0.1 $\mu g\ mL^{-1}$; arsenic, LoD 1 $\mu g\ mL^{-1}$ and LoQ 0.1 $\mu g\ mL^{-1}$.

The following reference values were considered (Table 2): lead, Pb < 10 $\mu g\ dL^{-1}$ environmental, Pb < 40 $\mu g\ dL^{-1}$ occupational; copper, Cu 60 to 140 $\mu g\ dL^{-1}$; manganese, Mn < 2.0 $\mu g\ dL^{-1}$; cadmium, Cd < 1.0 $\mu g\ dL^{-1}$; arsenic, As < 10.0 $\mu g\ dL^{-1}$; chromium, Cr < 5.0 $\mu g\ dL^{-1}$. The references of these concentrations on blood were according to National Institute of Health Science and Adolfo Lutz baselines.

The results were evaluated by the Kruskal-Wallis test, the Spearman correlation, the chi-square test, and Fisher's exact test with $p \leq 0.05$.

Results

For the heavy metal analysis, 546 analyses were performed on 91 blood samples collected. Lead, manganese, cadmium,

Table 2 Metals contamination baselines for blood according to NiH and Adolfo Lutz of Brazil baselines

Metal, element	Based on NIH and Adolfo Lutz Brazil, contamination baselines
Lead, Pb	Environmental lead: Pb < 10 $\mu g\ dL^{-1}$ Occupational: Pb < 40 $\mu g\ dL^{-1}$
Copper, Cu	Cu 60–140 $\mu g\ dL^{-1}$
Manganese, Mn	Mn < 2 $\mu g\ dL^{-1}$
Cadmium, Cd	Cd < 1 $\mu g\ dL^{-1}$
Arsenic, Ar	As < 10.0 $\mu g\ dL^{-1}$
Chromium, Cr	Cr < 5.0 $\mu g\ dL^{-1}$

arsenic, and chromium measurements can be observed in Table 3. The quantification for copper showed the following results (mean ± SD): 85.1 ± 18.5 for Group 1, 95.1 ± 12.6 for Group 2, and 95.4 ± 12.1 for Group 3; the comparison between these groups showed a significant difference by the Kruskal-Wallis test (*$p = 0.0223$) (Fig. 1).

The authors have tested the Regression test to see if there was any relationship between metal and smoke. The "p" of our findings were copper × time $p = 0.76$ / copper × number of cigarettes $p = 0.23$ / copper × year-cigarettes pack $p = 0.18$ / copper × age $p = 0.7$. There were no significant differences.

To evaluate the association between copper levels and the neoplasm stage, the groups were divided according to the average concentration of copper (Table 4). Stages I and II were classified as the initial stages of SCC, and stages III and IV were classified as advanced stages by TNM classification.

The chi-square test demonstrated no significant association between the groups of smokers with cancer ($p = 0.5945$), and the same was observed in the non-smoker group with cancer ($p = 0.4286$) by the Fisher exact test.

As for occupation, only 6 individuals reported professional activity that could justify a possible contamination risk by exposure: 1 chemist, 1 painter, and 4 metallurgists.

In Group 1 (smokers with cancer), 32 individuals (47%) smoked more than 20 cigarettes a day and were in the advanced stages. This association was significant ($p = 0.005526$), according to the chi-square test.

Discussion

Chronic exposure to heavy metals may occur as a result of cigarette smoke inhalation or via environmental and occupational agents. They are also described as a risk factor for lung and bladder cancer development, with a likely relationship between this exposure and malignant tumors of the nasal cavities. However, the direct relationship between oral, pharynx, and larynx malignant neoplasms remains controversial (Stewart and Wild 2014; Tiwari et al. 2016).

Table 3 Quantification of lead, manganese, cadmium, arsenic, and chromium in the peripheral blood for the SCC smoker (Group 1), non-smoker SCC (Group 2), and smokers only (Group 3)

Heavy metal	Group 1	Group 2	Group 3
Lead	$n = 6$ [5.5–8.4]	0	0
Manganese	$n = 11$ [1–1.7]	$n = 2$ [1.3–1.5]	0
Cadmium	0	0	0
Arsenic	0	0	0
Chrome	0	0	0

n = the number of cases where quantification was detectable [minimum value μg dL^{-1} − maximum value μg dL^{-1}]

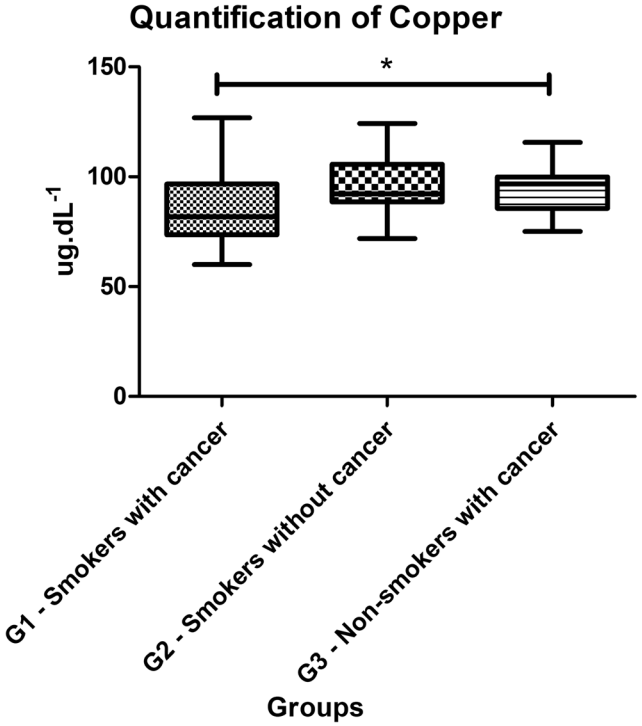

Fig. 1 Difference between copper levels in G1, G2, and G3 groups. Significant difference showed by the Kruskal-Wallis test with *$p = 0.0223$

Smoking is a chronic disease related to the use of nicotine and its action at the brain reward system (Fiore et al. 2008). In the oncology setting, tobacco use remains frequent, even if it is related to tumor recurrence, second primary tumor advent, or to an unfavorable therapeutic outcome (Gritz et al. 2014). Smoking patients with cancer have higher nicotine dependence, a fact that may justify the persistent use or relapse, even after initiation of cancer treatment ending (Chang et al. 2017).

In our study, we found higher nicotine dependence in the group of smokers with cancer, compared to the group of smokers without cancer, thinking on the copper increase on the smokers without cancer, it could be also explained by an increase of inflammatory cytokines on the respiratory tract (data not shown-measured) (Morrison et al. 1999). Although the duration of tobacco use was the same for the two groups, there was a difference in the smoking load (Table 1). The greater dependence of the cancer group justifies the larger number of cigarettes consumed per day and consequently raises the burden of tobacco consumed during their entire life.

Even patients at risk for occupational exposure were not observed increasing the amount of heavy metals in their blood samples. In the other patients, the greater exposure to tobacco use showed no difference in relation to the smoking groups, which could be attributed to the exposure of tobacco carcinogens.

Table 4 Association between the smoking SCC stage (Group 1), the non-smoker SCC (Group 2), and the amount of copper in the peripheral blood

Smoker's with SCC (Group 1)			
	Less than or equal to average	Higher than average	*p* value
Early stage (I and II)	9	11	0.5945*
Advanced stage (III and IV)	25	23	
Non-smokers SCC (Group 2)			
Early stage (I and II)	4	2	0.4286**
Advanced stage (III and IV)	0	2	

*Chi-square test; **Fisher's exact test

Serum copper levels were lower in the group of smokers with cancer compared to the group of non-smokers with cancer (Fig. 1), with no statistical difference between these two groups isolated, the only tendency of decrease on smokers. These findings corroborate to the study of Schuhmacher and authors, which they found no correlation with smokers and non-smokers on the concentration of copper (Schuhmacher et al. 1994). These results were contrary to the findings of a study that observed a high serum copper level in oral cancer patients (Baharvand et al. 2014) and smokers (Zhang et al. 2015). Likewise, another study demonstrated significant increases in nickel and chromium in blood samples from individuals with head and neck cancer (Khlifi et al. 2013). In our series, we did not observe changes in chromium levels.

When soil used for agricultural crops is contaminated, the harvested food can be also contaminated, increasing the risk of developing chronic diseases (da Silva et al. 2017; Dziubanek et al. 2015). Studies in Taiwan have shown the relationship between oral cancer, environmental exposure, and ingestion of food contaminated by chromium-laden soil (Chiang et al. 2011). In an Indian study, it was proved that the presence of arsenic in water was related to greater nuclear damage in cells in the oral mucosa of smokers (Roy et al. 2016).

An increase in blood chromium and nickel levels in patients with oral cancer was observed when compared to the controls. In these studies, the influence of smoking on the increased risk of exposure and carcinogenesis was not clear (Chiang et al. 2011; Yuan et al. 2011). In these studies, the influence of smoking on the increased risk of exposure and carcinogenesis was not clear (Chiang et al. 2011; Yuan et al. 2011). Although this is possible in our country, there is no information system to alert citizens that a certain region may be contaminated with high levels of heavy metals in the soil, which will consequently cause food contamination.

Tobacco leaves have a great ability to absorb heavy metals found in the soil. Thus, the variable concentration levels of these components in tobacco leaves may be related to higher indices of these metals, according to the geochemistry of the environment and/or anthropogenic action where they were cultivated (Khlifi and Hamza-Chaffai 2010; Regassa and Chandravanshi 2016).

A study of cigarette brands from different cities in China showed varying and high levels of heavy metals, suggesting that the use of fertilizers and pesticides may also contribute to these differences in the tobacco samples evaluated (O'Connor et al. 2015). High rates of oral cancer were found in regions contaminated with heavy metals (Yuan et al. 2011).

In a similar study, Viana et al. demonstrated that carcinogenic heavy metals levels (Pb, Cd, As, Ni, and Cr) represented an important source of exposure and risk for both active and passive smokers, although this may vary widely among the different brands of cigarettes sold in the country (Viana et al. 2011).

Thus, although heavy metals can be found in tobacco leaf products and are inhaled along with cigarette smoke or through occupational exposure, the concentration of manganese, arsenic, chromium, and cadmium levels in blood samples from smokers and non-smokers with SCC of the oral, pharynx, or larynx, nor in blood samples of cancer-free smokers were below the limit of detection.

The present study could only detect the concentration of copper, which was higher than the limit of detection. This could be explained by the knowledge of transitory aspects of blood on the toxicologic analysis because on the present work, almost 75% of smokers with cancer had already quit smoke since the diagnosis of cancer (Almeida et al. 2014). The study of Fontaine and authors corroborates with our findings as they concluded that the levels of lead and mercury decrease with the ending of occupational exposure, but the cadmium increased on the population as they increase the tobacco use (Fontaine et al. 2008). Cadmium concentration in blood and urine is higher on smokers, intermediate on rare smokers, and lower in former/non-smokers (Becker et al. 2002; Mannino et al. 2004). Kocyigit and authors found a correlation between higher concentration of Copper on plasma and higher presence of an enzyme called erythrocyte Cu-Zn SOD (an antioxidant enzyme) on tobacco smokers (Kocyigit et al. 2001), the authors have concluded that Cu-Zn SOD enzyme might have a correlation with inflammation of the respiratory tract and the studies that corroborated with this findings have circumstantial findings about this issue (Beshgetoor and Hambidge 1998; Perrin-Nadif et al. 1996). The authors' results could explain

the findings of the present study about the copper concentration. But by the data showed on the present study, it could be told that stopping the habit of smoking after being diagnosed with cancer could decrease the levels of heavy metals and increase the level of copper, which is correlated with release of corticosteroids and catecholamines (Lapenna et al. 1995) and the increase of copper could be by the absence of cadmium concentration, which it is well known that competes to copper on blood and could affect the copper homeostasis (Marklund 1986). On the present study, the cadmium concentration was below the limits of detection in all three groups. Also, the higher concentration of copper on the smokers without cancer and non-smokers with cancer compared with the smokers with cancer can be explained by an increase of the inflammatory activity (Morrison et al. 1999; Sher et al. 1999). On the present study, no measurements of enzymes were made.

Therefore, for the groups evaluated, it was not possible to prove the relationship between the studied metals in the development of the neoplasm. On the present study, the results of copper demonstrate a correlation between the smokers with cancer and lower values of copper. The groups that had smokers without cancer and non-smokers with cancer had higher values of copper. It is possible that even after the diagnosis of cancer, some smokers do not stop the habit because of the lower levels of copper circulating which might be correlated with the absence of inflammatory or respiratory tract.

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Compliance with ethical standards

The present work was approved by the ethics committee of ISCMSP (Protocol number 147/10) and complies with the international guidelines of human use on sciences. All the participants agreed to participate in the research and signed an Informed Consent Term (ICT).

Conflict of interest The authors declare that they have no competing interests.

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