Universidade Estadual Paulista “Júlio de Mesquita Filho”
Faculdade de Odontologia de Araçatuba (FOA – UNESP)
Programa de Pós-Graduação em Odontologia
Área de Concentração: Estomatologia

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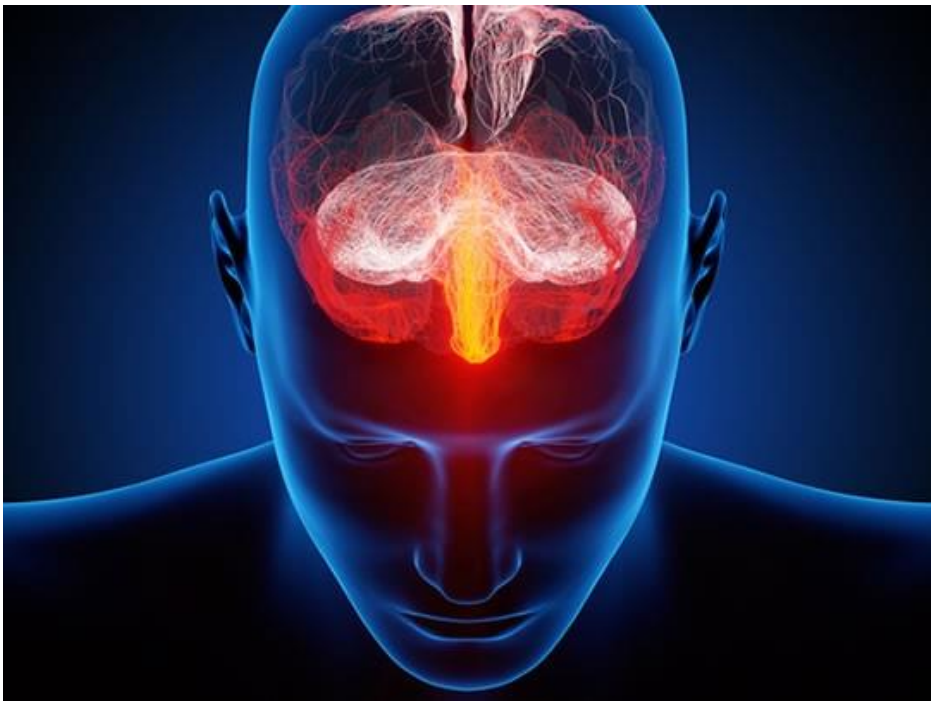

PLASMA CATECHOLAMINES LEVELS IN ORAL AND OROPHARYNGEAL CANCER PATIENTS AND THEIR ASSOCIATIONS WITH CLINICOPATHOLOGICAL VARIABLES AND ANXIETY SYMPTOMS

Araçatuba – São Paulo

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Dissertação apresentada à Faculdade de Odontologia do Campus de Araçatuba – Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, para obtenção do Título de MESTRE EM ODONTOLOGIA (Área de concentração em Estomatologia).

Orientador: Prof. Ass. Dr. Daniel Galera Bernabé

Coorientador: Prof. Tit. Glauco Issamu Miyahara

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Dedicatória

Eu dedico esta conquista às pessoas mais importantes da minha vida. Aqueles que fazem parte da minha história: ***minha família!***

"Família é onde a nossa história começa."

(Autor desconhecido)

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"Feliz é o homem que persevera na provação, porque depois de aprovado receberá a coroa da vida que Deus prometeu aos que o amam."

(Tiago 1:12)

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Sem dedicação, não há vitória, sem sacrifício, não há recompensa. A Dor é passageira, mas a Glória é eterna!

(Guilherme Antunes de Souza)

Resumo

Bastos, D.B. **Níveis plasmáticos de catecolaminas em pacientes com câncer de boca e orofaringe e sua correlação com as variáveis clinicopatológicas e sintomas de ansiedade.** [Dissertação]. Araçatuba: Faculdade de Odontologia da Universidade Estadual Paulista; 2017.

RESUMO

Objetivos: As catecolaminas podem regular diversos efeitos biológicos resultantes do estresse crônico. Estudos demonstram que as catecolaminas podem influenciar a progressão do câncer. No entanto, pouco se sabe sobre o perfil de secreção das catecolaminas em pacientes com câncer de cabeça e pescoço (CCP) e sua associação com as variáveis clinicopatológicas e psicológicas. O presente estudo investigou os níveis plasmáticos pré-tratamento das catecolaminas norepinefrina (NE) e epinefrina (E) em pacientes com câncer de boca e orofaringe e em pacientes com leucoplasia bucal, bem como sua associação com as variáveis clinicopatológicas, biocomportamentais e os sintomas de ansiedade. **Pacientes e métodos:** Um total de 71 pacientes com carcinoma espinocelular (CEC) de boca, 22 pacientes com CEC de orofaringe e 32 portadores de leucoplasia bucal foram submetidos à coleta de amostras de sangue. Os níveis plasmáticos das catecolaminas NE e E foram mensurados por meio de Cromatografia Líquida de Alta Eficiência com detecção eletroquímica (CLAE-ED) e os níveis psicológicos de ansiedade foram mensurados pelo Inventário de Ansiedade de Beck (IAB). As diferenças nos níveis hormonais entre os grupos foram avaliadas pelo teste ANOVA e análises univariadas e regressões múltiplas foram realizadas para avaliar as associações dos níveis hormonais com as variáveis clinicopatológicas, biocomportamentais e psicológicas. **Resultados:** As concentrações plasmáticas de NE e E foram significativamente maiores nos pacientes com câncer de boca e orofaringe em relação aos pacientes com leucoplasia bucal ($p<0,05$). Pacientes com CEC de boca mostraram níveis de NE (462.03±47.53 pg/mL) cerca de 6 vezes mais

elevados do que os pacientes com CEC de orofaringe (74.46±12.52 pg/mL) e 9 vezes maior em relação aos pacientes com leucoplasia (51.69±6.28 pg/mL). Os níveis plasmáticos de NE e E foram correlacionados positivamente nos pacientes com CEC de boca (p=0,0011), mas não nos outros dois. A análise de regressão múltipla mostrou que no grupo de pacientes com CEC de boca e no grupo composto por pacientes com CEC de boca e orofaringe juntos (grupo câncer de cabeça e pescoço - CPP), o histórico de maior consumo de álcool foi preditivo para níveis reduzidos de NE plasmática (CCP: β=-171,7, p=0,0002; CEC de boca: β=-119,2, p=0,0296). Os níveis globais de ansiedade medidos pelo IAB não foram significativamente correlacionados com os níveis de catecolaminas nos pacientes com câncer (p>0,05). Entretanto, os sintomas de ansiedade "tremor das mãos" (β=157,5; p=0,0377) e "coração acelerado" (β=15,88; p=0,0441), foram significativamente associados com níveis elevados de E no grupo de CCP e no grupo de CEC de orofaringe, respectivamente. A privação de sono e a má qualidade do sono na noite anterior à coleta de sangue foram variáveis preditivas para níveis elevados de NE em pacientes com leucoplasia bucal. O consumo excessivo de cigarro (β=1,54; p=0,0051) e níveis elevados de ansiedade (β=7,16; p=0,0003) foram preditores independentes para maiores níveis plasmáticos de E nos pacientes com leucoplasia bucal. **Conclusão:** Os pacientes com câncer de boca e orofaringe apresentam uma modulação na secreção plasmática de NE e E. Além disso, os níveis de catecolaminas nos pacientes com câncer de cabeça e pescoço e nos pacientes com leucoplasia podem ser influenciados por fatores biocomportamentais e psicológicos.

Palavras-chave: Neoplasia de cabeça e pescoço; Câncer de boca; Norepinefrina; Epinefrina; Ansiedade.

Abstract

Bastos, D.B. **Plasma catecholamines levels in oral and oropharyngeal cancer patients and their associations with clinicopathological variables and anxiety symptoms.** [Dissertation]. Araçatuba: UNESP - São Paulo State University; 2017.

ABSTRACT

Background: Catecholamines may regulate several biological effects resulting from chronic stress. Studies have shown that stress-related catecholamines may affect cancer progression. However, little is known about catecholamines secretion profile in head and neck cancer squamous cell carcinoma (HNSCC) patients and its association with clinicopathological and psychological variables. The present study investigated the pre-treatment plasma levels of catecholamines norepinephrine (NE) and epinephrine (E) in patients with oral and oropharyngeal SCC and patients with oral leukoplakia, as well as their associations with clinicopathological and biobehavior variables and anxiety symptoms. **Patients and methods:** A total of 71 patients with oral SCC, 22 patients with oropharyngeal SCC and 32 patients with oral leukoplakia were submitted to blood samples. Plasma levels of NE and E were measured by High Performance Liquid Chromatography with electrochemical detection (HPLC-ED) and psychological anxiety levels were measured by the Beck Anxiety Inventory (BAI). Differences in hormone levels among the groups were analyzed by ANOVA test. Univariate and multiple regression analyzes were performed to evaluate the associations of hormonal levels with clinicopathological, biobehavior and psychological variables. **Results:** Plasma NE and E concentrations were significantly higher in patients with oral and oropharyngeal cancer than oral leukoplakia patients ($p<0.05$). Oral SCC patients showed NE levels (462.03±47.53 pg/mL) about six times and nine times higher than patients with oropharyngeal SCC (74.46±12.52 pg/mL) and oral leukoplakia (51.69±6.28 pg/mL), respectively. Plasma NE and E levels were positively correlated in patients with oral SCC ($p=0.0011$), but not in the oropharyngeal SCC and oral

leukoplakia groups. Multiple regression analyses showed that in oral SCC group single and joined with oropharyngeal SCC patients (HNSCC group), the history of high alcohol consumption was predictive for reduced plasma NE levels (oral SCC: β=-119.2, p=0.0296; HNSCC: β=-171.7, p=0.0002). In the cancer groups, the overall anxiety score measured by BAI was not significantly correlated to catecholamines levels (p>0.05). However, anxiety symptoms measures with BAI such as “hands trembling” (β=157.5, p=0.0377) and “heart pouding/rancing” (β=15.88, p=0.0441) were significantly associated with higher plasma E levels in HNSCC and oropharyngeal SCC groups, respectively. Sleep deprivation and worse sleep quality in the previous night of blood collection were predictive variables for elevated NE levels in oral leukoplakia. In this patient group, severe tobacco consumption (β=1.54, p=0.0051) and higher anxiety levels (β=7.16, p=0.0003) were independent predictors for higher plasma E levels. **Conclusion:** Oral and oropharyngeal cancer patients display a modulation of plasma NE and E secretion. Furthermore, systemic catecholamines levels in patients with head and neck cancer and potentially malignant disorders may be influenced by biobehavior and psychological factors.

Keywords: Head and Neck Neoplasm; Oral cancer; Norepinephrine; Epinephrine; Anxiety.

Listas

°C = degree Celsius

€ = score per day of tobacco consumption

ł = score per day of alcohol consumption

% = percentage

¥ = Charlson Comorbidity Index numerical score

‡ = values are considered statistically significant at $p<0.05$

β = parameter estimate

µl = microliter (unit of measurement equivalent to 10^{-6} l)

ρg/mL = picogram per milliliter

Al_2O_5 = aluminium oxide

AM = ante meridiem

ANOVA = analyses of variance

ANS = autonomic nervous system

BAI = Beck Anxiety Inventory

CCI = Charlson Comorbidity Index

Ch = chemotherapy

CLAE-ED = de Cromatografia Líquida de Alta Eficiência com detecção eletroquímica

CNS = central nervous system

DHBA = internal standard, dihydroxybenzylamine

E = epinephrine

EDTA = ethylenediamine tetraacetic acid

eg. = for example

FAPESP = São Paulo State Research Foundation

g = gram

HNSCC = head and neck squamous cell carcinoma

HPA = hypothalamic pituitary adrenal

HPLC-ED = high performance liquid chromatography with electrochemical detection

IL-6 = interleukin-6

mg = milligram (unit of measurement equivalent to 10^{-3} g)

min = minute

mL = milliliter (unit of measurement equivalent to 10^{-3} l)

moles/L = moles per liter

NE = norepinephrine

ng = nanogram (unit of measurement equivalent to 10^{-9} g)

NK = natural killer

OSCC = oral squamous cell carcinoma

p = p-value

pH = hydrogen potential

rpm = rotations per minute

RT = radiotherapy

SAS = Statistical Analysis System

SCC = squamous cell carcinoma

SE = Standard error

SEM = structural equation modeling

SNS = sympathetic nervous system

SP = São Paulo

Sr = surgery

TNM = classification of malignant tumors

UNESP = São Paulo State University

v = volume

V = volts

VEGF = vascular endothelial growth factor

WHO = World Health Organization

Sumário

SUMÁRIO

Plasma catecholamines levels in oral and oropharyngeal cancer patients and their correlation with clinicopathological variables and anxiety symptoms

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Brief running title: Norepinephrine and epinephrine levels in head and neck patients.

Keywords: Head and Neck Neoplasm; Oral cancer; Norepinephrine; Epinephrine; Anxiety.

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*Normalização segundo a revista Head & Neck (ANEXO B)

Plasma catecholamines levels in oral and oropharyngeal cancer patients and their associations with clinicopathological variables and anxiety symptoms.

ABSTRACT

Background: Catecholamines may regulate several biological effects resulting from chronic stress. Studies have shown that stress-related catecholamines may affect cancer progression. However, little is known about catecholamines secretion profile in head and neck cancer squamous cell carcinoma (HNSCC) patients and its association with clinicopathological and psychological variables. The present study investigated the pre-treatment plasma levels of catecholamines norepinephrine (NE) and epinephrine (E) in patients with oral and oropharyngeal SCC and patients with oral leukoplakia, as well as their associations with clinicopathological and biobehavior variables and anxiety symptoms. **Patients and methods:** A total of 71 patients with oral SCC, 22 patients with oropharyngeal SCC and 32 patients with oral leukoplakia were submitted to blood samples. Plasma levels of NE and E were measured by High Performance Liquid Chromatography with electrochemical detection (HPLC-ED) and psychological anxiety levels were measured by the Beck Anxiety Inventory (BAI). Differences in hormone levels among the groups were analyzed by ANOVA test. Univariate and multiple regression analyzes were performed to evaluate the associations of hormonal levels with clinicopathological, biobehavior and psychological variables. **Results:** Plasma NE and E concentrations were significantly higher in patients with oral and oropharyngeal cancer than oral leukoplakia patients ($p<0.05$). Oral SCC patients showed NE levels (462.03±47.53 pg/mL) about six times and nine times higher than patients with oropharyngeal SCC (74.46±12.52 pg/mL) and oral leukoplakia (51.69±6.28 pg/mL), respectively. Plasma NE and E levels were positively correlated in patients with oral SCC ($p=0.0011$), but not in the oropharyngeal SCC and oral leukoplakia groups. Multiple regression analyses showed that in oral SCC group single and joined with oropharyngeal SCC patients (HNSCC group), the history of high alcohol consumption was predictive for reduced plasma NE levels (oral SCC: β=-119.2, $p=0.0296$; HNSCC: β=-171.7, $p=0.0002$). In the cancer groups,

the overall anxiety score measured by BAI was not significantly correlated to catecholamines levels ($p>0.05$). However, anxiety symptoms measures with BAI such as “hands trembling” ($\beta=157.5$, $p=0.0377$) and “heart pouding/rancing” ($\beta=15.88$, $p=0.0441$) were significantly associated with higher plasma E levels in HNSCC and oropharyngeal SCC groups, respectively. Sleep deprivation and worse sleep quality in the previous night of blood collection were predictive variables for elevated NE levels in oral leukoplakia. In this patient group, severe tobacco consumption ($\beta=1.54$, $p=0.0051$) and higher anxiety levels ($\beta=7.16$, $p=0.0003$) were independent predictors for higher plasma E levels. **Conclusion:** Oral and oropharyngeal cancer patients display a modulation of plasma NE and E secretion. Furthermore, systemic catecholamines levels in patients with head and neck cancer and potentially malignant disorders may be influenced by biobehavior and psychological factors.

Keywords: Head and Neck Neoplasm; Oral cancer; Norepinephrine; Epinephrine; Anxiety.

1. INTRODUCTION*

Despite recent advances in cancer treatment, disease's morbidity and psychological disorders remain affecting patients' quality of life.[1] In general, many patients experience wearing physical and symptoms from the time of suspected cancer, through diagnosis, treatment, and survivorship.[2,3,4] It is very common oncological patients display high levels of stress, anxiety, depression and lack of social support.[5-7] This often results in neuroendocrine changes which may influence the tumor progression.[6,8]

Central nervous system (CNS) perceptions of threat from environmental stressors when experienced chronically, lead to downstream activation of neuroendocrine pathways including hypothalamic pituitary adrenal (HPA) axis and the autonomic nervous system (ANS).[2,9] The HPA axis is governed by the hypothalamus and results in secretion of the hormone cortisol from the adrenals.[2,10,11] The sympathetic nervous system (SNS) is known for its role in the "fight-or-flight" stress response and secrets acetylcholine which activates the secretion of the catecholamines at nerve terminals by adrenal medulla.[2] SNS activation and subsequent release of catecholamines from sympathetic neurons and the adrenal medulla mediate ANS stress responses.[11] The main catecholamines involved in the stress response are epinephrine (E) and norepinephrine (NE).[9] Catecholamine levels have been found to be increased in individuals who experience acute or chronic stress and mediate ANS influences on cardiac, respiratory, vascular and other organ systems.[11,12] This ANS-mediated "macroenvironment" exerts a profound influence on the tumor microenvironment.[2,9]

*Normalização segundo a revista Head & Neck (ANEXO B)

Investigations have indicated that catecholamine derived from chronic stress and other emotional disorders like anxiety, may influence cancer progression.[11,13-15] Catecholamines may have modulatory effects on pathophysiological events which have crucial role in cancer progression, including immune impairment, angiogenesis, invasion, and modulation of inflammation.[10,12,16] For example, increased levels of NE and E can cause specific effects on the tumor-related immune response including reduction of natural killer (NK) cell numbers, the functional activity of these cells and dysregulation in the production of cytokines by lymphocytes.[13,17,18] Catecholamines derived from stress may induce increased levels of vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6) on the tumor microenvironment, molecules which can drive angiogenesis and tumor growth.[19-24] Other consequences of increasing catecholamines levels in the tumor microenvironment may be the activation of molecules associated with increased cellular migration and invasiveness (eg. metalloproteinases) of cancer cells and apoptosis inhibition mediated by anoikis.[15,22,25,26]

Clinical studies also have shown a direct association between catecholamines levels and psychological disorders in cancer patients. In a study with ovarian cancer patients, the authors showed an significant association between low subjective social support and higher intratumoral NE.[15] In other clinical investigation, plasma catecholamine levels were significantly correlated with anxiety scores in hepatocellular carcinoma patients, whereas the hormonal levels were associated with tumor differentiation.[27] Patients with head and neck squamous cell carcinoma (HNSCC) can have several psychological disorders.[28-30] *In-vitro* investigations have shown that stress-related hormones can influence

HNSCC cells behavior.[20] In previous study, for example, we have found that elevated NE levels may increase oral squamous cell carcinoma (OSCC) cells proliferation through a pathway dependent on beta-adrenergic receptors.[20] However, there are almost no studies which have measured catecholamines systemic levels in HNSCC patients and their associations with psychological and cilnicopathological variables. Xie et al.[14] found that circulating catecholamine levels were higher in oral cancer patients than those patients with benign oral tumor group and were associated with the depression and obsessive-compulsion symptoms.[14] However, it was not reported which were the histopathological diagnostics of the lesions which composed the oral cancer and oral benign tumor groups. In the present study, we have measured the pre-treatment plasma NE and E levels in patients with oral and oropharyngeal cancer and oral leukoplakia, as well their associations with clinicopathological and biobehavior variables. We also examined the relationship between catecholamine levels and anxiety symptoms in these three groups.

2. PATIENTS AND METHODS

2.1 Ethics statements

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by the human studies committee of the São Paulo State University (UNESP), School of Dentistry, Araçatuba, SP – Brazil (no. 01314/2011). Review board requirements for written informed consent were waived because all personal identifying information was removed from the dataset prior to analysis. Participants were not paid for their participation in the study.

2.2 Patients

Patients over 18 years of age with primary tumor of oral and oropharyngeal squamous cell carcinoma (SCC) and oral leukoplakia (an oral benign lesion but considered a potentially malignant disorder) were recruited at the Oral Oncology Center - São Paulo State University (UNESP), School of Dentistry, Araçatuba, SP, Brazil - between 2012 and 2016. Inclusion criteria for HNSCC groups were as follows: histopathological diagnosis of SCC without any previous treatment, primary tumor for oral SCC group to be located in the anterior tongue, floor of the mouth, gingiva, lip, hard palate, buccal mucosa, or retromolar area; and primary tumor for oropharyngeal SCC group to be located in soft palate, uvula, tonsils or base of tongue. Inclusion criteria for leukoplakia group was clinical and histopathological diagnosis of oral leukoplakia according to criteria of the World Health Organization (WHO).[31] HNSCC patients who had concomitant tumors in another organ site were excluded. Exclusion criteria for all groups were past medical history of cancer, current pregnancy, inability to perform peripheral blood collection and patients who did not consent to having the blood collected. A total of 125 patients (71 oral SCC, 22 oropharyngeal SCC and 32 oral leukoplakia) were included.

2.3 Clinicopathological and biobehaviors variables

Clinical and histopathological information were obtained from medical records. The clinical parameters evaluated were as follows: age range (0-45, 45-65, >65), gender, ethnicity, marital status, income, education, live with some relative, site of primary tumor, clinical TNM classification, clinical staging, histopathological grade, pain occurrence related to primary tumor or lesion at the moment of the

medical appointment and type of treatment. Information on preexisting comorbidity were too collected. The Charlson Comorbidity Index (CCI) was used to evaluate the comorbidity occurrence. The CCI is a widely used index and has been validated for head and neck cancer patients by Singh et al.[32] It contains 19 different medical conditions, each weighted according to its potential to impact on mortality.

Information on health behaviors such as tobacco and alcohol consumption, awareness of cancer diagnosis and hours and quality of sleep were obtained from medical records or provided by patient health report. Sleep self-report were obtained at the same day of blood collection and information such as the number of hours of sleep in the previous night of and quality of sleep experience over the last night and last week were reported. Five subscales were used to describe a quality of sleep as follows: great, good, regular, very bad and terrible.

2.4 Anxiety levels and symptoms

Psychological assessment was obtained by interview and was completed at the same day of the blood sampling was done, using Beck Anxiety Inventory (BAI). The BAI is a 4-point Likert-type scale self-report inventory developed by Beck to measure the frequency of anxiety symptoms.[33] The total score of the inventory ranges from 0 to 63. Thirteen questions evaluate physiological symptoms, 05 questions evaluate comprehension, and 03 questions evaluate somatic and comprehension symptoms.

2.5 Blood samples

All the patients were fasting on the morning before blood collection, in order to prevent the effects of changes in oral intake on hormonal levels. Blood samples were collected from HNSCC and oral leukoplakia patients in the same controlled environment, with reduced outside stress stimuli, between 08:00 and 10:00 hours AM. Peripheral blood was collected from the participants by syringe treated with EDTA to prevent clotting. Subsequently, the blood sample was centrifuged at 1500 rpm under refrigeration at 4°C for 20 minutes, and then plasma was separated and stored at −80°C until hormonal analysis was performed. In order to evaluate changes in plasma catecholamine levels during oncological follow-up of HNSCC patients, blood collections were repeated in 16 patients (15 with oral SCC and 1 with oropharyngeal SCC), days after they had been informed about cancer diagnosis; and in 6 other patients (4 with oral SCC and 2 with oropharyngeal SCC) after they had finished treatment. The data comparing the catecholamines levels pre- and post-awareness of cancer diagnosis, and pre- and post-treatment were graphically expressed as mean±SEM.

2.6 Measurement of catecholamines

Plasma catecholamines (NE and E) levels were determinate by high performance liquid chromatography using ion-pair reverse phase chromatography coupled with electrochemical detection (0.5 V) (HPLC-ED) as described by Di Marco et al.[34] and Naffah-Mazzacoratti et al.[35] The plasma volume of 1-3mL were added in 1mL Tris-buffer (pH 8.8) 2 moles/L and 40 µl (8 ng) DHBA (internal standard, dihydroxybenzylamine) plus 50 mg Al_2O_5. The suspension was vortex-mixed for 10 min and the precipitated alumina was washed three times. The

catecholamines were eluted with 400 µl perchloric acid after centrifugation for 3 min at 1,500g. The supernatant was filtered and injected into the reverse phase column in HPLC-ED. The NE and E concentrations were expressed as pg/mL.

2.7 Statistical analysis

All data were stored in Microsoft Office Excel 2013, and the statistical analysis were performed by SAS software v.9.3. Normality was tested for continuous variables using Shapiro-Wilk test. In case of normality comparisons among groups were made using ANOVA followed by multiple comparison Tukey test. Otherwise, comparisons were made using an adjustment for gamma distribution followed by multiple comparison Wald test. Chi-square and/or Fisher exact test was used to evaluate associations between groups and categorical variables (clinicopathological, biobehavior and psychological ones). Correlations between plasma NE and E levels in each group were tested using Pearson correlation test. These analyses were performed in each group separately and also in a group composed by oral and oropharyngeal SCC patients joined (HNSCC group). Multivariate regression analysis were performed via stepwise method of elimination considering the levels of plasma NE and E as a dependent variables and clinicopathological, biobehavior and psychological as explanatory. In addition, the median levels of plasma NE and E were considered and a logistic multivariate model was fitted using a stepwise method for elimination with the same explanatory variables. The results were presented in graphs as mean±SE of mean (SEM) and p value of <0.05 was considered statistically significant.

3. RESULTS

3.1 Epidemiologic and clinicopathological characteristics

One hundred and twenty five patients met inclusion criteria of whom 71 had oral SCC, 22 had oropharyngeal SCC and 32 had oral leukoplakia. The patient's clinicopathological characteristics are described in Table 1. Participants were primarily male, white and married or living with a partner. Age means were very similar for the oral SCC, oropharyngeal SCC and leukoplakia patients (57 years, 54 years and 59 years, respectively) and the most patients were in the age interval of 45 to 65 years (Table 1). The most common site of primary tumor for oral SCC patients were tongue (36.6%) and floor of the mouth (23.9%). For oropharyngeal SCC group, the most common sites were base of tongue (50%) and tonsils (22.7%).

The groups did not differ significantly with respect to age, marital status income, education, live with some relative, T classification, histopathological grade and treatment. Leukoplakia group had a higher proportion of female patients (31.2%) compared to oral SCC and oropharyngeal SCC patients (12.7% and 9.1%, respectively) (p=0.0373). Although a higher proportion of non-white patients (33.8%) have been observed in oral SCC group compared to oropharyngeal SCC (13.6%) and leukoplakia (6.3%) groups (p=0.0188), the majority of the patients from three groups had white ethnicity. The most of patients from the three groups had at least one comorbidity. Leukoplakia and oropharyngeal SCC patients (87.5% and 72.7%, respectively) had higher scores of comorbidities measured by CCI compared to oral SCC patients (52.1%) (p=0.0015). Oropharyngeal SCC patients showed higher regional metastasis occurrence (p=0.001) and advanced stage disease (p=0.0149) than oral SCC

patients. As expected, HNSCC patients reported higher pain occurrence related to primary tumor when compared to patients with oral leukoplakia (Table 1). For the oral SCC group, the majority of the patients received only surgery (46.5%), following by surgery with adjuvant radiotherapy (12.7%), only chemotherapy (12.7%) and chemotherapy with radiotherapy (12.7%). The most patients with oropharyngeal SCC were treated with only chemotherapy (27.3%), however the cancer groups did not differ significantly with respect to treatment (p=0.1289).

Table 1. Patients' epidemiological and clinicopathological characteristics.

Variables	Leukoplakia group 32 (%)	Oral SCC group 71 (%)	Oropharyngeal SCC group 22 (%)	p-Value
Age (years)				-
Mean (SD)	59.71 (8.81)	57.28 (11.69)	54.63 (7.17)	
Age range (years)				0.2984
0-45 years-old	1 (3.1%)	7 (9.9%)	1 (4.5%)	
45-65 years-old	24 (75.0%)	46 (64.8%)	19 (86.4%)	
>65 years-old	7 (21.9%)	18 (25.3%)	2 (9.1%)	
Gender				**0.0373‡**
Male	22 (68.8%)	62 (87.3%)	20 (90.9%)	
Female	10 (31.2%)	9 (12.7%)	2 (9.1%)	
Ethnicity				**0.0188‡**
White	21 (65.6%)	46 (64.8%)	19 (86.4%)	
Non-white	2 (6.3%)	24 (33.8%)	3 (13.6%)	
Unknown/Missing data	9 (28.1%)	1 (1.4%)	0	
Marital status				0.4956
Single	4 (12.5%)	20 (28.1%)	5 (22.7%)	
Married/living with a partner	19 (59.4%)	39 (55.0%)	14 (63.7%)	
Divorced/separated	6 (18.8%)	7 (9.9%)	1 (4.5%)	
Widowed	2 (6.2%)	5 (7.0%)	2 (9.1%)	
Unknown/Missing data	1 (3.1%)	0	0	
Income (R$/month)				0.3764
<1.000,00	2 (11.1%)	3 (4.3%)	1 (4.8%)	
0,00 – 1.000,00	5 (27.8%)	21 (30.0%)	4 (19.0%)	
1.000,00 – 5.000,00	7 (38.9%)	14 (20.0%)	5 (23.8%)	
>5.000,00	4 (22.2%)	32 (45.7%)	11 (52.4%)	
Education				0.7768
Illiterate	2 (6.2%)	3 (4.2%)	1 (4.5%)	
High school or less	16 (50.0%)	26 (36.6%)	10 (45.5%)	
College graduate	10 (31.3%)	38 (53.6%)	9 (41.0%)	
Postgraduate	1 (3.1%)	3 (4.2%)	1 (4.5%)	
Unknown/Missing data	3 (9.4%)	1 (1.4%)	1 (4.5%)	
Live with some relative				0.4464
Yes	5 (15.6%)	9 (12.7%)	5 (22.7%)	
No	20 (62.5%)	62 (87.3%)	17 (77.3%)	
Unknown/Missing data	7 (21.9%)	0 (0%)	0 (0%)	

CCI Score ¥				**0.0015‡**
0	4 (12.5%)	34 (47.9%)	6 (27.3%)	
1	12 (37.5%)	21 (29.6%)	11 (50.0%)	
2	4 (12.5%)	11 (15.5%)	4 (18.2%)	
3+	12 (37.5%)	5 (7.0%)	1 (4.5%)	
T – Classification				0.8393
T1	-	17 (23.9%)	3 (13.7%)	
T2	-	21 (29.6%)	7 (31.8%)	
T3	-	16 (22.6%)	7 (31.8%)	
T4	-	17 (23.9%)	5 (22.7%)	
Regional metastasis				**0.001‡**
N0	-	53 (74.7%)	8 (36.4%)	
N+	-	18 (25.3%)	14 (63.6%)	
Clinical stage				**0.0149‡**
I	-	17 (23.9%)	1 (4.6%)	
II	-	20 (28.2%)	5 (22.7%)	
III	-	15 (21.1%)	2 (9.1%)	
IV	-	19 (26.8%)	14 (63.6%)	
Histopathologic grade				0.8989
Grade I	-	11 (15.5%)	3 (13.6%)	
Grade II	-	42 (59.2%)	12 (54.5%)	
Grade III	-	2 (2.8%)	1 (4.6%)	
Unknown/Missing data	-	16 (22.5%)	6 (27.3%)	
Pain				**0.0024‡**
With pain	4 (12.5%)	35 (49.3%)	8 (36.4%)	
No pain	27 (84.4%)	36 (50.7%)	13 (59.1%)	
Unknown/Missing data	1 (3.1%)	0 (0%)	1 (4.5%)	
Treatment				0.1289
Surgery only	-	33 (46.5%)	3 (13.6%)	
Sur + RT	-	9 (12.7%)	2 (9.1%)	
RT	-	2 (2.8%)	1 (4.6%)	
Ch	-	9 (12.7%)	6 (27.3%)	
Ch + RT	-	9 (12.7%)	4 (18.2%)	
Sur + RT + Ch	-	4 (5.6%)	3 (13.6%)	
Other	-	5 (7.0%)	3 (13.6%)	

Abbreviations: SCC, Squamous cell carcinoma; CCI, Charlson Comorbidity Index; Sr, Surgery; RT, radiotherapy; Ch, Chemotherapy; STP, Second primary tumor.
¥ CCI: Each numerical score equals different medical conditions, each weighted according to its potential to impact on mortality.
‡ Values are considered statistically significant at $p<0.05$

3.2 Biobehavior and Psychological Factors

The patient's biobehavioral and psychological variables are presented in Table 2. Regarding the tobacco and alcohol consumption, patients from the oropharyngeal SCC group consumed more tobacco and alcohol than oral SCC and leukoplakia patients ($p=0.0019$ and $p=0.0014$ respectively). The mean sleep duration in the previous night of blood collection for all groups was very similar being approximately of 6 hours (Table 2). HNSCC patients showed worse sleep

quality during the previous night than leukoplakia patients (p=0.0464). Most of oral SCC (53.5%) and oropharyngeal SCC patients (68.2%) had awareness of cancer diagnosis at the moment of blood collection. In general, all patients showed lower anxiety levels measured by BAI. About eighty-seven percent of oral SCC, 85.7% of oropharyngeal SCC and 75.9% of leukoplakia patients displayed minimum and light scores of anxiety, while only 12.7%, 14.3% and 24.1%, respectively, presented moderate and severe anxiety scores. Total BAI mean score did not differ significantly among the three groups (p=0.3195).

Table 2. Patients' biobehavior and psychological characteristics.

Variables	Leukoplakia group 32 (%)	Oral SCC group 71 (%)	Oropharyngeal SCC group 22 (%)	p-Value
History of tobacco consumption €				**0.0019‡**
Non-smoker	5 (15.6%)	11 (15.5%)	1 (4.5%)	
Low	7 (21.9%)	31 (43.7%)	2 (9.1%)	
Moderate	3 (9.4%)	13 (18.3%)	6 (27.3%)	
Heavy	17 (53.1%)	16 (22.5%)	13 (59.1%)	
History of alcohol consumption ɬ				**0.0014‡**
Non-drinker	8 (25.0%)	10 (14.1%)	0 (0%)	
Low	9 (28.1%)	27 (38.0%)	4 (18.2%)	
Moderate	6 (18.8%)	16 (22.5%)	2 (9.1%)	
Heavy	9 (28.1%)	18 (25.4%)	16 (72.7%)	
Sleep duration (Mean - hours)	6.43	6.52	6.61	-
Sleep quality (previous night)				**0.0464‡**
Great	12 (37.6%)	14 (19.7%)	4 (18.2%)	
Good	17 (53.1%)	32 (45.1%)	10 (45.4%)	
Regular	2 (6.2%)	17 (23.9%)	2 (9.1%)	
Very bad	0 (0%)	5 (7.1%)	4 (18.2%)	
Terrible	1 (3.1%)	3 (4.2%)	2 (9.1%)	
Awareness of cancer diagnosis				0.2249
Yes	-	38 (53.5%)	15 (68.2%)	
No	-	33 (46.5%)	7 (31.8%)	
Beck Anxiety Inventory (BAI)				0.3195
Mean (SD)	11.55 (12.94)	8.35 (8.21)	10.95 (8.05)	
Minimum	20 (69.0%)	51 (71.8%)	12 (57.1%)	
Light	2 (6.9%)	11 (15.5%)	6 (28.6%)	
Moderate	5 (17.2%)	7 (9.9%)	3 (14.3%)	
Severe	2 (6.9%)	2 (2.8%)	0 (0%)	

€ Smoking: each category equals (per day) 10 cigarettes (manufactured, paper), two cigarettes (manufactured, straw), or two cigars or pipes of tobacco smoked.

ɬ Alcoholism: each category equals (per day) two doses of distilled alcohol, two bottles of beer, or two glasses of wine consumed.

‡ Values are considered statistically significant at p<0.05

3.3 Catecholamines

The mean plasma NE concentration of oral SCC patients, was approximately six times higher (462.03±47.53 pg/mL) than oropharyngeal SCC patients (74.46±12.52 pg/mL; p<0.0001), and almost nine times higher than leukoplakia patients (51.69±6.28 pg/mL; *p*<0.0001) (Figure 1A). Oropharyngeal patients showed higher plasma NE levels than those with leukoplakia (p=0.0382) (Figure 1A). The pattern of differences in plasma E levels among the groups was similar to that found with plasma NE levels. Plasma E levels in the oral SCC group were higher (49.94±4.10 pg/mL) compared to oropharyngeal SCC (31.39±4.51 pg/mL; p=0.0097) and leukoplakia (23.02±2.59 pg/mL; p<0.0001) groups (Figure 1B). In respect to oropharyngeal SCC patients, we also observed that patients had higher plasma E levels than patients with oral leukoplakia (p=0.0452) (Figure 1B). Plasma NE levels were correlated positively with E levels in oral SCC patients (p=0.0011), but not in oropharyngeal SCC and leukoplakia patients (p>0.05) (Figure 2).

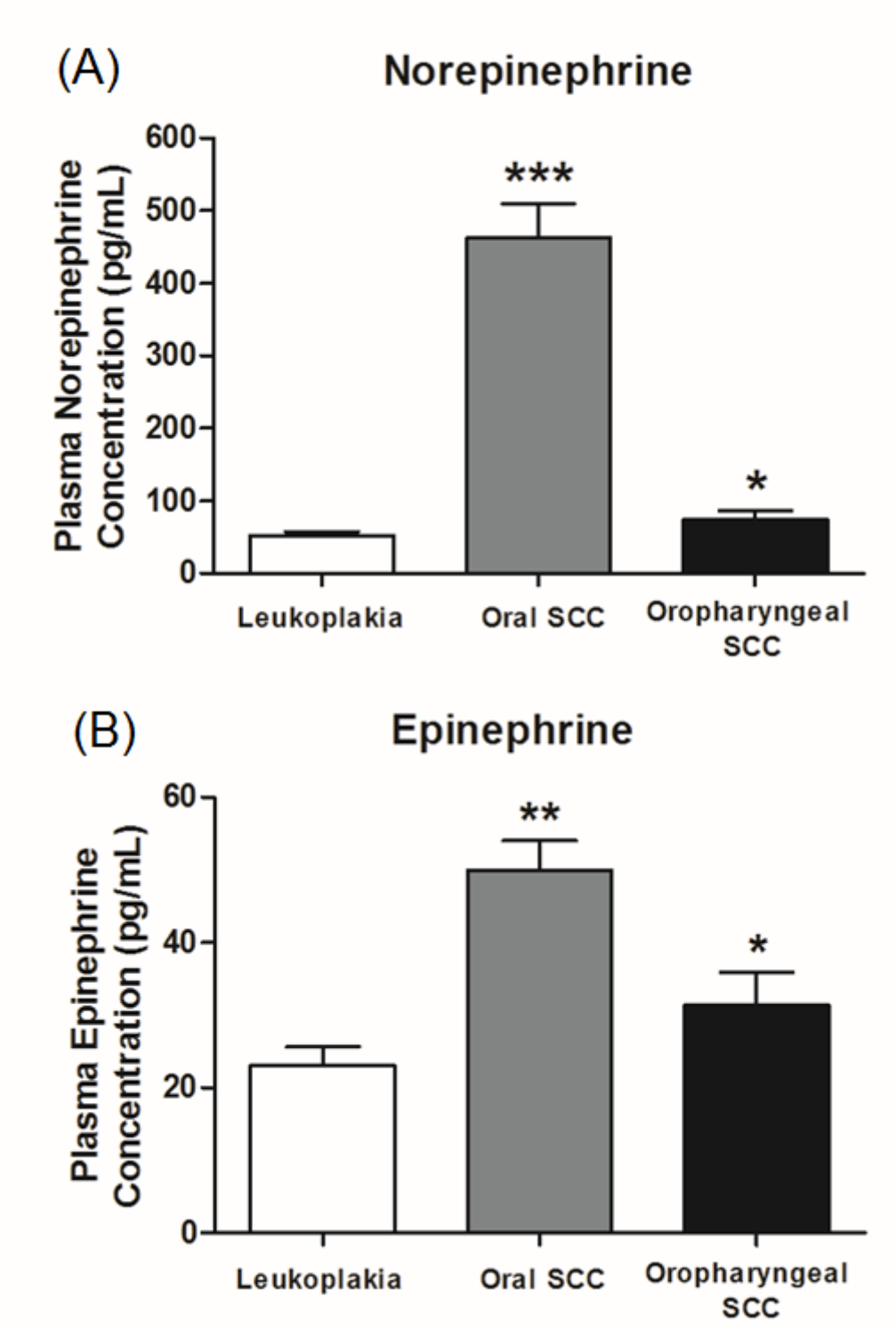

Figure 1. Plasma catecholamine concentrations: Plasma norepinephrine (A) and epinephrine (B) levels from oral leukoplakia (n=32), oral SCC (n=71) and oropharyngeal SCC patients (n=22) were measured by HPLC. Results are expressed as mean±SEM. ***p ≤0.0001: plasma NE levels from oral SCC group compared to oropharyngeal SCC and leukoplakia group; **p ≤0.001: plasma E levels from oral SCC group compared to oropharyngeal SCC and leukoplakia group; *p ≤0.05: plasma NE and E levels from oropharyngeal SCC group compared to leukoplakia group.

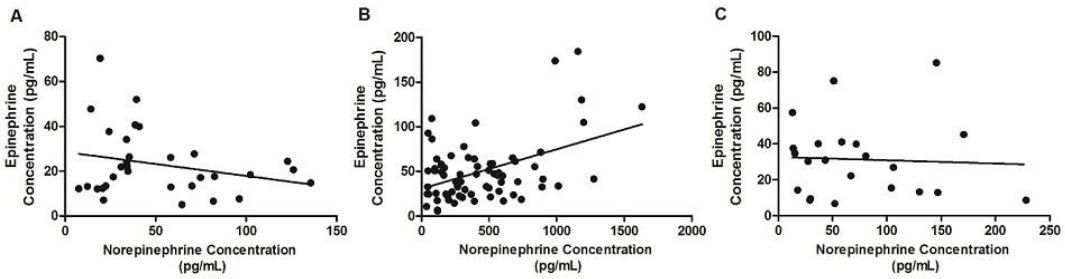

Figure 2. Correlation between plasma concentrations of norepinephrine and epinephrine. Correlations between plasma NE and E levels in oral leukoplakia (A), oral SCC patients (B) and oropharyngeal SCC patients (C) were analyzed using Pearson correlation test. Plasma NE levels were positively correlated with E levels in oral SCC patients (p=0.0011).

3.4 Associations between plasma catecholamine levels and clinicopathological variables

To investigate the association between plasma NE and E levels and clinicopathological variables, oral and oropharyngeal SCC patients sample were explored joined (HNSCC group) and separately. Univariate analysis revealed no associations between plasma catecholamine levels and clinicopathological variables in HNSCC group. However, patients with advanced stage (stage III and IV) showed a trend towards lower levels of plasma NE (p=0.0662). When HNSCC groups were analyzed separately, we found a significant association between plasma NE levels and income in oral SCC group (p=0.0217). Oral SCC patients who had a higher socioeconomic status showed higher plasma NE levels. There was a significant association between the patients' ethnicity and E levels in oropharyngeal SCC group. White patient showed higher E levels than non-white patients (p=0.0389). Oropharyngeal SCC patients with smaller tumors (T1 and T2) had higher E levels compared to patients with larger tumors (T3 and T4) (p=0.0156). Based on CCI score, univariate analysis also showed that leukoplakia patients who had no occurrence of comorbidities, had higher plasma

E levels than patients with one or more comorbidities (p=0.0031). Conversely to what was found in oral SCC group, leukoplakia patients who had a poor socioeconomic status had higher E levels compared to patients with better financial condition (p=0.0379).

Table 3 shows data from the multivariate analyze. When the oral and oropharyngeal group were analyzed together (HNSCC group), multivariate analysis showed no significant associations between the plasma NE and E and clinicopathological variables. Analyzing the groups separately, plasma catecholamines also were unrelated to any clinicopathological variables. Multivariate regression analysis showed that live with some relative (β=-4.44, p=0.0015) was an independent variable associated with higher plasma E levels in leukoplakia group (Table 3).

Table 3. Multiple regression coefficients of biobehavioral and psychological variables and plasma catecholamines measures for leukoplakia group.

Variables	**Leukoplakia group**		
	β	SE	p-Value
Plasma Norepinephrine			
Sleep Duration	-9.70	1.78	0.0029
Sleep Quality	69.53	9.66	0.0008
Plasma Epinephrine			
Live with some relative	-4.44	0.57	0.0015
Tobacco consumption	1.54	0.27	0.0051
BAI Score	7.16	0.61	0.0003

Abbreviations: SE, Standard error; BAI, Beck Anxiety Inventory.
β: Parameter estimate
All values are considered statistically significant at p<0.05

3.5 Associations among plasma catecholamine levels and biobehavioral variables and anxiety status

The results showed that HNSCC patients who had history of higher tobacco (p=0.0135) and alcohol consumption (p=0.001) showed lower plasma NE levels compared to patients who consumed these addictive substances in lower intensity. In univariate analysis, global anxiety scores measured by BAI symptom were not significantly associated with plasma NE and E levels in none of the groups. Then, we investigated the associations of each BAI subscales with the NE and E levels. The results showed that NE levels endorsed more negative symptomatology relative as difficulty in breathing to the HNSCC group as measured by the BAI (p=0.0366). When analyzing the groups separately, we also observed positive associations between this symptomatology with NE levels in oral SCC patients (p=0.0383) and with E levels in oropharyngeal SCC patients (p=0.026). Therefore, no association was observed between these symptoms and catecholamine levels in leukoplakia patients. (p>0.05).

Table 4 shows data from the multivariate analyze. Multivariate analysis showed that higher alcohol consumption was independent predictor for lower NE levels (β=-171.7, p=0.0002) in HNSCC group (Table 4). A similar result was found when oral SCC group was analyzed singly. Oral SCC moderate and severe drinkers displayed lower plasma NE than light drinkers (β=-119.2, p=0.0296) (Table 4). For leukoplakia group, multiple regression model analysis showed that fewer hours of sleep and worse sleep quality at the previous night were independent predictors for higher plasma NE (β=-9.70, p=0.0029; β=69.53, p=0.0008, respectively) (Table 3). Sleep quality during the last week was not correlated with catecholamine levels in any group. Severe tobacco consumption

(β=1.54, p=0.0051) and higher anxiety levels (β=7.16, p=0.0003) were independent predictors for higher plasma E levels (Table 3). When analyzing separately each subscales symptom measured by BAI, the results showed that "hands trembling" symptom (β=157.5, p=0.0377) in HNSCC group was an independent predictor for higher plasma NE levels (Table 4). In oropharyngeal SCC group, "heart pounding/racing" symptom independently predicted higher plasma E levels (β=15.8, p=0.0441) (Table 4).

Table 4. Multiple regression coefficients of patients' biobehavior and psychological variables and plasma catecholamine levels for cancer groups.

Variables	**HNSCC group**			**Oral SCC group**			**Oropharyngeal SCC group**		
	β	SE	p-Value	β	SE	p-Value	β	SE	p-Value
Plasma Norepinephrine									
Alcohol consumption	-171.7	44.1	0.0002	-119.2	53.2	0.0296	-	-	-
Plasma Epinephrine									
Hands trembling	157.5	74.2	0.0377	-	-	-	-	-	-
Heart pounding / Racing	-	-	-	-	-	-	15.8	6.9	0.0441

Abbreviations: SE, Standard error.
β: Parameter estimate
All values are considered statistically significant at p<0.05

3.6 Catecholamines levels in oncological follow-up

To assess changes in plasma catecholamines levels during oncological follow-up, we repeated the measurements of plasma NE and E levels of some HNSCC patients after awareness of cancer diagnosis and after treatment. There were no significant differences in plasma NE and E levels between pre- (NE: 482.4±88.74 pg/mL; E: 63.94±13.47 pg/mL) and post-awareness of cancer diagnosis (NE: 541.1±123 pg/mL; E: 51.13±9.12 pg/mL) ($p>0.05$) (Figures 3A and 3B). Some HNSCC patients had higher NE and E concentrations after awareness of cancer diagnosis, while in others the hormonal levels decreased after being aware of disease diagnosis. HNSCC patients had lower plasma levels of both NE (110.7±37.87 pg/mL) and E (24.11±3.4pg/mL) in post-treatment compared to basal pre-treatment phase (NE: 234.7±105.7 pg/mL; E: 36.12±14.56 pg/mL), but these results did not reach statistical significance ($p>0.05$) (Figures 3C and 3D).

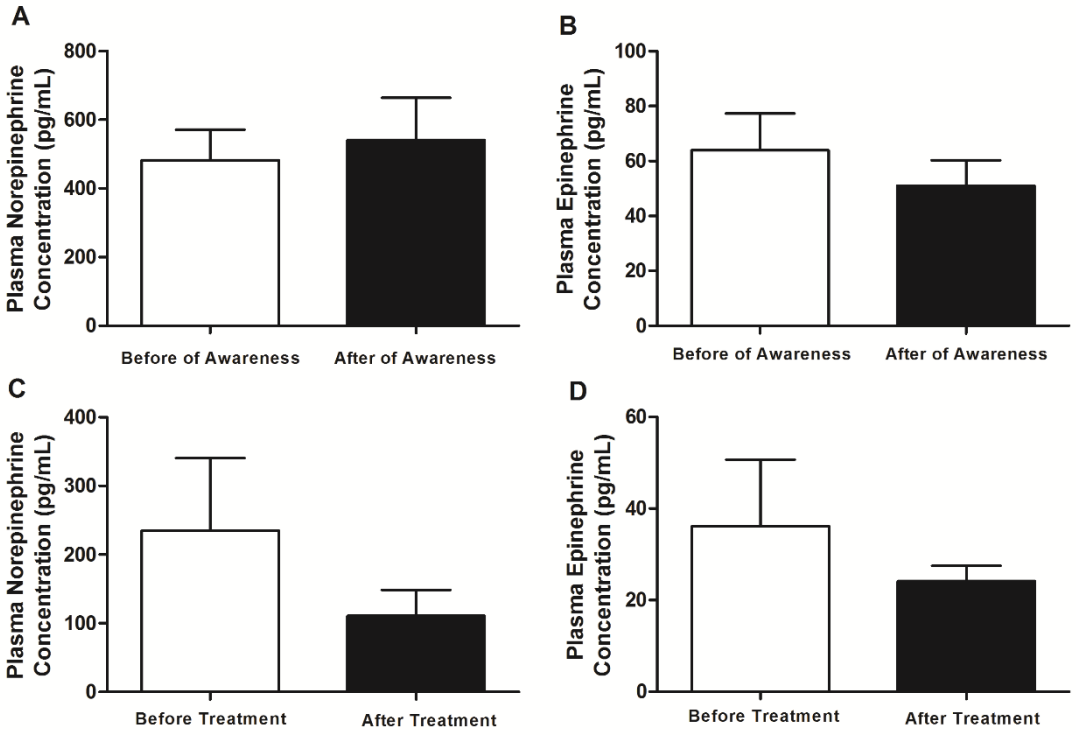

Figure 3. Plasma catecholamine levels in HNSCC patients at different phases of oncological treatment. Plasma Norepinephrine (A) and Epinephrine (B) levels from sixteen HNSCC patients before and after they had been informed about cancer diagnosis. Six HNSCC patients were also investigated for NE (C) and E (D) levels before and after cancer treatment. Results are expressed as mean±SEM. There were no significant differences in plasma NE and E levels considering pre- and post periods in the both conditions ($p>0.05$).

4. DISCUSSION

A growing number of clinical and pre-clinical studies have demonstrated that hormonal alterations resulting from chronic stress and other behavioral conditions may influence cancer progression.[20,22,23,24,26,36] Among the stress-related hormones, catecholamines, such as NE and E, have played a central role in the relationship between stress-related biological events and cancer progression. There are a solid set of evidences from *in-vitro* and pre-clinical investigations showing that, increased catecholamines levels in blood and tumor may stimulate cancer cells proliferation and invasion, angiogenesis, apoptosis inhibition and immune response impairment against the tumor.[20,22,23,24,26,36] However, few clinical studies have explored the catecholamine levels in cancer patients and their impact on disease prognosis, as well as which variables would affect hormonal secretion. The present study investigated plasma catecholamine levels in oral and oropharyngeal cancer patients before treatment and their association with clinicopathological variables and anxiety symptoms. We also evaluated plasma catecholamine levels in patients with oral leukoplakia, a benign lesion potentially malignant. Our results showed that patients with oral and oropharyngeal SCC exhibited plasma catecholamine (NE and E) levels significantly higher than leukoplakia patients. Interesting, highest difference peak was observed for NE concentration in oral SCC patients, who displayed nine times higher hormonal levels than leukoplakia patients. Some other investigations have reported increasing rates of systemic catecholamine compared non-cancer individuals. Drott et al.[37] showed that urine excretion of E was significantly increased in a small sample of cancer patients suffering from malnutrition compared with control patients equally malnourished. Similarly to our

finding, in the only previous study analyzing plasma catecholamine levels in HNSCC patients, Xie et al.[14] also found that oral cancer patients had higher plasma NE and E levels compared to patients with oral benign tumor.[14] However, in this study, only forty oral cancer patients were included and the authors did not specify which were the clinical and histopathological diagnoses of lesions made up the oral benign tumor and cancer groups. On the other hand, two studies, one with breast cancer patients[38] and other exploring patients with different types of cancer,[39] could not find differences of urinary catecholamine levels when cancer patients were compared to those observed in health controls. There are many factors that may interfere with measurement of catecholamine levels in cancer patients and make it difficult to compare the results from clinical studies, such as the type of sample and its method of collection and storage, the method used for hormone determination, the sample size and clinicopathological profile of cancer and control groups, and the stage of oncological follow-up which the patients were accessed.[40,41] Surprisingly, our results showed that the mean concentrations of plasma catecholamines in oral SCC patients were higher than those found in oropharyngeal SCC patients. This difference were also more expressive for plasma NE, whose levels in oral SCC patients were approximately six times higher compared to oropharyngeal SCC patients. It is not easy to explain the hormonal differences found between two HNSCC groups. In a way, oral and oropharyngeal tumors exhibit relatively similar characteristics, including common risk factors such as smoking and alcoholism. In our sample, there were no significant differences between the two HNSCC groups for most of the clinicopathological and biobehavior variables. However, oropharyngeal SCC patients displayed at the time of diagnosis a higher occurrence of regional

metastasis and history of increased consume of tobacco and alcohol. Although our findings have shown increased alcohol consumption as the main independent variable associated with lower NE levels in HNSCC patients, it is presumptuous to point out this variable as the only one responsible for the lower NE levels observed in oropharyngeal cancer patients compared to oral cancer patients. Due to small sample of oropharyngeal SCC patients, other studies with a larger sample and exploring other influencing variables are required.

When the multivariate analysis was performed to evaluate which variables had a predictive value to influence plasma catecholamine levels, our results showed that higher alcohol consumption was an independent factor for lower plasma NE levels in HNSCC group (oral and oropharyngeal SCC groups joined) and oral SCC group single. Moderate and severe drinkers displayed lower plasma NE than light drinkers. Few studies have explored the association between alcohol consumption and catecholamine levels in cancer patients. In two of these studies[14,15], one with ovarian cancer patients and another with oral cancer patients, no significant associations were found between alcohol consumption and plasma NE and E levels, although in both studies detailed alcohol consumption profile did not been reported. Investigations in humans and animals have shown that alcohol consumption can be associated with increased levels of catecholamine secretion.[42-45] Parlesak et al.[45] found that patients with alcoholic liver disease had increased urinary and plasma NE and E compared to healthy patients. Interestingly, the authors have found a reversibility of increased urinary NE levels in alcoholic patients after two weeks of abstinence. In an experimental study, Kovács et al.[46] observed that ethanol administration in animals promotes prompt increase in plasma NE and E and hormonal levels tend

to also increase during tolerance/dependence phase. In our study, blood collections from HNSCC patients were performed before or shortly after the disease diagnosis. Information regarding alcohol consumption were obtained from the medical records. This information reports the history of alcohol consumption throughout the patient's life and it does not take into account the last days or weeks before blood collection. Then, can not be ruled out that in the days prior to blood collection, the patients were consuming alcohol in a different way from reported in the medical interview. We hypothesize that due to the tumor-related symptoms, the alcohol and tobacco consumption may decrease or even cease. Therefore, it is reasonable to think that a part of our alcohol-dependent patients could be in acute or chronic abstinence from alcoholism, which would have direct impact on catecholamines secretion. Investigations have reported evidences of those abstinent alcohol-dependent patients can display a significant reduction in the plasma levels of NE or its metabolites compared to actively drinking individuals.[42,47,48] Ehrenreich et al.[47], for example, found lower plasma NE levels and a significantly decreased NE/E ratio in abstinent alcoholics than healthy controls. These findings reinforce the importance of cross-sectional studies with cancer patients using retrospective data, updating their clinicopathological and biobehavior data up to the time of blood collection.

In the present study, univariate analysis showed that history of higher tobacco consumption and advanced clinical staging were associated with lower NE levels in HNSCC patients, although these results have not remained as independent variables in the multiple regression model. In a study evaluating the response to vagal stimulation in duodenal ulcer patients when smoking versus non-smoking, Lindell et al.[49] found that plasma concentrations of NE decreased

during the smoking of a single cigarette, whereas those of E were increased on smoking days. However, in general, clinical studies with non-cancer smokers have demonstrated that smoking leads to increased systemic levels of NE and E, which are strongly reduced after smoking withdrawal.[50-52] Regarding clinical staging, in a previous study we have found increased plasma and salivary levels of other stress-related hormone (cortisol) in SCC oral patients compared to controls and the hormonal levels were associated with advanced clinical staging.[53] In the present study, using a sample of oral cancer patient with similar profile to that, advanced clinical staging tended to be correlated with lower NE levels. These findings may suggest a different endocrine response for cortisol and NE in HNSCC patients with advanced clinical staging. Two different studies with ovarian cancer patients, did not find significant associations between disease's stage and plasma catecholamine levels.[15,43] Interesting, in one of these studies, Lutgendorf et al.[15] found higher intratumoral NE levels in advanced stage and higher grade tumors. Already Xie et al.[14] and Liu et al.[27] exploring patients with oral and hepatocellular carcinoma, respectively, found associations between increased peripheral blood catecholamines levels and advanced clinical staging. These results indicate a dysfunction of the ANS in cancer patients with advanced clinical staging. For example, cancer patients may have nutritional deficits, whereas cancer-induced cachexia has been associated with modulation of the SNS.[37,39] Chauhan et al.[39] reported a case series which advanced cancer cachectic patients displayed reduced sympathetic and parasympathetic responses demonstrated by the overall decrease in heart rate variability, although urinary NE and E levels were not different when compared to healthy subjects.

Some clinical investigation with cancer and healthy patients have shown an association between systemic catecholamines levels and psychological disorders.[4,14,54] In the present study, we evaluated the anxiety levels in patients with oral and oropharyngeal cancer through the BAI questionnaire. Our hypothesis was that anxiety status could be correlated with plasma NE and E levels. However, only 14% of the total sample of oral and oropharyngeal patients had high levels of anxiety, classified as moderate and severe degree. In addition, no differences were found in overall anxiety levels between patients with oral and oropharyngeal cancer and patients with oral leukoplakia. Our results showed no significant association between the anxiety scores on the BAI and plasma catecholamines levels. These findings are consistent with some studies, which did not identify significant association between catecholamines levels and global anxiety measures in cancer patients.[15,38,43] However, a recent study, Liu et al., found a significant positive correlation between anxiety scores and catecholamine levels in a sample of patients with liver cancer.[27] The low occurrence of high global anxiety levels in our HNSCC sample may have disrupted the intersection of hormonal and psychological data. Multiple regression analysis showed that BAI subscale of “hands trembling” in HSNCC group and “heart pounding/racing” in oropharyngeal SCC group were independent predictors for plasma NE and E levels, respectively. Although we have not analyzed which clinicopathological variables could have affected anxiety scores, and whether catecholamines levels were influencing factors for anxiety symptoms in HNSCC patients, our findings may suggest a possible relationship of anxiety symptoms and alcohol and tobacco consumption and withdrawal in these patients. Anxiety-related symptoms, such as shakiness and heart racing,

may predict larger abstinence-induced increases in fatigue and depressive affect.[55] Others findings corroborate with our results and have shown associations between psychological subscales measures and catecholamine levels.[4,14] Besides that, other symptoms or psychological disorders could be correlated with modulation of catecholamine secretion in head and neck cancer patients. Depressive symptoms, mood changes, distress, social isolation, and fatigue have been shown to be possibly influenced by the SNS in cancer patients.[4,14,15,54] Our results also did not show a correlation between the awareness of cancer diagnosis and catecholamines levels. Even when we have compared NE and E levels between pre- and post-awareness, hormone levels were not significantly changed. When assessed individually, some HNSCC patients had increased catecholamines levels after being aware of the disease's diagnosis, while others showed a decrease in the hormonal levels.

In contrast to observed in multiple regression models for oral and oropharyngeal SCC groups, different independent variables predicting NE and E levels remain in the final model for oral leukoplakia group. This fact may be due the absence of some variables (eg. clinical staging) and the lower intensity of some relevant influencing factors for catecholamine levels such as tobacco and alcohol in leukoplakia group. Epinephrine levels in oral leukoplakia group were positively associated with tobacco consumption and to live with some relative. Moreover, fewer hours of sleep and worse sleep quality at the previous night of blood collection were independent predictors for higher plasma NE. This is the first study exploring the relationship between sleep disturbances and catecholamine levels in HNSCC and oral leukoplakia patients. Sleep disruption and privation have been reported in cancer patients.[38,56,57] Carlson et al.[38]

showed that breast cancer patients had worse sleep quality compared to healthy controls, however sleep markers were not correlated with urinary NE and E levels. Other study also did not find association between plasma catecholamines and sleep deprivation in women with ovarian cancer.[15] In our findings, worst sleep quality and sleep deprivation in the night before the blood collection were the only predicted biobehavioral variables for NE levels in leukoplakia, but they were not associated with hormonal concentrations in HNSCC groups. Sleep disturbance has been linked to deregulated catecholamine secretion in non-cancer individuals. For example, Mezick et al.[58] observed higher variability in sleep measures among healthy patients with both high NE levels and high negative affect and anxiety. Poor sleep continuity has been also related to systemic increased NE levels in individuals under chronic stress.[59,60] Physiological stress, as indexed by heightened nocturnal activity of the sympatho-adrenal medullary system, leads to unstable and deprivation sleep patterns.[59,60] In our findings, multiple regression analysis defined higher anxiety scores as an independent psychological variable for E levels in leukoplakia group. The fact that both sleep disturbance and elevated anxiety raises had been associated with systemic catecholamine levels in oral leukoplakia patients group suggest that catecholamines could have a relevant role in bidirectional pathways involving psychological factors and sleep patterns. Prospective cohort studies using appropriate tests for sleeping behavior, associated with stress hormones measurements in a larger sample of oral leukoplakia can bring a new understanding regarding impact of biobehavioral factors and SNS disruption on the oral SCC onset and progression.

In the present study, oral and oropharyngeal cancer patients showed increased plasma NE and E levels compared to non-cancer patients. Moreover, we have indentified history of alcohol consumption and anxiety symptoms as the main variables influencing the hormonal levels in HNSCC patients. These findings suggest that HNSCC patients display a modulation of SNS, what could have relevant impact on disease progression. Although intratumoral catecholamines concentrations have not been measured, elevated hormonal levels in peripheral tissues including tumor microenvironment may reflect those found in the blood. In our hypothesis, increased concentrations of NE and/or E in the tumor microenvironment could induce tumor progression through adrenergic receptors activation impacting HNSCC prognosis. Liu et al.[27] showed that plasma catecholamine levels were independent prognostic predictors of overall survival and tumor reccurrence in hepatocelullar carcinoma patients. In our study, we were not able to assess whether the plasma NE and E levels had effects on prognostic factors of HNSCC survival due to the patient's short follow-up time period. Thus, we reinforce the idea of evaluating post-treatment catecholamine levels in a larger patient sample and investigating the impact of these hormones on the HNSCC prognosis. The present study is the first to examine the plasma catecholamines levels and their association with clinicopathological variables and anxiety symptoms in oral and oropharyngeal SCC. Our findings have potential implications for clinical care in head and neck patients, whereas biobehavioral and psychological variables are able to influence catecholamine levels, which have been implicated in tumor progression.

CONFLICTS OF INTEREST

All authors declare they have no conflicts of interest.

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Anexos

ANEXO A – Certificado de aprovação do Comitê de Ética em Pesquisa (CEP)

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PROC. 1314/2011
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"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

COMITÊ DE ÉTICA EM PESQUISA

CERTIFICADO

Certificamos que o Projeto ***"Avaliação da correlação entre fatores psicológicos e mediadores relacionados ao estresse em pacientes com lesões benignas e malignas de cabeça e pescoço e sua influência sobre a progressão da doença e tratamento"***, sob a responsabilidade do Pesquisador **DANIEL GALERA BERNABÉ**, está de acordo com os Princípios Éticos em Pesquisa e foi aprovado por este Comitê, conforme o Processo FOA-01314/2011.

Araçatuba, 19 de dezembro de 2011.

ANA CLÁUDIA DE MELO STEVANATO NAKAMUNE
Coordenadora do CEP

ACMSN/wbm.

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ANEXO B – Normas para publicação

Head & Neck

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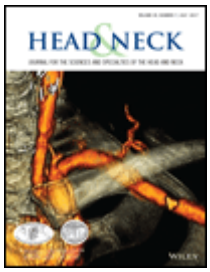

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