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DYSREGULATION IN THE SECRETION OF THE STRESS-RELATED CATECHOLAMINES IN ORAL CANCER PATIENTS: PREDICTORS AND PROGNOSTIC VALUE

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2022

DANIELA BRITO BASTOS

DYSREGULATION IN THE SECRETION OF THE STRESS-RELATED CATECHOLAMINES IN ORAL CANCER PATIENTS: PREDICTORS AND PROGNOSTIC VALUE

Tese apresentada à Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do título de "Doutora em Odontologia" - Área de Concentração Estomatologia.

Orientador: Prof. Ass. Dr. Daniel Galera Bernabé

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Dedicatória

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“Deus me dê Serenidade para aceitar as coisas
que não posso mudar, Coragem para mudar
aquilo de que sou capaz e Sabedoria
para ver a diferença.”

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"Não deixe de fazer algo que gosta,
devido à falta de tempo,
pois a única falta que terá,
será desse tempo que
infelizmente não voltará mais".
(Mário Quintana)

Epígrafe

“...Nobody said it was easy

No one ever said it would be this hard...”

(The Scientist – Coldplay)

Resumo

Bastos DB. **Desregulação da secreção das catecolaminas relacionadas ao estresse em pacientes com câncer de boca: valor preditivo e prognóstico** [tese]. Araçatuba: Faculdade de Odontologia da Universidade Estadual Paulista; 2022.

RESUMO

Introdução: Pacientes com câncer podem apresentar uma hiperativação do Sistema Nervoso Simpático (SNS). Como consequência, uma desregulação na secreção das catecolaminas relacionadas ao estresse norepinefrina (NE) e epinefrina (EPI) têm sido implicadas na progressão de alguns tipos de tumores malignos. No entanto, a associação entre os níveis sistêmicos das catecolaminas e o prognóstico do câncer, bem como seus fatores preditores são pobremente conhecidos. Pacientes e métodos: O presente estudo investigou os níveis plasmáticos de NE e EPI em 168 pacientes com carcinoma espinocelular (CEC) de boca, 70 pacientes com leucoplasia bucal e 54 voluntários saudáveis; bem como sua associação com variáveis demográficas, clínicopatológicas, biocomportamentais e psicológicas nos pacientes oncológicos. Os níveis plasmáticos de NE e EPI foram medidos por cromatografia líquida de alta eficiência com detecção eletroquímica (CLAE-ED) e o estado psicológico de humor foi avaliado pela Escala de Humor de Brunel (BRUMS). Análise de Kaplan-Meier e regressão de Cox foram usadas para investigar as associações entre os níveis de NE e EPI e a sobrevida global e específica após 5 anos de follow-up em 82 pacientes com câncer de boca. Os níveis sistêmicos das catecolaminas também foram avaliados em 32 pacientes após o tratamento da doença e comparados com os níveis pré-tratamento. Resultados: As concentrações plasmáticas de NE e EPI foram significativamente maiores em pacientes com CEC de boca em comparação aos pacientes com leucoplasia bucal ($p<0,0001$ e $p=0,024$, respectivamente). Os pacientes com CEC de boca também apresentaram níveis plasmáticos de NE mais elevados do que os voluntários saudáveis ($p= 0,0002$). As concentrações plasmáticas de NE nos pacientes com leucoplasia bucal foram menores do que os voluntários saudáveis ($p= 0,032$). Análises de regressão múltipla mostraram que níveis aumentados de NE foram significativamente associados com uma sensação de “sentir-se sem energia”, um descritor medido pelo BRUMS (OR= 2,9; $p= 0,023$). Baixa escolaridade e ocorrência de comorbidades com maior severidade foram independentemente associados a altos níveis de EPI (OR= 4,0; $p= 0,004$ e OR= 7,2; $p= 0,005$; respectivamente). Além disso, a presença de um estado de humor de raiva e “não estar em alerta” foram associados a altos níveis plasmáticos de EPI (OR= 2,6; $p= 0,039$ e OR= 3,2; $p= 0,010$, respectivamente). Os resultados mostraram que os pacientes com CEC de boca não casados e que tinham pior renda familiar foram preditivos para menor sobrevida global dos

pacientes com CEC de boca (p<0,05). Estágio clínico avançado foi associado à diminuição da sobrevida global (HR= 5,2; p= 0,000) e específica do câncer (HR= 4,2; p= 0,000). A análise de regressão de Cox também evidenciou que níveis sistêmicos aumentados tanto de NE como de EPI foram associados à menor sobrevida global (HR= 3,4; p= 0,002 e HR= 2,8; p=0,006, respectivamente). Os pacientes com CEC de boca apresentaram níveis plasmático de NE mais baixos após o tratamento do câncer em comparação com a fase pré-tratamento (p= 0,0008). Conclusão: Os resultados do presente estudo mostram que pacientes com câncer de boca podem apresentar níveis sistêmicos elevados das catecolaminas norepinefrina e epinefrina, e que esta desregulação hormonal associada com fatores clinicopatológicos e psicológicos é preditiva para pior sobrevida global.

Palavras-chave: Neoplasias de cabeça e pescoço. Câncer. Câncer de boca. Carcinoma de células escamosas. Estresse. Catecolaminas. Norepinefrina. Epinefrina. Desordens emocionais. Humor. Sobrevida. Leucoplasia bucal.

Abstract

Bastos DB. **Dysregulation in the secretion of the stress-related catecholamines in oral cancer patients: predictor and prognostic value** [thesis]. Araçatuba: UNESP - São Paulo State University; 2022.

ABSTRACT

Introduction: Cancer patients may have a hyperactivation of the Sympathetic Nervous System (SNS). Therefore, dysregulation in the secretion of the stress-related catecholamines norepinephrine (NE) and epinephrine (EPI) have been implicated in the progression of some types of cancer. However, the association between systemic levels of catecholamines and cancer prognosis, as well as its predictors, are poorly understood. Patients and methods: The present study investigated the plasma NE and EPI levels in 168 patients with oral squamous cell carcinoma (OSCC), 70 patients with oral leukoplakia and 54 healthy volunteers; as well as its association with demographic, clinicopathological, biobehavioral and psychological variables in cancer patients. Plasma NE and EPI levels were measured by high performance liquid chromatography with electrochemical detection (HPLC-ED) and psychological mood states was assessed by the Brunel Mood Scale (BRUMS). Kaplan-Meier analysis and Cox regression were used to investigate associations between the systemic levels of catecholamines and overall and specific survival after 5 years of follow-up in 82 patients with oral cancer. Systemic levels of catecholamines were also evaluated in 32 patients after oral cancer treatment and were compared with pre-treatment levels. Results: Plasma NE and EPI concentrations were significantly higher in patients with oral SCC compared to patients with oral leukoplakia ($p<.0001$ and $p=.024$, respectively). Patients with oral SCC also displayed higher plasma NE levels than healthy volunteers ($p=.0002$). Patients with oral leukoplakia had lower plasma NE levels than healthy volunteers ($p=.032$). Multiple regression analyzes showed that increased NE levels were associated with a feeling of “no energy”, a descriptor measured by BRUMS (OR= 2.9; p= .023). Low education and the occurrence of severe comorbidities were independently associated with high levels of EPI (OR= 4.0; p= .004 and OR= 7.2; p= .005; respectively). Furthermore, the presence of an angry mood state and denial of “being on alert” were associated with high EPI levels (OR= 2.6; p= .039 and OR= 3.2; p= .010, respectively). The results showed that non-married oral SCC patients and those who had a lower family income had lower overall survival in patients with oral SCC ($p<0.05$). Advanced clinical stage was associated with decreased overall (HR=5.2; p=.000) and cancer-specific (HR=4.2; p=.000) survival. Cox regression analysis also showed that increased levels of both NE and EPI were associated with worse overall survival (HR=3.4; p=.002 and HR=2.8; p=.006, respectively). Patients with oral SCC had lower plasma

NE levels after cancer treatment compared to the pre-treatment period (p=.0008). Conclusion: The results of the present study reveal that patients with oral cancer may have high systemic levels of the catecholamines norepinephrine and epinephrine, and that this hormonal dysregulation is associated with clinicopathological and psychological factors and predictive for worse overall survival.

Keywords: Head and neck neoplasm. Cancer. Oral cancer. Squamous cell carcinoma. Stress. Catecholamines. Norepinephrine. Epinephrine. Emotional disorders. Mood. Survival. Oral leukoplakia.

Listas

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LISTA DE ABBREVIATURAS, SÍMBOLOS E SIGLAS

% percentage

¥ Charlson Comorbidity Index numerical score

€ score per day of tobacco consumption

< less

> superior

°C degree Celsius

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

ACCI age-adjusted Charlson Comorbidity Index

AJCC American Joint Commission on Cancer

Al_2O_5 aluminium oxide

AM ante meridiem

ANOVA analyses of variance

BRUMS Brunel Mood Scale

CA California

CCI Charlson Comorbidity Index

CEC carcinoma espinocelular

Ch chemotherapy

CI confidence interval

CLAE-ED Cromatografia Líquida de Alta Eficiência com detecção eletroquímica

CSS cancer-specific survival

DHBA internal standard, dihydroxybenzylamine

EDTA thylenediamine tetraacetic acid

eg. for example

EPI epinephrine

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

HR hazard ratio

ɬ score per day of alcohol consumption

IBM International Business Machines

ICD International Classification of Diseases

IL-6 interleukin-6

Inc. Incorporated

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

min minute

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

MMP matrix metalloproteinases

mo month

moles/L moles per liter

n number

NE norepinephrine

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

OR odds ratio

OS overall survival

OSCC oral squamous cell carcinoma

p p-value

pH hydrogen potential

Ph.D. doctorate degree

r correlation coefficient

R$ Real

rpm rotations per minute

RT radiotherapy

SCC squamous cell carcinoma

SD tandard deviation

SE Standard error

SEM structural equation modeling

SNS sympathetic nervous system

SP São Paulo

SPSS Statistical Package for the Social Science

SPT second primary tumor

Sr surgery

TNF-α tumor necrosis factor

TNM classification of malignant tumors

UNESP São Paulo State University

USA United States of America

V volts

v volume

VEGF vascular endothelial growth factor

WHO World Health Organization

A alfa

β beta

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

π R$1.00 equals approximately U$6.00

ρg/mL picogram per milliliter

Sumário

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DYSREGULATION IN THE SECRETION OF THE STRESS-RELATED CATECHOLAMINES IN ORAL CANCER PATIENTS: PREDICTORS AND PROGNOSTIC VALUE*

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* Normalização segundo a revista Cancer Research (ANEXO B)

INTRODUCTION

Stress is a complex event that encompasses psychological and environmental factors triggering a cascade of reactions in the central and peripheral nervous systems (1). Animal research and epidemiological humans studies provide evidence that chronic stress can disrupt the endocrine system and seriously compromise the immune and physiological functions (1-3). The two main pathways that mediate the effects of chronic stress are the hypothalamic-pituitary-adrenal (HPA) axis, with a consequent increase in the production of glucocorticoids, and the sympathetic nervous system (SNS) (2-4). Stress-related hyperactivation of the SNS results in dysregulation of secretion of the catecholamines norepinephrine (NE) and epinephrine (EPI) (5,6). Both NE and EPI are chemically similar catecholamines synthesized by the adrenal medulla and act as hormones when secreted into the bloodstream (7). The catecholamines may interact with adrenergic receptors (types α- and β) present on the membrane of cancer cells and other cell types in the tumor microenvironment, leading to specifics activations of signaling pathways reflecting in cancer progression (8,9).

The increase in catecholamine levels due to states negative psychological effects also can have specific effects on the cancer imune response (1,3,10) disrupting the production of several types of cytokines, chemokines, and growth factors which are related to cancer progression (1,11-13). Several studies have shown that stress-related catecholamines plays a role in the progression of several malignant tumor types including head and neck (8,14,15), ovarian (16), breast (17), prostate (18), and skin (19) by increasing cell proliferation, angiogenesis, invasion, and metastasis. Cancer patients tend to have high levels of emotional disorders at some point during the diagnosis and tretament (20-24). Recently, in a study from our group, systemic levels of the NE e EPI were associated with anxiety symptoms in head and neck cancer (HNC) patients (5), suggesting that psychological disorders could be correlated with the secretion of stress-related catecholamines in cancer patients. Mood states have an impact on negative people acts

affected by cancer treatment (24,25). A study with breast cancer patients demonstrated that they had significantly more overall mood disturbance compared to women without the disease (26).

The oral region is the anatomical site of basic functions for an individual, such as speech, swallowing, and breathing, besides being related to social interaction (27,28). HNC is a public health problem of which at least 90% these tumors are represented by squamous cell carcinomas (SCCs) (29). Head and neck squamous cell carcinoma (HNSCC) is a highly heterogeneous malignant tumor, which can originate from various anatomical sites in the upper airway and digestive tract, including oral cavity, oropharynx, larynx, pharynx, paranasal sinuses, nasal cavity, and lip (29,30). Oral squamous cell carcinoma (OSCC) is the most frequent type of HNC whose etiology is correlated with exposure to tobacco-derived carcinogens and excessive alcohol consumption (29,31). Although the overall survival rate for OSSC has improved over the last decades, its prognosis is still very poor, with patients having to deal with the impact of their treatment on functional and aesthetic aspects (27,31). Some pre-clinical evidence have revealed that increased levels of the stress-related catecholamines could affect the OSSC progression. High concentrations of NE that simulate stress conditions enhance OSSC cells proliferation and invasion (14,32). Xie et al. (33), observed that chronic stress resulted in greater tumor size, increased expression of cancer progression-related molecules and increased invasive growth of OSCC cells in a pre-clinical model. In a recent study by our laboratory, increased NE concentrations in the microenvironment of chemically induced OSCC in rats were predict to oral carcinogenesis and also associated with cancer growth and immune response (34). In previous study, we have demonstrated that the stress-related catecholamines NE and EPI was elevated in head and neck SCC patients compared to non-cancer patients (5).

Few studies have analyzed the systemic levels of catecholamines in cancer patients and their association with psychological changes and prognostic factors. To the best of our knowledge, only one study investigated the association of systemic catecholamines levels and

survival in cancer patients. Liu et al., showed that abnormal serum catecholamine levels was associated with shorter survival in hepatocellular cancer patients (35). Although evidence attest that catecholamines can affect the behavior of OSCC cells and other types of tumors, there are no studies which have analyzed whether the systemic concentrations of these stress hormones are associated with mood states and prognosis in oral cancer patients. In the current study, we evaluated the plasma NE and EPI levels in oral cancer patients, their correlation with clinical and mood-related psychological variables, as well as their influence on the disease prognosis.

PATIENTS AND METHODS

Study design and Ethics statements

This cross-sectional study was conducted between 2012 and 2021 at the Oral Oncology Center, São Paulo State University (UNESP), School of Dentistry, Araçatuba, SP–Brazil, a public reference center for head and neck cancer treatment. The present study was performed according to the ethical standards of the World Medical Association Declaration of Helsinki and was approved by the human studies committee under number 28860420.5.0000.5420. Moreover, before analysis, requirements for written informed consent were obtained from all patients and volunteers participating in this study.

Patients

A total of 292 patients and volunteers were enrolled in this study. 168 patients who had been diagnosed with oral SCC by histopathological exam were recruited. Seventy patients with oral leukoplakia (a potentially malignant oral disorder) and 54 healthy age- and gender-matched individuals were also recruited as a control group. Individuals were eligible if they had primary oral SCC tumors who had not received any prior oncological treatment and inclusion criteria for this group were patients with tumors located in the anterior tongue, the floor of the mouth, gingiva, lip, hard palate, buccal mucosa, alveolar ridge or retromolar area. Inclusion criteria for the oral leukoplakia patients were clinical and histopathological diagnosis of oral leukoplakia according to the criteria of the World Health Organization (WHO) (36) and lesions located in the same sites previously reported for oral SCC patients. Exclusion criteria for all groups were patients with the inability to perform peripheral blood collection, patients who did not consent to have the blood collected, pregnancy patients, and previous medical history of cancer.

Demographic, clinicopathological and biobehavioral variables

For all groups, variables such as age, sex, ethnicity, marital status, family income, education level, presence of comorbidity, tobacco and alcohol consumption, and sleep quality during the last week before the medical appointment were accessed. For cancer group, data regarding site of the primary tumor, clinical TNM classification, clinical staging, histopathological grade, and awareness of cancer diagnosis were also collected. All this information's were obtained from medical records. Information about preexisting comorbidity was extracted from the medical records of all the patients coded according to International Classification of Diseases, 10^{Th} revision (ICD-10) (37). The occurrence and severity of the comorbidities were classified in without comorbidity (CCI 0), with few comorbidities (CCI 1 or 2), or with many comorbidities (CCI 3+) according to the Charlson Comorbidity Index (CCI) (38). The age-adjusted CCI (ACCI) scores were calculated with additional points added for age (39). ACCI scores were also categorized according to comorbidity severity. Tumor stages were measured by using the American Joint Commission on Cancer (AJCC) staging classification system (40), and the intensity of the history of tobacco and alcohol consumption were assessed using a score scale from 1 to 4 points, according to the amount of cigarette consumption or doses of alcoholic drink a day (41). The patients were also asked about their sleep quality in the last week using a Likert-type scale as follows: 1 = very good, 2 = good, 3 = regular, 4 = bad and 5 = terrible (5).

Psychological measures

The mood was assessed by a psychologist from our center using the Brunel Mood Scale (BRUMS) a 24-item mood adjective checklist, with six transitory subscales mood states (i.e., tension, depression, anger, vigor, fatigue, and confusion) of four items each (42,43). The BRUMS subscales tension, depression, anger, vigor, fatigue, and confusion include descriptors such as nervous and panicky, downhearted, and miserable, bitter, and annoyed, energy and alert, tired

and worn out, and mixed up and muddled, respectively. Each item was rated on a 5-point Likert scale of 0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, and 4 = extremely, with total possible subscale scores ranging from 0–16. The response timeframe was “How do you feel right now?” The results were calculated using the mean of the items in each subscale.

Plasma sample collection

Fasting blood samples were collected from each participant before treatment and were collected under the same conditions to prevent changes in hormonal levels. In every case, a blood sample was obtained between 08:00 and 10:00 hours AM with a syringe treated with ethylenediaminetetraacetic acid (EDTA) and immediately centrifuged at 1500 rpm under refrigeration at 4°C for 20 minutes. The plasma was separated from whole blood and the aliquots were kept frozen at −80°C and then thawed shortly before determination of catecholamine levels.

Measurement of norepinephrine and epinephrine levels *by HPLC-ED*

Quantification of plasma NE and EPI levels were performed using high-performance liquid chromatography with electrochemical detection (HPLC-ED) following the method described by Bastos *et al*. (5) and Di Marco *et al*. (44) The HPLC-ED separation of NE and EPI were performed using ion-pair reverse-phase chromatography coupled with electrochemical detection (0.5 V). A plasma volume of 1 mL was added in 1mL Tris-buffer (pH 8.8, 2 moles/L) and 40 µl (8ng) DHBA (internal standard, dihydroxybenzylamine), plus 50mg Al_2O_5. The suspension was vortex mixed for 10 min and the precipitated alumina was washed three times. After centrifugation (3min at 1,500g) the NE and EPI were eluted with 400µl perchloric. Then, the supernatant was filtered and injected into the reverse phase column in HPLC-ED. The NE and EPI concentrations were expressed as pg/mL.

Prognostic variables

Post-treatment local recurrence of the primary tumor, second primary tumor (SPT) and overall and cancer-specific survival were considered as the prognostic variables. To determine whether the systemic levels of NE and EPI have prognostic value they are correlated with tumor recurrence, occurrence of SPT and survival durations. The time for overall survival (OS) was defined as the time of diagnosis to death of any cause. Cancer-specific survival (CSS) was calculated from the date of diagnosis to the date of death attributed to oral SCC progression.

Catecholamine levels pre- and pos-oncological treatment

To assess changes in plasma hormone levels before and after oncological treatment, blood collection and plasma measurements of NE and EPI were repeated in 32 oral cancer patients after they had been treated for cancer disease.

Statistical analysis

GraphPad Prism version 8.2 (GraphPad Software Inc., San Diego, CA, USA) and SPSS software, version 21.0 (IBM SPSS Statistics, Armonk, New York), were used to perform the statistical tests. Continuous variables were tested with the Shapiro-Wilk test to assess normality. In presence of normality, the mean comparisons were performed by the analysis of variance (One-way ANOVA) with post-test Tukey–Kramer multiple comparison analysis was performed to determine whether if there were NE and EPI differences between the groups. The Pearson correlation coefficient was used to assess correlations between the levels of NE and EPI in oral cancer and leukoplakia patients as also healthy volunteers. Associations between hormone levels and other categorical parameters (demographic, clinicopathological, biobehavioral, and psychological) were analyzed using Chi-square or Fisher exact test. In addition, the median of plasma NE and EPI levels was used as the cutoff point. Plasma NE and EPI levels below and above the median were used to

define lower and higher catecholamine levels, respectively. A logistic regression model was fitted using a stepwise method for analyzing which variables were predictors for the stress-related catecholamines levels. For this, demographic, clinicopathological, biobehavioral, and psychological variables were considered as independent and NE and EPI levels as response variables. The association between prognostic variables (oncological treatment, local recurrence, occurrence of second primary tumor and survival) and systemic catecholamine level were analyzed through the Kaplan-Meier method. Univariate analysis was carried out using the log-rank test. Forward multivariate Cox proportional hazard models were used to determine whether the systemic levels of NE and EPI independently predict the overall and disease-specific survival of patients with oral cancer in a 5-years follow-up period. Cox proportional hazards regression models were created to compute hazard ratios (HR) with 95% confidence intervals (CIs). Multicollinearity among the independent predictor variables was observed. Oncological treatment, though significant in the univariate analysis, was not included in the Cox model as it was found to violate the multicollinearity assumption. The student's *t*-test was used to determine differences in the mean of NE and EPI levels between pre-and post-oncological treatment in oral SCC patients. The data were shown in graphs as mean±SEM (standard error of the mean). The level of statistical significance was set at a *p*-value less than 0.05 ($p<0.05$) for all statistical tests.

RESULTS

Demographic, clinicopathological, biobehavioral and psychological characteristics

A total of 54 healthy volunteers, 70 oral leukoplakia, and 168 oral cancer patients were recruited for this study. The patient's demographic profile and clinicopathological parameters are described in Table 1. Table 2 shows the biobehavioral and psychological data. Mean ages were similar for healthy, leukoplakia, and oral SCC patients (58 years old). Most patients were between 45 to 65 years old, were male, had white ethnicity, and were married or living with a partner. Tongue (36.9%) and floor of the mouth (23.2%) were the most common sites of OSCC. For the three groups, most patients had at least one comorbidity, and 27.8% healthy, 27.1% leukoplakia and 22.0% oral SCC patients had higher scores of comorbidities measured by CCI. The buccal mucosa (30.0%) was the most affected area in the leukoplakia patients, being that 40.5% of them had a microscopic diagnosis of epithelial dysplasia (mild, moderate, or severe) (data not shown). Although 48.2% of OSCC patients were diagnosed with advanced disease stages (III and IV), only 25.9% had regional metastasis at the time of diagnosis. Most oral tumors (71.1%) were microscopically classified as moderately differentiated, while only 4.1% were poorly differentiated. The cancer patients were predominantly treated with surgery only (45.2%) or surgery followed by radiotherapy and chemotherapy (12.7%). As observed in Table 1, only 15 patients (9.1%) with oral SCC had a second primary tumor (SPT) and 28 (17.1%) had tumor recurrence.

Table 1. OSCC patients' demographic and clinicopathological profile.

Patient characteristics	Healthy group n (%)	Leukoplakia group n (%)	Oral SCC group n (%)
Age (years)			
Mean (SD)	58.83 (10.84)	58.50 (9.59)	58.70 (12.57)
Age range (years)			
0-45 y	7 (13.0)	7 (10.0)	19 (11.3)
45-65 y	32 (59.2)	49 (70.0)	99 (58.9)
>65 y	15 (27.8)	14 (20.0)	50 (29.8)
Sex			
Male	42 (77.8)	40 (57.1)	128 (76.2)
Female	12 (22.2)	30 (42.9)	40 (23.8)
Ethnicity [a]			
White	19 (79.2)	32 (80.0)	116 (69.5)
Non-white	5 (20.8)	8 (20.0)	51 (30.5)
Marital status [a]			
Married	37 (84.1)	35 (57.4)	100 (60.2)
Non- Married	7 (15.9)	26 (42.6)	66 (39.8)
Family income (R$/month) [a,b]			
R$<1000/mo	-	7 (36.8)	52 (35.4)
R$1000 – 5000/mo	-	7 (36.8)	78 (53.1)
R$>5000/mo	-	5 (26.4)	17 (11.5)
Education [a]			
Illiterate	-	17 (39.5)	61 (37.2)
Elementary or high school	-	16 (37.2)	68 (41.5)
University or Postgraduate	-	10 (23.3)	35 (21.3)
CCI Score [c]			
0	12 (22.2)	11 (15.7)	53 (31.6)
1	12 (22.2)	24 (34.3)	42 (25.0)
2	15 (27.8)	16 (22.9)	36 (21.4)
3+	15 (27.8)	19 (27.1)	37 (22.0)
ACCI Score			
0	12 (22.2)	11 (15.7)	53 (31.6)
1	4 (7.4)	2 (2.9)	7 (4.1)
2	5 (9.3)	14 (20.0)	23 (13.7)
3+	33 (61.1)	43 (61.4)	85 (50.6)
Tumor site			
Retromolar area	-	3 (4.3)	16 (9.5)
Floor of the mouth	-	6 (8.6)	39 (23.2)
Gingiva	-	3 (4.3)	4 (2.4)
Lip	-	5 (7.1)	21 (12.5)
Tongue	-	9 (12.9)	62 (36.9)
Buccal mucosa	-	21 (30.0)	8 (4.8)
Hard palate	-	8 (11.4)	2 (1.2)
Soft palate	-	8 (11.4)	-
Alveolar ridge	-	7 (10.0)	16 (9.5)

T – Classification [a]			
T1	-	-	45 (27.1)
T2	-	-	49 (29.5)
T3	-	-	37 (22.3)
T4	-	-	35 (21.1)
Regional metastasis [a]			
N0	-	-	123 (74.1)
N+	-	-	43 (25.9)
Clinical stage [a]			
I	-	-	45 (27.1)
II	-	-	41 (24.7)
III	-	-	39 (23.5)
IV	-	-	41 (24.7)
Histopathologic grade [a]			
Well-differentiated	-	-	30 (24.8)
Moderately differentiated	-	-	86 (71.1)
Poorly differentiated	-	-	5 (4.1)
Oncological Treatment [a]			
Surgery only	-	-	75 (45.2)
Sur + RT	-	-	18 (10.8)
RT	-	-	7 (4.3)
Ch	-	-	15 (9.0)
Ch + RT	-	-	15 (9.0)
Sur + RT + Ch	-	-	21 (12.7)
Other	-	-	15 (9.0)
Second Primary Tumor (SPT) [a]			
Yes	-	-	15 (9.1)
No	-	-	149 (90.9)
Recurrence [a]			
Yes	-	-	28 (17.1)
No	-	-	136 (82.9)

Abbreviations: SCC, Squamous cell carcinoma; CCI, Charlson Comorbidity Index; ACCI, Age Charlson Comorbidity Index; Sr, Surgery; RT, Radiotherapy; Ch, Chemotherapy; Second Primary Tumor, SPT.
a Variables with missing data.
b R$1.00 equals approximately U$6.00.
c CCI: Use to evaluate the comorbidity severity. Each numerical score equals different medical conditions, each weighted according to its potential to impact on mortality.

In respect to tobacco consumption, most leukoplakia and oral SCC participants had a severe smoking habit (60% and 55.3%, respectively), while only 31.5% of healthy volunteers had severe tobacco consumption (Table 2). Regarding alcohol consumption, almost 50% of oral SCC patients were severe alcoholics and only 11.1% and 24.3% of health and oral leukoplakia patients drank severely, respectively. Most research participants reported great or good sleep quality at the last week. Ninety-three (56.7%) of oral SCC patients had awareness of cancer diagnosis at the time

of the research and the most prevalent mood profile was vigor (82.4% healthy, 66.7% leukoplakia, and 61.6% oral SCC patients) according to BRUMS subscales (Table 2).

Table 2. Patients' biobehavioral and psychological characteristics.

Patient characteristics	Healthy group n (%)	Leukoplakia group n (%)	Oral SCC group n (%)
Tobacco consumption [a]			
Non-smoker	22 (40.7)	14 (20.0)	29 (17.3)
Light	9 (16.7)	9 (12.9)	24 (14.3)
Moderate	6 (11.1)	5 (7.1)	22 (13.1)
Heavy	17 (31.5)	42 (60.0)	93 (55.3)
Alcohol consumption [b,c]			
Non-drinker	20 (37.0)	22 (31.4)	28 (16.9)
Light	21 (38.9)	19 (27.1)	37 (22.3)
Moderate	7 (13.0)	12 (17.2)	31 (18.7)
Heavy	6 (11.1)	17 (24.3)	70 (42.1)
Sleep quality (last week) [c,d]			
Very good	12 (22.2)	15 (22.0)	22 (13.2)
Good	21 (59.3)	35 (51.5)	93 (55.6)
Regular	9 (16.7)	14 (20.6)	32 (19.2)
Bad	0 (0)	3 (4.4)	13 (7.8)
Terrible	1 (1.9)	1 (1.5)	7 (4.2)
Awareness of cancer diagnosis [c]			
Yes	-	-	93 (56.7)
No	-	-	71 (43.3)
BRUMS [c]			
Tension	4 (7.8)	8 (15.7)	28 (17.1)
Depression	1 (2.0)	4 (7.8)	16 (9.8)
Fatigue	4 (7.8)	3 (5.9)	12 (7.3)
Anger	0 (0)	2 (3.9)	5 (3.0)
Confusion	0 (0)	0 (0)	2 (1.2)
Vigor	42 (82.4)	34 (66.7)	101 (61.6)

Abbreviations: Brunel Mood Scale, BRUMS; SCC, Squamous cell carcinoma.
a Smoking: each category equals (per day) 10 cigarettes (manufactured, paper), two cigarettes (manufactured, straw), or two cigars or pipes of tobacco smoked.
b Alcoholism: each category equals (per day) two doses of distilled alcohol, two bottles of beer, or two glasses of wine consumed.
c Variables with missing data.
d Sleep self-report were obtained at the same day of blood collection and information such as quality sleep experience over the last week were reported.

Plasma NE and EPI levels in healthy volunteers and oral leukoplakia and cancer patients

To observe the differences among plasma catecholamines levels in cancer and no cancer patients, we measurement circulating NE and EPI levels in a group of 54 healthy volunteers, 70 oral leukoplakia patients, and 168 oral SCC patients. The results showed that oral SCC patients (364.7 ± 24.22 pg/mL) had higher NE plasma concentration compared to healthy volunteers (192.1 ± 21.04 pg/mL) and oral leukoplakia patients (110.6 ± 8.92 pg/mL) ($p=.0002$ and $p<.0001$, respectively) (Figure 1A). Plasma NE levels in healthy patients were higher compared to leukoplakia patients ($p=.032$). There was no statistical difference between the mean plasma EPI concentration from oral SCC patients (124.8 ± 9.31 pg/mL) and healthy volunteers (86.84 ± 9.11 pg/mL) ($p =.489$). However, plasma EPI concentrations did differ among the oral SCC patients and oral leukoplakia patients (83.02 ± 8.97 pg/mL) ($p=.024$) (Figure 1B).

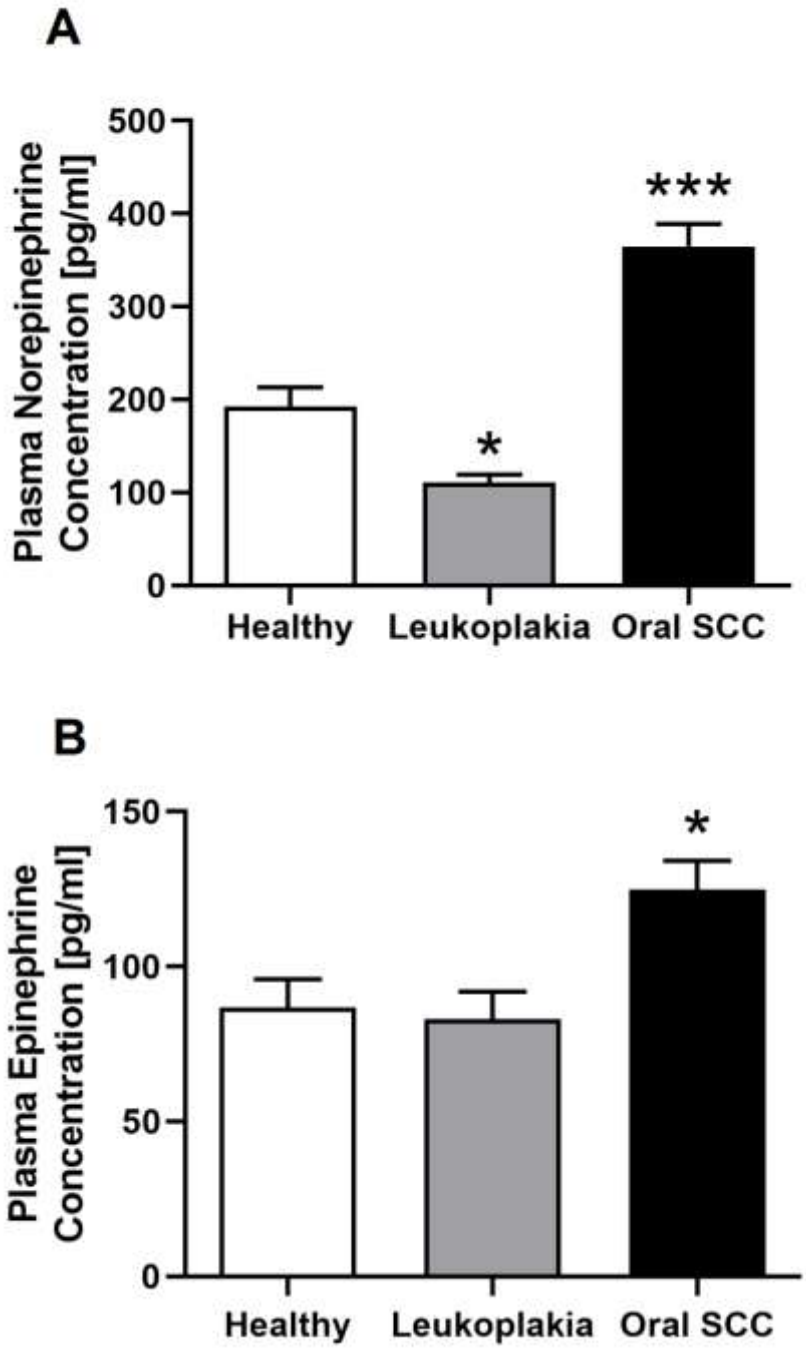

Figure 1. ANOVA test between the pre-treatment plasma norepinephrine (NE) and epinephrine (EPI) levels in healthy, leukoplakia and oral SCC patients. Plasma NE (A) and EPI (B) from healthy, leukoplakia and oral SCC patients. Results are expressed as mean±SEM. *$p \leq .05$: plasma NE levels from leukoplakia patients compared to healthy volunteers and plasma EPI levels from oral SCC compared to leukoplakia patients. ***$p \leq .0001$: plasma NE levels from oral SCC patients compared to healthy and leukoplakia patients.

Correlation between plasma norepinephrine and epinephrine

It was found a positive correlation between the plasma NE and EPI levels in healthy vollunteers (r=0.321; p=.017). The results also showed a positive correlation between the NE and EPI in oral leukoplakia patients (r=0.657; p<.0001). However, the correlation between these catecholamines in oral SCC patients did not reach statistical significance (r=0.139; p=.072). These data are summarized in figure 2.

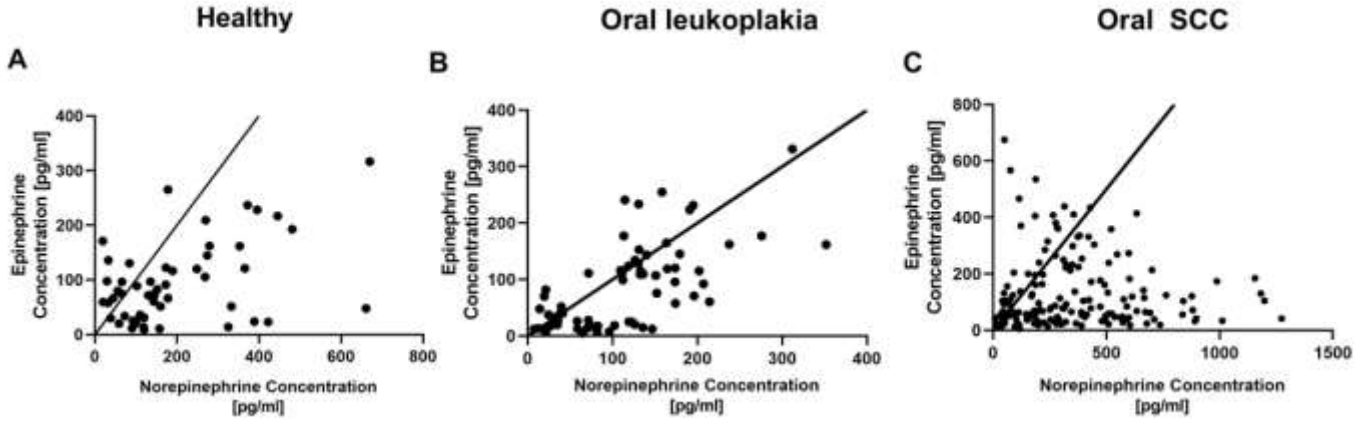

Figure 2. Correlation between plasma concentrations of norepinephrine and epinephrine. Correlations between plasma NE and EPI levels in healthy vollunteers (A), leukoplakia (B) and oral SCC patients (C) were analyzed using Pearson correlation test. Plasma NE levels were positively correlated with EPI levels in healthy and leukoplakia patients (p>.05).

Predictors for plasma NE and EPI levels in oral cancer patients

To identify which variables are predictive of systemic NE and EPI levels in oral cancer patients' logistic regression analyses were performed, and the results of the univariate and multivariate regression models are presented in Tables 3 and 4, respectively. Univariate analysis showed worse sleep quality during the last week (p=.014) and sensation of "no energy" (p=.010) by the BRUMS descriptor were significantly associated with high plasma NE levels in OSSC patients. When the associations between all variables and plasma EPI levels were explored, the results showed that worse education (p=.005) and better socioeconomic condition (p=.008) were associated with higher and lower plasma EPI levels, respectively. Univariate also showed that

higher CCI (p=.005) and ACCI (p=.023) scores and drinking alcohol at heavy intensity were associated with high EPI levels (p=.04). Moreover, oral SCC patients who had higher scores of tension measured by BRUMS status and who feeling not to be “on alert” measured by BRUMS descriptors displayed higher plasma EPI concentrations (p=.03 and .004, respectively).

Multivariate analysis showed no significant associations between plasma NE levels and demographic, clinicopathological, biobehavioral variables and mood status. However, when analyzing each BRUMS descriptors, the results showed that higher pre-treatment plasma NE levels were significantly associated with a sensation of “no energy” (OR=2.9; p=.023). Low education and higher CCI scores were independently associated with higher plasma EPI levels (OR=4.0; p=.004 and OR=7.2; p=.005; respectively). Regarding psychological evaluation, one mood symptom was predictive for systemic EPI levels in the multivariate analysis. OSCC patients who reported an angry mood (a BRUMS mood status) had higher plasma EPI levels (OR=2.6, p=.039). Although alertness is not be considered a mood state categorized by BRUMS, the feeling of “not to be on alert” (a mood descriptor) was also associated with higher EPI levels (OR=3.2, p=.010).

Table 3. Significant associations between demographic, clinicopathological, biobehavioral and mood psychological variables and plasma norepinephrine and epinephrine levels in oral SCC patients from the univariate analysis.

Variables[a]	**Univariate Model Results**
	***p*-Value**
Norepinephrine	
Sleep quality - last week (worse)	.014
Mood descriptor	-
"No energy"	.010[b]
Epinephrine	
Education (Illiterate)	.005
Income (R$ >5000/mo)	.008
CCI Score (high)	.005[c]
ACCI Score (high)	.023[c]
Alcohol consumption (heavy)	.04[d]
Mood status	-
Tension (high)	.03[b]
Mood descriptor	-
"On alert" (no)	.004[b]

Abbreviations: CCI, Charlson Comorbidity Index; ACCI, Age Charlson Comorbidity Index.
[a]The variables` associations with plasma norepinephrine and epinephrine levels reached statistical significance.
[b]Values were measured with the binary measure (yes or no).
[c]Values were measured with the severity categories (low or high).
[d]Non-drinker, light, moderate, or heavy consumption.
All values are considered statistically significant at p<.05.

Table 4. Predictive value of demographic, clinicopathological, biobehavioral and mood psychological variables for high plasma norepinephrine and epinephrine levels in oral SCC patients from the multivariate analysis.

Variables[a]	**Multivariate Model Results**		
	OR	**95% CI**	***p*-Value**
Norepinephrine			
Mood descriptor			
"No energy"	2.9	0.130 – 0.858	.023[b]
Epinephrine			
Education (Illiterate)	4.0	0.096 – 0.650	.004[c]
CCI Score (high)	7.2	1.825 – 28.895	.005[c]
Mood status			
Anger (yes)	2.6	1.053 – 6.783	.039[b]
Mood descriptor			
"On alert" (no)	3.2	0.125 – 0.756	.010[b]

Abbreviations: CCI, Charlson Comorbidity Index; CI, Confidence interval; OR, Odds Ratio; SE, Standard error.
[a]The variables` associations with high plasma norepinephrine and epinephrine levels reached statistical significance.
[b]Values were measured with the binary measure (yes or no).
[c]Values were measured with the severity categories (low or high).
All values are considered statistically significant at p<.05.

Prognostic value of the stress-related catecholamine levels in oral cancer patients

To determine whether the systemic levels of stress-catecholamines have a prognostic value in oral cancer patients, the pre-treatment concentrations of plasma NE and EPI were correlated with the occurrence of second primary tumor, tumor recurrence, and survival. Eighty-two oral SCC patients with a cutoff of at least 5 years of follow-up were included in the prognostic analysis. Depending on cut-off levels for plasma NE and EPI levels, the oral SCC patients were divided according to higher or lower plasma catecholamine levels. Kaplan-Meier analysis showed that plasma NE and EPI levels were not significantly associated with second primary tumor and recurrence (p>.05).

At the end of 5 years of clinical follow-up, survival information was available for all patients. The median follow-up duration was approximately 30 (range 1–139) months. On univariate analysis higher plasma concentrations of NE was significantly associated with OS (log-rank p=.044) (Figure 3A), but not with CSS (log-rank p=.303) (Figure 3B). Thereby, patients with higher systemic NE levels exhibited a significantly shorter survival time to death to any cause compared to patients with lower hormonal levels. Kaplan-Meier analysis also demonstrated that higher EPI levels was a significant independent predictor for poor OS (log-rank p=.026) and CSS (log-rank p=.047) (Figure 3C and D, respectively).

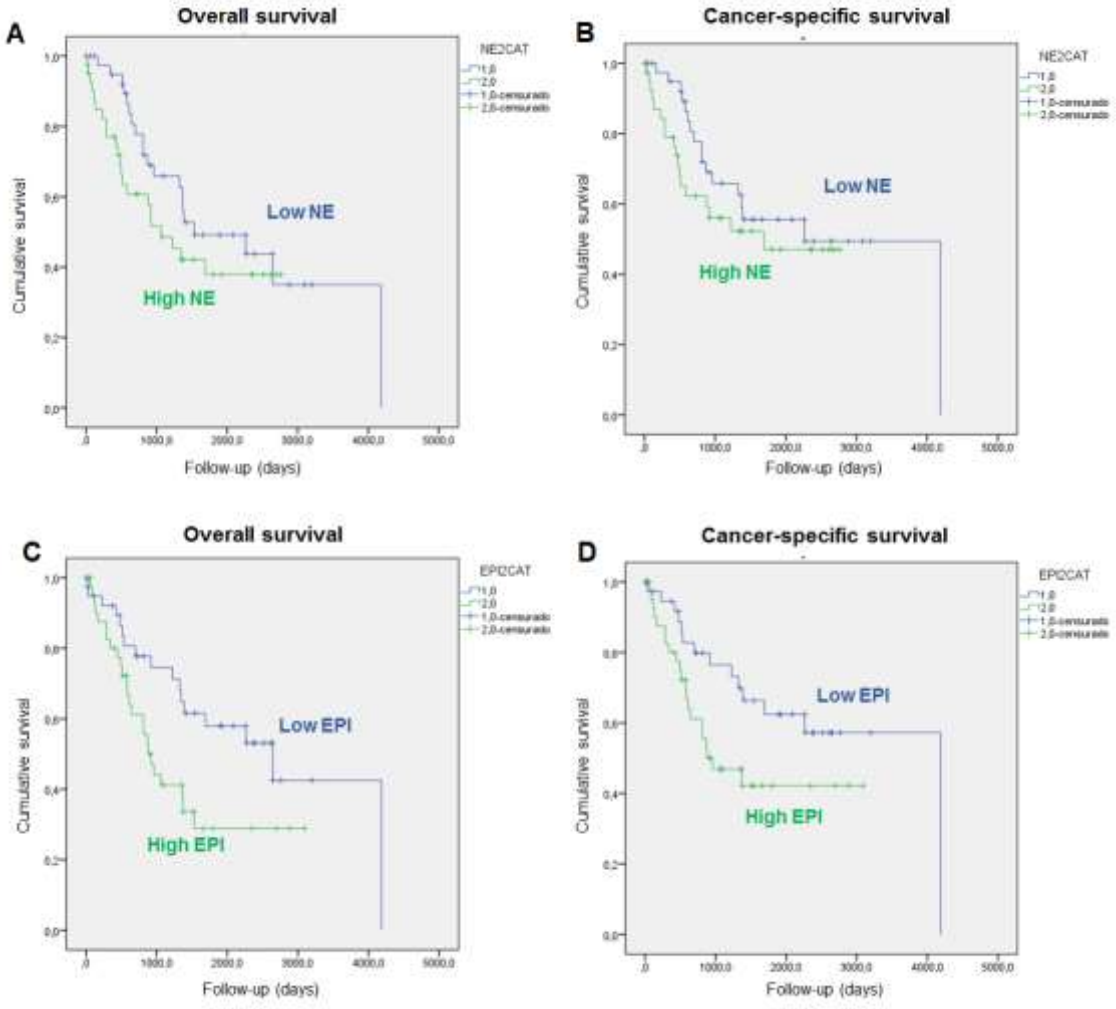

Figure 3. Higher norepinephrine and epinephrine levels were associated with decreased survival time in oral SCC patients. (A and B) Kaplan-Meier survival curve of the overall and cancer-specific survival for NE levels of oral SCC patients, respectively; (C and D) Kaplan-Meier survival curve of the overall and cancer-specific survival for EPI levels of oral SCC patients, respectively.

As shown in table 5, in terms of the demographic, clinicopathological and biobehavioral features, univariate survival analysis revealed that ethnicity, marital status, family income, tumor size, regional metastasis, clinical stage, and tobacco and alcohol consumption had a significant influence on worse overall survival rates through the Kaplan-Meier analysis and log-rank tests. In the univariate analysis, age, ethnicity, family income, tumor size, regional metastasis, clinical stage, and tobacco and alcohol consumption were also significantly associated with worse cancer-specific survival of oral SCC patients (Table 5). These variables were also included in the following multivariate Cox regression analysis.

Table 5. Univariate analysis of demographic, clinicopathological and biobehavioral variables for overall survival and cancer-specific survival from oral SCC patients.

Variables	**OS**		**CSS**	
	95% CI	***p*-Value**	**95% CI**	***p*-Value**
Age				
45-65 y	-	-	366.6 – 1553.3	.044
Ethnicity				
Non-white	357.7 – 1484.2	.002	83.1 – 1836.8	.012
Marital status				
Non- Married	265.3 – 1654.6	.015	-	-
Family income				
R$ <1000/mo	361.2 – 1480.7	.003	110.3 – 1809.6	.011
Tumor size				
T3/T4	415.3 – 872.6	.000	335.9 – 952.0	.000
Regional metastasis				
N+	413.8 – 752.1	.000	416.7 – 749.2	.002
Clinical stage				
III/IV (advanced)	438.1 – 783.8	.000	473.2 – 814.7	.000
Tobacco consumption				
Heavy smoker	680.9 – 1155.0	.035	310.5 – 1525.4	.027
Alcohol consumption				
Heavy drinker	543.3 – 1194.6	.039	595.4 – 1240.5	.039

Abbreviations: OS, overall survival; CSS, cancer-specific survival; 95% CI, 95% confidence interval.
All values are considered statistically significant at p<.05.

For the multivariate Cox proportional model (Table 6), oncological treatment was excluded because of high collinearity with other variables. Considering the clinical data, we observed that non-married OSCC patients were 2.8 times more likely to display poor OS (HR=2.8; p=.010). A worse financial condition was also a significant risk factor for dead for any cause (OS) (HR=6.2; p=.021). In addition, oral SCC patients with early-clinical stage had better OS and CSS compared to those with advanced stage (HR=5.2; p=.000 and HR=4.2; p=.000, respectively) (Table 6). On Cox regression, results evidenced that oral cancer patients who had higher NE plasma levels had significantly worse OS (HR=3.4; p=.002) as compared to individuals with lower NE plasma levels. Additionally, as also determined by the Kaplan–Meier method, the multivariate Cox regression analysis revealed that having higher pre-treatment EPI systemic levels was an independent prognostic factor for predicting poorer OS in oral SCC patients (HR=2.8; p=.006). However, higher NE and EPI levels were not significantly associated with shorter cancer-specific survival (p>.05).

Table 6. Outcome predictions in Cox regression analyses from oral SCC patients.

Variables	**OS**				**CSS**			
	HR	**SE**	**95% CI**	***p*-Value**	**HR**	**SE**	**95% CI**	***p*-Value**
Marital status (non-married)	2.8	.40	1.28 – 6.24	.010	-	-	-	-
Income (<1.000.00)	6.2	.79	.03 - .75	.021	-	-	-	-
Clinical stage (III/IV)	5.2	.43	2.23 – 12.32	.000	4.2	.38	1.99 – 9.00	.000
Norepinephrine (high)	3.4	.40	1.55 – 7.51	.002	-	-	-	-
Epinephrine (high)	2.8	.37	1.35 – 5.97	.006	-	-	-	-

Abbreviations: OS, overall survival; CSS, cancer-specific survival; HR, Hazard Ratio; 95% CI, 95% confidence interval; SE, Standard error.
[b]Values were measured with the binary measure (yes or no).
[c]Values were measured with the severity categories (low or high).
All values are considered statistically significant at p<.05.

Plasma stress-related catecholamines levels pre and post oncological treatment

To investigate changes in the stress-related catecholamines levels between diagnosis and post-treatment periods, 32 OSCC patients underwent to plasma NE and EPI measurements before and after oncological treatment. There was a significant difference in plasma NE levels between pre-and post-cancer treatment. Oral SCC patients had lower plasma levels of NE in post-treatment (214.1 ± 12.8 pg/mL) compared to the basal pre-treatment phase (369.3 ± 39.7 pg/mL) (p=.0008) (Figure 4A). Oral SCC patients showed lower plasma EPI levels (178.2 ± 30.8 pg/mL) in post-treatment compared to basal pre-treatment phase (131.0 ± 13.7 pg/mL). However, this result did not reach statistical significance (p=.379) (Figure 4B).

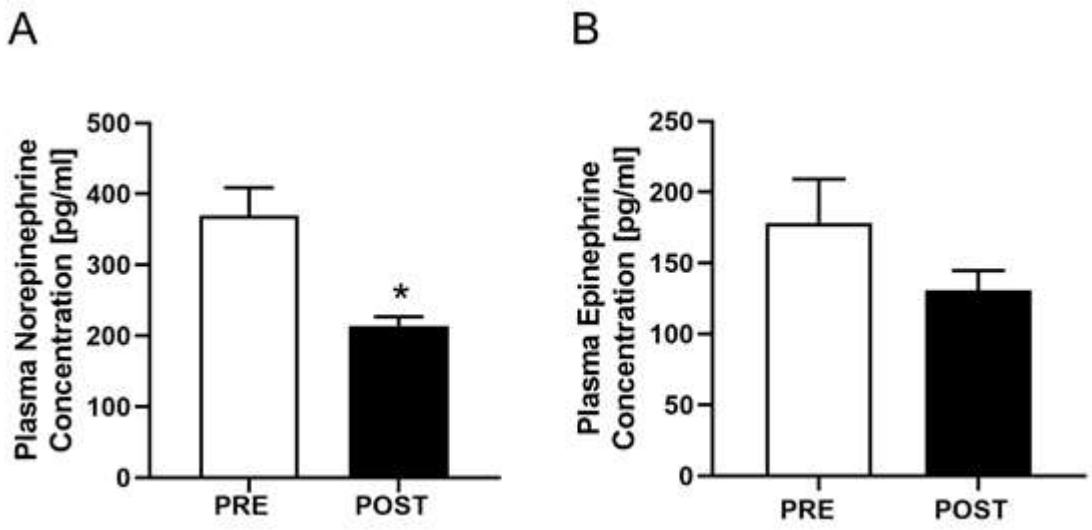

Figure 4. Plasma norepinephrine and epinephrine levels in oral SCC patients pre-and post-cancer treatment. Plasma norepinephrine (A) and epinephrine (B) levels from thirty-two oral SCC patients before and after they have completed cancer treatment. Results are expressed as mean±SEM. Student's *t* test; **p*=.0008: lower plasma norepinephrine levels in oral SCC patients after treated of cancer disease.

DISCUSSION

Recently, chronic stress-related catecholamines have been a common research focus. However, research evaluating the levels of stress-related hormones in cancer patient is very scarce. In the present study, we have explored the systemic secretion profile of the catecholamines NE and EPI in oral cancer patients, as well as their predictors and prognostic value. First, we have found increased NE levels in OSCC patients. Plasma NE levels in oral SCC patients were higher than oral leukoplakia patients and in healthy volunteers. Besides that, plasma EPI levels in OSCC patients were also higher than oral leukoplakia patients. This clinical finding is supported by our previous results demonstrating that head and neck cancer patients display higher plasma catecholamines levels than patients with no cancer (5). Other recent studies have also observed increased levels of stress-related catecholamines in oral (45), gastric (46), and breast (26) cancer patients. Recently, growing evidence has indicated that stress-related mediators and other behavioral conditions can be predictive of cancer progression (1,12-14,47). For example, Lutgendorf et al. (7,13) showed that NE stimulates vascular endothelial growth factor (VEGF) secretion and enhance the expression of matrix metalloproteinases (MMPs) by ovarian cancer cell lines. In a study from our laboratory, we have demonstrated that NE at stress levels upregulates IL-6 expression (a pro-inflammatory cytokine to OSCC growth) in OSCC cells, mechanisms by which catecholamines may increase cancer cell proliferation (14). Thereby, our findings reinforce an idea that the occurrence of oral cancer is associated with a dysregulation in the secretion of stress-related catecholamines, which could be associated with cancer progression. Furthermore, it is feasible that increased systemic catecholamines concentrations could also reflect increased levels of these hormones in the tumor microenvironment. In turn, these catecholamines in increased concentrations in the tumor could bind to beta-adrenergic receptors (β-ARs), inducing a cascade of events related to tumor progression. As demonstrated by previous studies, OSCC cells express β-ARs, whose activation by catecholamines could

induce a series of molecular events associated with tumor growth (14, 48). Our results also reveal a strong positive correlation was found between NE and EPI levels in both oral leukoplakia and healthy volunteers. Surprisingly, there was no correlation between NE and EPI levels in the oral cancer patients. We speculate that the SNS dysregulation found in cancer patients explains the lack of correlation in systemic levels of both catecholamines.

After observing that catecholamine levels were elevated in oral cancer patients, we have evaluated which variables were predictive for higher circulating catecholamines levels in these patients. Low education level from the OSCC patients was a predictor for systemic increased levels of EPI. We hypothesized that, in less educated cancer patients, chronic stress plays a more important role in their psychological well-being increasing plasma EPI levels. According to Sharif et al. (49), cancer patients with higher educational levels may turn to other coping strategies when faced with this major illness. They could be searching for more information, seek expert opinion and learn more about the illness to understand the illness better and to cope (49). Comorbidities are relatively prevalent among HNSCC patients (50,51,52). Omura et al. (52) observed that HNSCC patients with more severe comorbidity had significantly worse overall and disease-specific survival than those without comorbidity. Our results showed that the association in high CCI scores with high plasma EPI levels was significant in the expected direction in multivariate analysis. Although there are no studies that have showed association between the CCI scores with EPI levels in cancer patients, Feres et al. (53) showed that urinary NE and EPI levels were significantly associated with hypertension when compared with healthy nonsmoking volunteers. Hypertension may result from the inappropriate activation of the sympathetic nervous system and/or from chronically elevated levels of circulating catecholamines (54). In our oral cancer patients, hypertension was the most frequently comorbidity (38.2%). We have suspected that hypertension and other comorbidities from the OSSC patients could be associated with increased

EPI levels, which are involved in the regulation of tumor progression through neurohumoral mechanisms.

Another aspect highlighted by our study was the implications of mood states in the stress-related catecholamines levels in oral cancer patients. To the best of our knowledge, this is the first study that investigated an association between mood states and catecholamine levels in oral cancer and leukoplakia patients. The BRUMS inventory evidence changes in specific mood states (42,43). Our results demonstrated that vigor was the most prevalent mood in OSCC patients. At first glance, this finding might seem counterintuitive, as one could expect of fear of death, perception of disfigurement associated with cancer and its treatment would create a negative mood symptom. In a recent study, Acquaye et al. (25) investigated mood states in brain tumors patients. The authors showed that patients who were undergoing cancer treatment reported a level of mood disturbance and a negative mood symptom, presented as a more intense negative mood state when compared to patients who were only under medical follow-up (25). In the multivariate analysis, our results showed that angry mood measured by the BRUMS was associated with high EPI levels. Earlier evidence reveals that anger reflects negative affect (55). Lehto et al. (56) showed an association between anger and depressive symptoms shortly after diagnosis in breast cancer patients. Besides that, Akaho et al. (57) showed that anger was significantly associated with overall survival and disease-free survival in patients with leukemia. When we evaluated each descriptor used to assess the BRUMS subscales, our results also showed that sensation of “no energy” and feeling “not to be on alert” were independent predictors for high NE and EPI levels, respectively. There are a lack of studies investigating the mechanisms involved in systemic catecholamine levels related to these sensations and feelings in oncological patients. However, we can associate the sensation of “no energy” with a state of tiredness and fatigue, for example. This could resemble a state of exhaustion, a consequence of psychological stress. Furthermore, we can hypothesize that a state of “no energy” could also be associated with

depressive symptoms. Other studies demonstrate that psychological stress initiates a response of the SNS, which raises catecholamine levels and interacts with biological components of tumor cells through certain signaling pathways (2-5). In our hypothesis, negative mood, as angry for example, and others sentimental states could dysregulate the secretion of catecholamines affecting oral cancer prognosis. These findings indicate the importance of effective psychological interventions to control emotions (e.g., anger) in cancer patients not only for a better patient's quality of life, but also to prevent detrimental effects of neurohormonal dysregulation.

As with other types of cancer, the advanced clinical stage is a prognostic factor for HNSCC (58-60). In addition to clinical staging, an expected predictor for the survival of patients with oral cancer, the multivariate Cox regression analysis demonstrated that marital status and income were independent predictors for overall survival. Unmarried patients had a shorter survival compared to married patients. In another study evaluating marital status and survival in patients with several types of cancer, the authors showed that married patients were less likely to present with metastatic disease and to die as a result of their cancer than unmarried patients (61). Supporting the idea of Aizer et al. (61) our results suggest that features of marriage, particularly social support, can have on cancer detection, treatment, and survival (61). It raises the possibility that investments in targeted social support interventions aimed at vulnerable populations, such as unmarried patients, could significantly improve the likelihood of achieving a cure (61). In addition to our results showing that a low family income is associated with worse overall survival in oral cancer patients, in other studies, social disparities were found to contribute worse additional risk for death in people with cancer (62,63).

Multiple biomarkers have been implicated in the HNC progression (5,45,64). Stress-related catecholamines NE and EPI bind to beta-adrenergic receptors on the cell membrane and activates several oncogenic signaling pathways (65,66). Studies have shown an association between the use of beta-adrenergic antagonists and reduced rates of cancer progression (66). In

a preclinical study by our group, propranolol (“a b-blocker”) treatment not only reduced the occurrence and growth of OSCC, but also decreased the concentrations of pro-inflammatory cytokines IL-6 and TNF-α in the tumor microenvironment (66). Moreover, beta-adrenergic receptors expression was a significant predictor of tumor differentiation, lymph node metastasis and reduced overall survival rate in patients with tongue SCC (67). In particular, it is speculated that NE and EPI are relevant factors in predicting survival. However, to date only one study has evaluated the association between catecholamine levels with survival in cancer patients, Liu et al. (35) showed that increased serum NE and EPI levels were independent prognostic predictors of overall survival and tumor recurrence in patients with hepatocellular carcinoma. To investigate possible mechanisms behind the impact of catecholamine on oral cancer survival, here we demonstrated that the high plasma NE and EPI levels has been regarded as an independent risk factor for poor overall survival for oral SCC patients. As a stress hormones, NE and EPI seems to be associated with mortality in cancer and no cancer patients. As mentioned, secreted catecholamines resulted in the activation of certain functions involved in tumor proliferation, angiogenesis, metastasis and inflammation (14,48,66-68). An possible explanation for the patient survival-associated catecholamine high levels might be NE and EPI could stimulate these mechanisms in oral tumor cells leading to a shorter survival. The SNS is known for its role in the “fight-or-flight” stress response (5). While these rapid responses may be necessary for survival, sustained elevation of circulating catecholamines for prolonged periods of time can also produce others pathological conditions (69). The reduced parasympathetic nervous system and increased SNS throught stress catecholamines, can cause heart failure and hypertension (70). Latini et al. showed that higher plasma NE was associated with increased mortality in patients with heart failure (71). These data suggest that stress-related catecholamines may be involved in a worse overall survival in oral cancer patients, as they are also associated with other preexisting medical conditions. In the present study, the plasma catecholamines levels were reduced after oral cancer

treatment. Some studies have reported increased levels of the catecholamines in cancer patients but did not quantify changes post-treatment (5,45). Since higher catecholamine levels are associated with psychological stress, we hypotetized that these stress-related hormones are decrease after cancer treatment because the patient could had lower emotional distress, anxiety and depression symptoms, inherent to the end of treatment and feeling of healing. Although post-tretament NE and EPI systemic levels yet to be tested as prognostic predictors, the findings of the current study could serve as the foundation of studies exploring the stress-related catecholamine levels to monitor response to treatment in oral cancer patients.

This study has some limitations. Although elevated hormonal levels in peripheral tissues including tumor microenvironment may reflect those found in the blood, tumoral catecholamine levels were not measured. Moreover, we were not able to demonstrate whether in tumor catecholamines levels have impact on OSCC patients prognostic. In addition, we reinforce the idea of evaluating post-treatment catecholamines levels in a larger patient sample investigating their impact on the OSCC prognosis. In conclusion, our results provide insight into the potential specific role of NE and EPI in oral cancer. To our knowledge, the current study is the first to demonstrate that patients with oral cancer have a dysregulation in the secretion of the stress-related catecholamine NE, and that both plasma NE and EPI levels were associated with worse overall survival. This research suggests that catecholamine levels represent new and promising prognostic biomarkers for OSCC. This, in turn, could provide a direction for the development of novel therapeutic methods involving psychological and pharmacological interventions for oral cancer treatment.

CONFLICTS OF INTEREST

All authors declare they have no conflicts of interest.

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Anexos

ANEXO A – Parecer do CEP

UNESP - FACULDADE DE ODONTOLOGIA-CAMPUS DE ARAÇATUBA/ UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Níveis sistêmicos e tumorais de mediadores do estresse em pacientes com câncer de boca e sua associação com as alterações de humor e prognóstico da doença

Pesquisador: DANIELA BRITO BASTOS
Área Temática:
Versão: 4
CAAE: 28860420.5.0000.5420
Instituição Proponente: Faculdade de Odontologia do Campus de Araçatuba - UNESP
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.854.141

Apresentação do Projeto:
Trata-se de apresentação de emenda para incluir metodologia no projeto. Na proposta inicial do projeto de pesquisa, como parte de um dos objetivos secundarios, foi proposto avaliar os niveis plasmaticos de norepinefrina (NE), epinefrina (E) e IL-6 em pacientes portadores de CEC de boca. Os pesquisadores relatam nesta submissão a necessidade de uma nova realizacao experimental, onde e possivel obter resultados complementares ao da proposta
anteriormente solicitada. Varios estudos in-vitro, pre-clinicos em animais, como tambem estudos clinicos em humanos, tem demonstrado que a expressao dos receptores adrenergicos 1 e 2 e tambem da citocina IL-6, exibem papeis semelhantes aquelas expostas pelos niveis plasmaticos de NE e E nos pacientes com cancer. Com isso, com o intuito de explorar melhor estes mecanismos e nao so investigar os niveis plasmaticos de NE, E e IL-6, propoe-se tambem avaliar a expressao dos receptores -adrenergicos 1 e 2 e da citocina IL-6 no tecido tumoral parafinado destes pacientes com cancer de boca.

Objetivo da Pesquisa:
Objetivo Primario:
Avaliar a associacao das concentracoes plasmaticas de mediadores relacionados ao estresse com

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as variacoes de humor em pacientes com CEC de boca e sua influencia sobre o prognostico da doenca. Objetivo Secundario: Avaliar os niveis plasmaticos de norepinefrina, epinefrina e IL-6 em pacientes portadores de CEC de boca, leucoplasia bucal e voluntarios saudaveis sem cancer.• Comparar os niveis plasmaticos de norepinefrina, epinefrina e IL-6 dos pacientes portadores de CEC de boca entre as diferentes fases do tratamento oncologico. • Comparar as concentracoes de norepinefrina, epinefrina e IL-6 no microambiente tecidual entre as amostras de CEC de boca, leucoplasia e mucosa bucal normal.• Investigar a associacao entre as caracteristicas emocionais relacionadas a variacao de humor e as concentracoes plasmaticas e teciduais de norepinefrina, epinefrina e IL-6 em pacientes com cancer de boca.• Analisar a influencia dos niveis plasmaticos pre-tratamento de norepinefrina, epinefrina e IL-6 sobre o prognostico do cancer de boca.

Avaliação dos Riscos e Benefícios:

Riscos:

A participacao nesta pesquisa oferece riscos minimos, pois sua metodologia e: coleta de sangue, cuja tecnica e conhecida e aplicada em laboratorios de diagnostico. Neste projeto, a coleta de sangue sera realizada por profissionais capacitados. O teste psicologico que sera utilizado e validado na populacao brasileira e sera aplicado por profissional treinado. Nenhum dos procedimentos que serao realizados ira oferecer riscos a dignidade do paciente. Todas as informacoes coletadas neste estudo serao estritamente confidenciais.

Beneficios:

Esta pesquisa nao ira fornecer nenhum beneficio direto ao paciente. Entretanto, sera oferecida ao grupo controle uma avaliacao clinica intrabucal com o objetivo de realizar o diagnostico precoce de lesoes benignas e malignas. Caso for identificada alguma lesao bucal o voluntario sera encaminhado para tratamento na FOA/UNESP. Alem disso, o paciente portador sera encaminhado a profissionais ou centros psicologicos se houver necessidade. Esperamos que os resultados do presente estudo possam trazer maiores evidencias sobre a possivel participacao de fatores psicologicos ainda nao investigados na etiopatogenia do cancer de boca. Alem disso, os resultados poderao, em longo prazo, estimular o desenvolvimento de abordagens terapeuticas mais completas para o tratamento do cancer, que integrem a atencao aos fatores emocionais e suas manifestacoes clinicas.

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Comentários e Considerações sobre a Pesquisa:

Trata-se de emenda para incluir nova analise da expressao dos receptores -adrenergicos 1 e 2 e de IL-6 por imuno-histoquimica no tecido parafinado.

Considerações sobre os Termos de apresentação obrigatória:

Adequados.

Recomendações:

Nada a declarar.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Considerações Finais a critério do CEP:

Não havendo pendências, o CEP propõe a aprovação do projeto de pesquisa salientando que, de acordo com a Resolução 466 CNS de 12/12/2012 (título X, seção X.1., art. 3, item b, e, título XI, seção XI.2., item d), há necessidade de apresentação de relatórios semestrais, devendo o primeiro relatório ser enviado até 16/01/2022. O CEP reitera a necessidade de entrega de uma via (não cópia) do TCLE ao sujeito participante da pesquisa e solicita ao pesquisador responsável leitura da carta circular 003/2011 CONEP/CNS antes do início do projeto.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1785049_E2.pdf	30/06/2021 11:12:23		Aceito
Solicitação registrada pelo CEP	EMENDA_METODOLOGIA_Daniela_Bastos.pdf	30/06/2021 11:11:45	DANIELA BRITO BASTOS	Aceito
Folha de Rosto	Folha_de_rosto.pdf	10/02/2020 09:55:25	DANIELA BRITO BASTOS	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	29/01/2020 08:56:34	DANIELA BRITO BASTOS	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoDetalhado.pdf	29/01/2020 08:56:28	DANIELA BRITO BASTOS	Aceito
Cronograma	Cronograma.pdf	29/01/2020 08:56:19	DANIELA BRITO BASTOS	Aceito

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Continuação do Parecer: 4.854.141

Outros	QuestionarioBRUMS.pdf	29/01/2020 08:54:51	DANIELA BRITO BASTOS	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

ARACATUBA, 16 de Julho de 2021

Assinado por:
Aldiéris Alves Pesqueira
(Coordenador(a))

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ANEXO B – Normas de Publicação da Cancer Research

Cancer Research Online ISSN: 1538-7445

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