UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

ANA LÍVIA SANTOS SOUSA

PSYCHOLOGICAL AND CLINICAL VARIABLES ARE ASSOCIATED WITH EXPRESSION OF β1- AND β2-ADRENERGIC RECEPTORS IN ORAL SQUAMOUS CELL CARCINOMA

ARAÇATUBA
2023

ANA LÍVIA SANTOS SOUSA

Psychological and clinical variables are associated with expression of β1- and β2-adrenergic receptors in oral squamous cell carcinoma

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho", como parte dos requisitos para obtenção do título de Mestre em Odontologia. Área de concentração: Estomatologia e Psiconeuroimunologia.

Orientador: Prof. Tit. Glauco Issamu Miyahara
Coorientador: Prof. Ass. Daniel Galera Bernabé

ARAÇATUBA
2023

Catalogação na Publicação (CIP)

Diretoria Técnica de Biblioteca e Documentação – FOA / UNESP

Sousa, Ana Lívia Santos.
S729e Expression of β1- and β2-adrenergic receptors in oral squamous cell carcinoma and association with clinicopathological and psychological variables / Ana Lívia Santos Sousa. - Araçatuba, 2023
41 f. : il. ; tab.

Dissertação (Mestrado) – Universidade Estadual Paulista, Faculdade de Odontologia de Araçatuba
Orientador: Prof. Glauco Issamu Miyahara
Coorientador: Prof. Daniel Galera Bernabé

1. Head and neck neoplasms 2. Psychosocial distress 3. Receptors, Adrenergic, beta I. T.

Black D6
CDD 617.63

Claudio Hideo Matsumoto CRB-8/5550

Dedico este trabalho aos meus pais, ***Cristiane dos Santos Sousa*** *e* ***Wagno de Sousa****, por todo suporte, amizade e amor que sempre me conferiram.*

AGRADECIMENTOS

Inicialmente, devo minha gratidão a **Deus** e a **Espiritualidade**, por todo o amparo que me concederam durante esta jornada. Tenho ciência do quanto meus caminhos são iluminados e de toda força e esperança que me conferem.

O fim de um ciclo traz muitos resultados além do objetivo inicialmente almejado. Hoje, vejo que o título de Mestre não é meu principal ganho durante essa jornada, mas sim um resultado secundário diante de todo amadurecimento, crescimento pessoal e muito aprendizado que esse percurso me proporcionou. Ter passado anos longe de casa me mostrou que a possibilidade de cursar a pós-graduação em minha própria cidade é um privilégio imensurável, e os últimos anos me mostraram que nada é mais importante do que poder estar com a minha família todos os dias.

Dessa forma, sou imensamente grata aos meus pais, **Cristiane dos Santos Sousa** e **Wagno de Sousa**, por serem minha fonte inesgotável de amor, carinho, amparo e afeto que eu tanto precisei durante estes dois anos. Mesmo quando eu não conseguia enxergar nada além das lágrimas diante dos problemas e dificuldades, vocês me abraçaram, acolheram e me concederam forças para superar todos os obstáculos.

À minha irmãzinha, **Ana Flávia Santos Sousa**, por ser minha amiga e companheira durante toda a vida. Você é meu bem mais precioso, a coisinha que eu mais amo no mundo, e minha maior fonte de orgulho. É muito importante para mim poder acompanhar seu crescimento de perto, e poder ver a mulher independente que você está se tornando.

Aos meus avós **Lenita Maria dos Santos** e **José Francisco dos Santos**, por serem os avós mais carinhosos que eu poderia sonhar, e por acreditarem sempre em mim. Tenho muita sorte por ter vocês comigo. Às minhas primas, **Bianca dos Santos Oliveira**, **Emanuelle dos Santos Oliveira** e **Giovana dos Santos Oliveira**, e aos meus tios, **Elaine dos Santos**

Oliveira e **Vanderlei de Oliveira**. Muito obrigada por todo o amor, suporte e apoio em todas as decisões que tomei. Sou eternamente grata a Deus por ter me concedido uma família como a nossa. Nossos cafés da tarde e almoços em família são os melhores momentos que tenho nessa encarnação, e sei que a minha família é a coisa mais importante que essa vida me conferiu.

Ao meu amigo de longa data, **Carlos Alberto Pessin de Souza,** que esteve comigo durante as principais etapas da minha vida e acompanhou minhas melhores e piores decisões. Não tenho como agradecer a Deus por ter colocado uma amizade como a sua no meu caminho, e que sobreviveu às intempéries do tempo e às más escolhas da vida. Espero um dia poder dar a você a ajuda e o suporte que você sempre me deu.

Aos meus amigos **Ícaro Hial Rodrigues** e **Jonatas Monteiro da Silva**, por me mostrarem o real significado de amizade e companheirismo. Não tenho como agradecer todo o carinho e cuidado que vocês têm comigo, por todos os conselhos, por todos os abraços e puxões de orelha. Vocês são o melhor grupo de apoio!

Aos meus amigos que a pós-graduação me concedeu e tornaram esse caminho muito mais leve. À **Tamara Fernandes** e **Vitória "Laros"**, muito obrigada por todas as conversas, conselhos e risadas que sempre damos quando estamos juntas. Vocês me ensinaram muito mais além da Estomatologia. Ao **Nilton** e ao **Pedro Victor,** por alegrarem meus dias, por todas as nossas conversas e pela nossa amizade**,** e ao **Lukas** e **Winícius** por terem me acolhido tão bem**.** O curso de Mestrado foi muito mais leve com a ajuda e a amizade de vocês.

Agradeço à aluna de doutorado **Giseli Mitsuy Kayahara**, por toda ajuda durante o curso de mestrado, incluindo conselhos, ajuda no laboratório, discussão de resultados, estatística e os seus sábios conselhos. Não sou capaz de mensurar o quanto fui capaz de aprender com você, você é uma professora fantástica! E agradeço à **Daniela Brito Bastos** por

ter iniciado o presente trabalho como parte de sua tese e por ser uma ótima amiga durante todo esse percurso.

Aos meus orientadores, **Prof. Glauco Issamu Miyahara**, e, em especial, ao **Prof. Daniel Galera Bernabé**, por todo aprendizado que me conferiram durante essa jornada. Sou grata por todo ensinamento e por todas as dicas, conselhos, broncas e correções. Vocês foram uma peça essencial para todo o meu crescimento pessoal e profissional durante esses dois anos. Tenho uma enorme admiração por vocês.

Ao **Centro de Oncologia Bucal** e à **Faculdade de Odontologia de Araçatuba**, pela estrutura necessária para a realização desta pesquisa.

À **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES)** - Código de Financiamento 001, pela concessão da bolsa de mestrado. **À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)**, Processo nº 18/06928-9, pela concessão do auxílio à pesquisa e por todo suporte financeiro indispensável para a realização deste estudo.

Por fim, e não menos importante, meu profundo agradecimento vai a todos os pacientes que participaram desse estudo.

Sousa ALS. Variáveis clínicas e psicológicas são associadas com a expressão de receptores beta-adrenérgicos tipo 1 e 2 em carcinoma espinocelular de boca [dissertação]. Araçatuba: Faculdade de Odontologia da Universidade Estadual Paulista; 2023.

RESUMO

Introdução: Estressores físicos e psicológicos têm sido relacionados à progressão tumoral por meio da ativação de receptores beta-adrenérgico (β-AR) em diferentes tipos de câncer. Objetivo: Este estudo teve como objetivo investigar as expressões de β_1-AR e β_2-AR e sua associação com variáveis psicológicas e clínico-patológicas de pacientes com carcinoma espinocelular (CEC) em boca. Métodos: Foram coletadas amostras tumorais de pacientes diagnosticados com câncer de boca. As amostras foram submetidas à reação imuno-histoquímica para detectar a expressão de β_1-AR e β_2-AR. As variáveis psicológicas foram avaliadas por testes psicológicos aplicados por um psicólogo antes de qualquer tratamento oncológico. Resultados: Um total de 99 amostras de CEC de boca foram incluídas. Análises univariadas revelaram que maior expressão de β_1-AR foi associada a maior consumo de álcool (p=0,032), maior escolaridade (p=0,042), pior qualidade do sono (p=0,044) e maiores níveis de dor relacionadas ao tumor primário (p<0,001). Maior expressão de β_2-AR foi associada, principalmente, a metástase regional (p=0,014), aumento dos níveis de dor relacionados ao tumor primário (p=0,044), sintomas ansiosos (p<0,001) e depressivos (p=0,010) e maior pontuação para humor raiva (p=0,010) e fadiga (p=0,010. A análise multivariada identificou o estadiamento clínico avançado como um fator independentemente associado à expressão de β_1-AR (OR = 0.145, 95%, IC = 0.025-0.828, p=0.003), enquanto maior sintoma de ansiedade e maior humor fadiga são fatores independentes para a expressão de β_2-AR (OR = 4.256, 95% IC = 1.439-12.606, p=0.009; OR = 3.816, 95% CI = 1.258-11.573, p=0.018, respectivamente). Conclusão: A expressão de β-ARs em CEC de boca é influenciada por características clinicopatológicas e variáveis psicológicos. Esses achados encorajam mais estudos para elucidar o mecanismo de atuação receptores adrenérgicos e sua expressão no câncer de boca.

Palavras-chave: Neoplasias de Cabeça e Pescoço. Estresse Psicológico. Receptores Beta Adrenérgicos.

Sousa ALS. Psychological and clinical variables are associated with expression of β1- and β2-adrenergic receptors in oral squamous cell carcinoma [dissertation]. Araçatuba: UNESP – São Paulo State University; 2023.

ABSTRACT

Introduction: Physical and psychological stressors have been related to tumor progression through the activation of beta-adrenergic receptors (β-AR) in different types of cancer. Objective: This study aimed to investigate the expressions of β1-AR and β2-AR and their association with psychological and clinical-pathological variables in patients with oral squamous cell carcinoma (OSCC). Methods: Tumor samples were collected from patients diagnosed with oral cancer. The samples were submitted to immunohistochemical reaction to detect the expression of β1-AR and β2-AR. Psychological variables were assessed by psychological tests applied by a psychologist before any oncological treatment. Results: 99 oral SCC samples were included. Univariate analyzes revealed that higher expression of β1-AR was associated with higher alcohol consumption (p=0.032), higher education (p=0.042), worse sleep quality (p=0.044) and higher levels of pain related to the primary tumor (p<0.001). Higher expression of β2-AR was mainly associated with regional metastasis (p=0.014), increased levels of pain related to the primary tumor (p=0.044), anxious (p<0.001) and depressive symptoms (p=0.010) and higher scores for angry mood (p=0.010) and fatigue (p=0.010. Multivariate analysis identified advanced clinical staging as a factor independently associated with β1-AR expression (OR = 0.145, 95%, CI = 0.025-0.828, p=0.003). Higher anxiety symptoms and higher mood fatigue are independent factors for β2-AR expression (OR = 4256, 95% CI = 1439-12606, p=0.009; OR = 3816, 95% CI = 1258 -11,573, p=0.018, respectively) Conclusion: The expression of β-ARs in OSCC tumor are influenced by clinicopathological and psychological variables. These findings encourage further studies to elucidate the mechanism of action of adrenergic receptors and their expression in oral cancer.

Keywords: Head and neck neoplasms. Psychosocial distress. Beta Adrenergic Receptors.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS, SÍMBOLOS E SIGLAS

%	Percentage
≤	Minor or equal
>	Superior
<	Minor
4NQO	4-Nitroquinoline 1-oxide
AJCC	American Joint Committee on Cancer
BDI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
BRUMS	Brunel Mood Scale
CCI	Charlson Comorbidity Index
EMT	Epithelial–mesenchymal transition
Epi	Epinephrine
HPA	Hypothalamic–pituitary–adrenal
LC	Locus coeruleus
MDD	Major depressive disorder
mM	Milimolar
NE	Norepinephrine
No.	Number
NY	New York
OSCC	Oral squamous cell carcinoma
PBS	Phosphate-buffered saline
SNS	Sympathetic nervous system
SPSS	Statistical Package for the Social Sciences
UNESP	São Paulo State University
USA	United States of America
β-AR	Beta-adrenergic receptors
μm	Micrometer

SUMÁRIO

PSYCHOLOGICAL AND CLINICAL VARIALES ARE ASSOCIATED WITH EXPRESSION OF β1- AND β2-ADRENERGIC RECEPTORS IN ORAL SQUAMOUS CELL CARCINOMA

Ana Lívia Santos-Sousa[1], Giseli Mitsuy Kayahara[1], Daniela Brito Bastos[1], Bruna Amélia Moreira Sarafim-Silva[1], Marcelo Macedo Crivelini[2], Vítor Bonetti Valente[1], José Eduardo Corrente[3], Jose Candido C. Xavier-Júnior[4]; Glauco Issamu Miyahara[1], Daniel Galera Bernabé[1]*.

[1] Psychosomatic Research Center, Oral Oncology Center, São Paulo State University (UNESP), School of Dentistry, Araçatuba, São Paulo, Brazil

[2] Department of Diagnosis and Surgery, Araçatuba Dental School, São Paulo State University (UNESP), Araçatuba, São Paulo, Brazil.

[3]Bioscience Institute, Biostatistics Department, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil.

[4] School of Medicine, UNISALESIANO, Araçatuba, São Paulo, Brazil.

***Correspondence:**

Daniel Galera Bernabé

Psychosomatic Research Center and Oral Oncology Center

Araçatuba Dental School - São Paulo State University - UNESP

Rua José Bonifácio, 1193, Araçatuba- São Paulo – Brazil, CEP.15050-015

Tel. +55 (18) 36363268 / 36363275.

E-mail address: daniel.bernabe@unesp.br

1 INTRODUCTION*

Cancer patients usually live under chronic stress, caused by diagnosis-related strong emotional experience, disease progression and depression (Batty et al., 2017). The high stress-levels in cancer patients are associated with cancer progression, invasion and metastasis (Dai et al., 2020). Cancer patients can display increased stress levels when compared with health individuals (Bastos et al., 2018; Bernabé et al., 2012). Stress is related to disorder of hypothalamic–pituitary–adrenal axis (HPA) axis and hyperactivation of sympathetic nervous system (SNS) (Antoni et al., 2006; Cole et al., 2015). These activations result in the release of cortisol and catecholamines from adrenal glands and sympathetic neurons (Glaser and Kiecolt-glaser, 2005). Catecholamines such as norepinephrine (NE) and epinephrine (Epi) are hormones that act in the cancer microenvironment through G protein–coupled adrenergic receptors (types α- and β) located on the surface of tumor cells, fibroblasts, and immune cells (Eng et al., 2014). Several studies have shown that β-adrenergic signaling may affect the progression of different types of cancer, by modulating the angiogenesis, tumor invasion, epithelial–mesenchymal transition (EMT), and tumor growth-related genes.(Bernabé et al., 2011; Cole and Sood, 2012; Tjioe et al., 2022; J. Zhang et al., 2016).

Oral squamous cell carcinoma (OSCC) is the main type of oral cancer, accounting for about 90-95% of all head and neck cancers (Johnson et al., 2021). In 2020, more than 377,000 new cases and 177,000 deaths from oral cancer were recorded worldwide (IARC, 2022). Patients with OSCC display high occurrence of emotional disorders throughout diagnosis and treatment (Aarstad et al., 2005; Figueira et al., 2022). In head and neck cancer, high levels of anxiety and depression are associated with shorter overall survival, regional lymph node metastasis and worsen prognosis (Aarstad et al., 2005; Rieke et al., 2017). Although chronic tobacco and alcohol consumption are the main risk factor for OSCC, recent studies have

Normalização segundo a revista Psychoneuroendocrinology - https://www.sciencedirect.com/journal/psychoneuroendocrinology

shown that neuroendocrine changes derived from chronic stress may influence the tumor progression (Thaker et al., 2006; Verza et al., 2021). Similarly, adrenergic antagonists inhibit tumor progression, otherwise, in in the absence of stress, adrenergic agonists accelerate tumor progression and metastasis in ovary, breast and oral cancer (Shang et al., 2009; Smith et al., 2019). Cecílio et al. revealed that rats treated with carcinogen 4NQO and propranolol, a non-selective β-blocker, display lower occurrence and progression of OSCC than those rats from the sham group (Cecilio et al., 2020). Therefore, beta-adrenergic receptor (β-AR) blockers have been associated with better survival and reduced the risk of recurrence in different types of cancer (De Giorgi et al., 2013; Shaashua et al., 2017; Zhong et al., 2016).

Previous studies reported the expression of β-ARs in different malignant tumors, including OSCC (Pérez-Sayáns et al., 2012), lung cancer (Al-Wadei et al., 2012), melanoma (Moretti et al., 2013) and prostate carcinoma (M. Zhang et al., 2020). In ovarian cancer, β-ARs expression were correlated with anxiety and depression symptoms (Huang et al., 2016). Clinical studies demonstrated a correlation between higher levels of β-AR and worse prognosis in gastric cancer (Koh et al., 2021), head and neck cancer (Kwon et al., 2021) and breast cancer (Powe et al., 2011). Previous clinical studies in OSCC patients reported that higher expression of β-AR are correlated with low survival, advanced clinical stage and nodal metastasis (Dong et al., 2017; Krishna et al., 2022; Liu et al., 2018). However, to our knowledge, no study has investigated the correlation of tumor expression of β-AR with psychological factors in OSCC patients. Therefore, the current study analyzed the expression of β1- and β2-ARs in OSCCs and their association with clinicopathological, biobehavioral, and psychological variables.

2 PATIENTS AND METHODS

2.1 Patients

To evaluate the expression of β1- and β2-ARs in OSCCs, paraffin tumor tissue samples from OSCC patients diagnosed and treated at the Oral Oncology Center, São Paulo State University (UNESP), School of Dentistry, Araçatuba, São Paulo, Brazil between 2012 and 2020. The tissue samples used were extracted from biopsy for diagnostic purposes or from oncological surgery to treat the primary tumor. The inclusion criteria were histopathological diagnosis of OSCC, primary tumor located in the anterior tongue, floor of the mouth, gingiva, lip, hard palate, buccal mucosa, or retromolar area. Patients with prior cancer diagnosis and treatment, current pregnancy, and patients who did not consent to participate in the study were excluded. This study was approved by the Committee of Human Studies of the São Paulo State University (UNESP), School of Dentistry, Araçatuba, SP–Brazil (n°. 35314720.9.0000.5420) and conducted according to the guidelines in the Declaration of Helsinki.

2.2 Demographic, clinicopathological and biobehavioral variables

Demographic, clinicopathological and biobehavioral data were extracted from the patient's clinical records. Demographic variables (age, sex, marital status, living alone, education, and family income), clinicopathological variables (comorbidity, pain related to the primary tumor, clinical staging, primary tumor size (T), presence of regional metastases (N), tumor histological grade and type of treatment) and biobehavioral data (sleep quality and tobacco and alcohol consumption) were obtained in OSCC patients' admission. Comorbidities were assessed according to the Charlson Comorbidity Index (CCI) (Charlson et al., 1987). Clinical staging and classification of histological grades were examined according to the AJCC Staging Manual and the World Health Organization Classification of Tumors (Amin et al.,

2017). Visual Analog Scale was used to estimate primary tumor-related pain intensity and classified in a scale from 0 (absence of pain) to 10 (severe pain). The patient's self-reports of sleep quality in the previous week were classified as very good, good, regular, bad, and terrible (Bastos et al., 2018; Sarafim-Silva et al., 2018). Tobacco and alcohol consumption intensity was assessed using a score scale of 1–4 points: non-smoker/drinker, light use (1–10 cigarettes/1–2 drinks per day), moderate use (11–20 cigarettes/3–4 drinks per day), and heavy use (more than 20 cigarettes/ more than 4 drinks per day)(Sarafim-Silva et al., 2018).

2.3 Psychological variables

All the patients included in the sample underwent psychological tests applied by a psychologist. Anxiety and depression symptoms were assessed using the Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI) (Beck et al., 1996, 1988; Sarafim-Silva et al., 2018). BAI and BDI are a Likert-type scale questionnaires that measure the occurrence of anxiety and depressive symptoms (Beck et al., 1996, 1988). For both scales, severity of each anxiety or depression symptoms scores were graded in four levels: minimal (0–10 for BAI; 0–11 for BDI), mild (11–19; 12–19), moderate (20–30; 20–35) and severe (31–63; 36–63). The Brunel Mood Scale (BRUMS) is a 24-item mood scale that measure six identifiable affective mood states: anger, confusion, depression, fatigue, tension and vigor (Lane and Terry, 2000; Terry et al., 2003, 1999). All psychological tests were performed before any oncologic treatment.

2.4 Immunohistochemistry and staining evaluation

Histological sections of 4μm thickness were deparaffinized and rehydrated to immunohistochemistry evaluation. Heat-induced epitope retrieval was performed with 10mM citrate buffer, pH 6.0, in microwave for 12 minutes. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 20 min. The sections were incubated with the primary antibody

anti-β_1 adrenergic receptor antibody (dilution 1:500; Abcam, ab3442) and Anti-β_2 adrenergic receptor antibody (dilution: 1:600; Abcam, ab137494) for 3h at room temperature. Then, sections were incubated with Histofine antibody polymer conjugated with horseradish peroxidase (Histofine® Simple Stain MAX PO (MULTI), Nichirei Biosciences Inc., Tokyo, Japan) for 40 minutes. The slides were washed with PBS buffer and the chromogenic substrate (3,3′,5,5′-tetramethylbenzidine; Spring Bioscience, Pleasanton, USA) was incubated for 5 minutes. Harris's hematoxylin was used for counter-staining. Kidney and stomach samples were used as positive and negative controls for immunohistochemistry staining of β_1-AR and β_2-AR, respectively.

Qualitative and quantitative analyses of β_1- and β_2-ARs expressions were accomplished in the tumor section including the invasion front at a 400x magnification. To qualitative evaluation, staining intensity of tumor cells was scored in negative/weak and moderate/strong intensity (Wang et al., 2012). To quantitative evaluation, the percentage of positively stained cells tumor in the invasive front was categorized in $\leq$ 50% and > 50% (Z. F. Zhang et al., 2016). Two independent observers (GMK and ALSS) who were blinded to the clinicopathological features of the patients performed all analyses.

2.5 Statistical Analysis

SPSS software (version 24.0, NY, USA) was used to perform the statistical analysis. The BAI and BDI were grouped in no/minimum symptoms and low/moderate/high symptoms. The six-mood profile obtained by the BRUMS test were grouped in higher scores or lower scores for each mood type according to the median. The comparison between the different categories of β-ARs expression and clinicopathological, biobehavioral and psychological variables were performed by Chi-square test or Fisher's exact test. Multivariate regression analysis was performed by the stepwise logistic regression method, considering the β-ARs expression levels as a dependent variable, and the clinicopathological, biobehavioral and psychological

characteristics as exploratory variables. The level of statistical significance was set at 5% ($p<0.05$) for all statistical tests.

3 RESULTS

3.1 Epidemiological and clinicopathological profile

Ninety-nine patients with OSCC met the inclusion criteria and were included in the current study. Table 1 shows the demographic, biobehavioral, and clinicopathological features from OSCC patients. Most of the patients were men (75.8%), middle-aged (68.7%), and married (61.2%). The principal education level reported was incomplete primary school (30.9%), and most of the patients had a family income of R$ 1000-5000 per month (48.3%). The majority (72.4%) of patients reported at least one comorbidity and 42.4% of them related having hypertension. The main type of antihypertensive used was angiotensin receptor blocker (18.9%), followed by angiotensin-converting enzyme inhibitors (13.1%), diuretics (12.6%) and beta-blockers (11.6%) (Table 1). Most OSCC patients had the disease classified in initial clinical stage (I and II) (56.2%), and tumors microscopically graded as moderately differentiated (71.6%). Regional metastasis occurred in 37.8% of patients. Almost 43% of OSCC patients reported pain related to the primary tumor, most of them had moderate pain (18.4%), followed by 13.3% with intense pain and 11.2% with minimum pain. The clinical stage are also shown in Table 1. Data concerning tobacco and alcohol consumption, and sleep quality in last week are presented in Table 2.

Table 1: Demographic and clinicopathological variables of patients with oral squamous cell carcinoma.

Variable	OSCC
	No. (%)
Age	
Mean age (years, SD)	59 (± 10.9)
0-45y	7 (7.1)
46-65y	68 (68.7)
>65y	24 (24.2)
Sex	
Male	75 (75.8)
Female	24 (24.2)
Ethinicity	
White	66 (66.7)
Non-white	33 (33.3)
Marital status*	
Single	19 (19.4)
Married	60 (61.2)
Divorced	11 (11.2)
Widowed	8 (8.2)
Living alone*	
Yes	13 (13.7)
No	82 (86.3)
Education*	
Illiterate	6 (6.2)
Incomplete primary school	30 (30.9)
Primary school	15 (15.5)
Elementary school	28 (28.9)
High school	14 (14.4)
University	4 (4.1)
Family income*	
R$0/mo	3 (3.3)
<R$ 1000/mo	35 (38.5)
R$1000-5000/mo	44 (48.3)
>R$5000/mo	4 (9.9)
Comorbidity*	
Yes	71 (72.4)
No	27 (27.6)
CCI score*	
0	27 (27.5)
1	28 (28.6)
2	25 (25.5)
3+	18 (18.4)
Comorbidity type	
Hypertension	42 (42.4)
Diabetes	15 (21.1)
Depression*	10 (14.1)

Others	5 (7.1)
Antihypertensive use	
ARB	18 (18.9%)
ACEI	13 (13.1%)
Diuretics	12 (12.6%)
Beta blocker	11 (11.6%)
Pain*	
Yes	42 (42.9)
No	56 (57.1)
Pain intensity*	
No pain	56 (57.1)
Minimum	11 (11.2)
Moderate	18 (18.4)
Intense	13 (13.3)
T classification*	
T1	28 (28.6)
T2	33 (33.7)
T3	18 (18.3)
T4	19 (19.4)
Regional metastasis*	
N0	61 (62.2)
N+	37 (37.8)
Clinical stage*	
I	28 (28.6)
II	27 (27.6)
III	22 (22.4)
IV	21 (21.4)
Histopathological grade*[a]	
Grade I	18 (24.3)
Grade II	53 (71.6)
Grade III	3 (4.1)

Abbreviation: CCI, Charlson Comorbidity Index; ARB, Angiotensin Receptor Blocker; ACEI, Angiotensin-Converting Enzyme Inhibitors;
*Variables with missing data.
[a]Grade I, well differentiated; grade II, moderately-differentiated; grade III, poorly-differentiated.

Table 2: Biobehavioral variables of patients with oral squamous cell carcinoma.

Variable	OSCC
	No. (%)
Sleep quality*	
Great	17 (17.3)
Good	47 (48.0)
Regular	23 (23.5)
Bad	6 (6.1)
Terrible	5 (5.1)
Smoking	
No	18 (18.2)
Current smoker	66 (66.6)
Ex-smoker	15 (15.2)
Tobacco intensity	
Non-smoker	18 (18.2)
Light	17 (17.2)
Moderate	14 (14.1)
Heavy	50 (50.5)
Alcohol consumption	
Non-drinker	19 (19.2)
Current drinker	52 (52.5)
Ex-drinker	28 (28.3)
Alcohol intensity	
Non-drinker	19 (19.2)
Light	15 (15.2)
Moderate	22 (22.2)
Heavy	43 (43.4)

*Variables with missing data.

3.2 Psychological profile

The psychological variables of the OSCC patients are described in Table 3. Most of the patients displayed minimum levels of anxiety (55.6%) and depressive (55.6%) symptoms, followed by mild (25.6%, BAI; 18.9%, BDI), moderate (7.8%, BAI; 5.6%, BDI) and severe symptoms (2.2%, BAI, 4.4%, BDI). The majority of the patients displayed confusion as preponderant humor (57.7%), followed by tension (19.8%) and vigor (13.2%).

Table 3 Psychological features of patients with oral squamous cell carcinoma.

Variable	OSCC
	No. (%)
Anxiety symptoms	
No	8 (8.9)
Minimum	50 (55.6)
Mild	23 (25.6)
Moderate	7 (7.8)
Severe	2 (2.2)
Depression symptoms	
No	14 (15.6)
Minimum	50 (55.6)
Mild	17 (18.9)
Moderate	5 (5.6)
Severe	4 (4.4)
Predominant humor*	
Anger	4 (4.4)
Confusion	55(57.7)
Depression	1 (1.1)
Fatigue	4 (4.4)
Tension	18 (19.8)
Vigor	12 (13.2)

*Variables with missing data

3.3 Associations between β_1-AR expression with clinicopathological, biobehavioral and psychological characteristics

The cancer cells were positive for β_1-AR and the immunostaining were observed in the cytoplasm and plasma membrane of tumor cells, with a diffuse reactivity (Figure 1). The β_1-AR staining pattern in tumor cells was weaker in invasive front than in the tumor extension. Considering β_1-AR qualitative assessment, univariate analysis demonstrated stronger straining intensity in tumor samples from the patients who reported higher anger humor ($p=0.010$). Higher positivity for β_1-AR expression in OSCC samples were found in OSSC patients who were drinker and former drinkers ($p=0.032$), had higher educational levels ($p=0.042$) and worse sleep quality ($p=0.044$) (Table 4). The patients' smoking habits and other clinicopathological characteristics were not associated with β_1-AR expression in OSCC samples ($p>0.05$). β_1-AR expression was not significantly correlated with anxiety and depression symptoms measured by BAI and BDI. In addition, there was no correlation between β_1-AR expression and antihypertensive use ($p>0.05$).

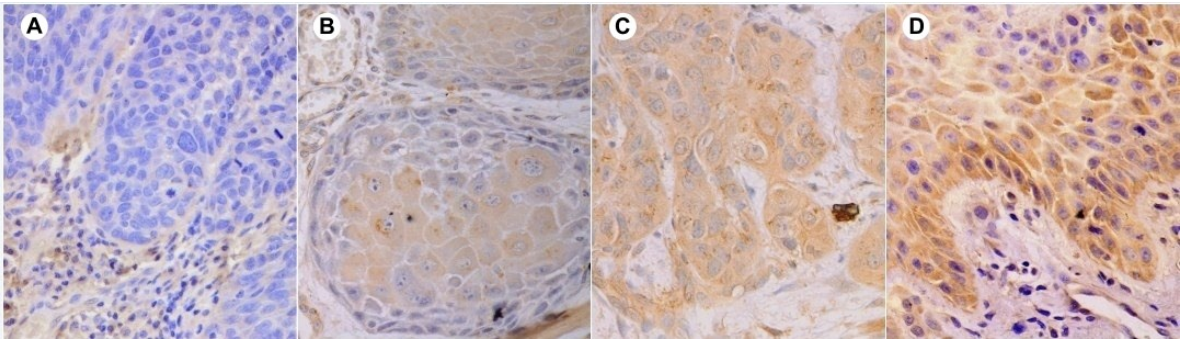


Figure 1. Expression pattern of β_1-AR in OSCC specimens. Qualitative assessment showed negative **(A)**, weak **(B)**, moderate **(C)** and strong **(D)** β_1-AR expression. (Original magnification x400).

3.4 Associations between β_2-AR expression and clinicopathological, biobehavioral and psychological characteristics

β_2-AR expression was observed in the cytoplasm and plasma membrane of OSCC cells, with granular staining pattern (Figure 2). Strong β_2-AR staining was also observed in salivary duct glands, arterial smooth muscle fibers and inflammatory cells. Considering the clinicopathological and biobehavioral features, stronger intensity of β_2-AR expression was associated with non-married marital status ($p=0.027$), living alone ($p=0.010$), occurrence of regional metastasis ($p=0.014$), and higher pain levels related to primary tumor ($p=0.044$). In quantitative assessment, associations of β_2-AR tumor expression with clinicopathological and biobehavioral characteristics do not reach significance.

In univariate analysis, anxiety and depression symptoms measured by BDI and BAI were correlated with stronger intensity of β_2-AR expression in tumor cells ($p=0.010$ and $p=0.004$, respectively). There were significant associations between β_2-AR tumor expression and four mood profiles assessed by BRUMS test (depressive mood, anger, fatigue and vigor; $p<0.05$). Similar to that found with depression symptoms assessed by BDI, higher scores of depressive mood were correlated with higher intensity and positivity of β_2-AR expression in OSCC cells ($p=0.036$ and $p=0.042$, respectively). Higher fatigue scores were correlated with stronger intensity and higher positivity of β_2-AR expression ($p=0.010$ and $p=0.026$, respectively). Vigor score measured by BRUMS was negatively correlated with β_2-AR expression intensity ($p=0.037$) (Table 4).

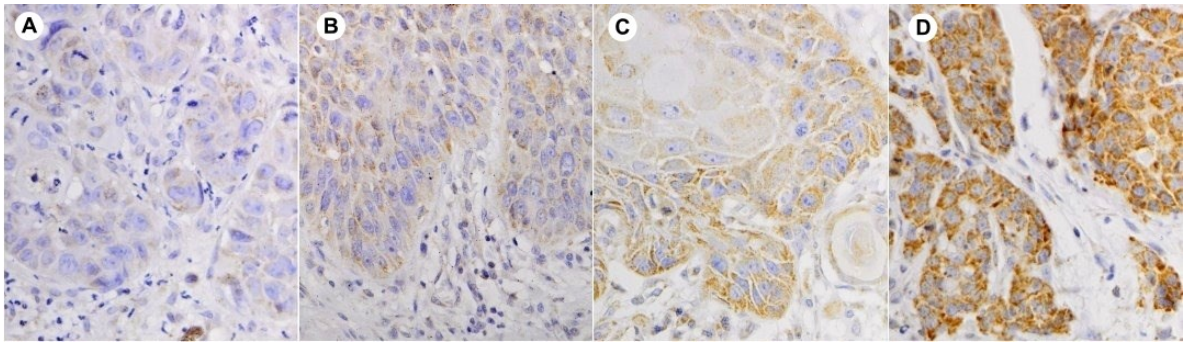


Figure 2. Expression pattern of β_2-AR in OSCC specimens. Qualitative assessment showed negative **(A)**, weak **(B)**, moderate **(C)** and strong **(D)** intensity of β_2-AR expression (Original magnification x400).

Table 4 Associations of β_1-AR and β_2-AR expression with clinicopathological, biobehavioral, and psychological characteristics in patients with OSCC.

Variables[a]	Qualitative assessment (*P-Value*)	Quantitative assessment (*P-Value*)
	β_1-AR expression	
Alcohol consumption[c]	.876	.032[b]
Higher education level	.259	.042[b,c]
Worse sleep quality[d]	.850	.044[b,c]
Anger[e]	.010[b,c]	.309
	β_2-AR expression	
Non-married marital status	.027[b,d]	.449
Living alone	.010[b,d]	.091
Regional metastasis	.014[b,d]	.205
Higher pain levels	.044[b,d]	.103
Higher depressive symptoms	.010[b,d]	.097
Higher anxiety symptoms	.004[b,d]	.519
Higher depressive mood[e]	.036[b,d]	.042[b,d]
Higher fatigue[e]	.010[b,d]	.026[b,d]
Lower vigor[e]	.037[b,d]	.068

[a]The variables' associations with the tumor expression of β1-AR reached statistical significance.
[b]Values were considered statistically significant at $p < .05$ (chi-square test or Fisher's exact test).
[c]No alcohol consumption, current drinker, or former drinker.
[d]Values were measured with the binary measure (yes or no, good or bad).
[e]Values evaluated according to population median.

3.5 Independent predictive factors for β_1- and β_2-ARs expression

To identify which variables are predictive for β_1-AR and β_2-AR expression in OSCC patients, logistic regression analyses were performed considering qualitative and quantitative expression in the OSCC samples. Multivariate analysis showed that advanced clinical staging was a protective factor for β_1-AR expression. Patients with advanced clinical stage had 86% less chance of having stronger intensity of β_1-AR expression (OR = 0.145, 95% CI = 0.025-0.828, p=0.003) (Table 5). For β_2-AR, multivariate analysis showed that anxiety symptoms measured by BAI and fatigue mood assessed by BRUMS were a risk factor for its expression in OSCC cells. Patients with no and minimum levels of anxiety symptoms had 4.2-fold increase in the chance of displaying stronger β2-AR staining intensity (OR = 4.256, 95% CI = 1.439-12.606, p=0.009) compared to OSCC patients with lower score of anxiety. Patients with higher scores for fatigue mood were 3.8 times more likely to display higher β_2-AR staining positivity (OR = 3.816, 95% CI = 1.258-11.573, p=0.018) than OSCC patients with lower score for this mood type (Table 5).

Table 5 Significant results from stepwise logistic regression analyses considering β_1-AR and β_2-AR expression as response variable and clinicopathological, biobehavioral, and psychological data from OSCC patients as independent variables.

Independent variable	Dependent Variable: β_1-AR expression					
	Qualitative evaluation (2 categories)			Quantitative evaluation (2 categories)		
	OR	95% CI	*P value*	OR	95% CI	*P value*
Clinical stage	-	-	-	0.145	0.025 – 0.828	0.03
	Dependent Variable: β_2-AR expression					
Higher anxiety symptoms [a]	4.259	1.439 - 12.606	0.009	-	-	-
Higher fatigue[b]	-	-	-	3.816	1.258 - 11.573	0.018

[a] Variable assessed by Beck Anxiety Inventory.
[b] Variable assessed by Brunel Mood Scale.

4 DISCUSSION

In the current study, our results revealed that initial clinical stage was independently associated with β_1-AR tumor expression. Furthermore, they also indicated that higher levels of anxiety and fatigue mood were independent predict factors of β_2-AR tumor expression. Chronic stress-derived catecholamines such as NE and Epi play a pivotal role in the cancer progression (Huang et al., 2011; Jeong et al., 2022). In the tumor microenvironment, catecholamines act through G protein-coupled adrenergic receptors (subtypes α_1, α_2, β_1, β_2 and β_3) located on the surface of tumor cells, endothelial cells, fibroblasts, immune cells, and other cell types (Bernabé, 2021). The adrenergic signaling activation in tumor cells can promote malignant transformation and cancer progression (Eng et al., 2014). Several studies have investigated the relationship between the expression of β-ARs in different types of tumors specimens and clinicopathological features. For example, increased tumor expression of β_2-AR was significantly associated with advanced clinical stage and occurrence of regional metastasis (Krishna et al., 2022; Liu et al., 2018). The β_2-AR expression are further correlated to OSCC patients' prognosis: stronger expression is associated with higher overall and specific survival (Bravo-Calderón et al., 2011). However, to our knowledge, no study has evaluated the association of tumor expression of β-AR with biobehavioral, clinicopathological and psychological features in OSCC patients.

OSCC patients with higher anxiety symptoms had 4.2 times more chance to have stronger β_2-AR tumor expression compared to patients with lower levels of anxiety. Previous studies have shown the importance of β_2-AR and NE in emotional and stress response (MacCormack et al., 2021). Evidences in preclinical models denote that the locus coeruleus (LC) is involved in stress response and is linked to anxiety and stress behavior (Morris et al., 2020). Chronic or repeated stress enhances LC activity releasing NE in regions related with

psychiatry disorders, including amygdala, hippocampus and prefrontal cortex, inducing a β_2-AR response (Zhang et al., 2022). Optogenetic activation of β_2-AR in medial prefrontal cortex can induce anxiety-like behavior, and the receptor lockdown generate an anxiolytic effect (Lei et al., 2022). Huang et al. evaluated β_2-AR expression in ovarian cancer samples, which results also indicate a positive correlation between β_2-AR expression and anxiety symptoms in multivariate analysis (Huang et al., 2016). Nonetheless, one of the limitations of the present study is the psychometric instrument utilized. The Beck Anxiety Inventory are not the optical measure instrument for anxiety in cancer patients. The BAI do not report clinical cut-offs specifically for cancer patients, and may identify false positives in clinical anxiety symptoms (Shunmugasundaram et al., 2020).

In multivariate analysis, our results indicated that OSCC patients with higher mood fatigue score had 3.8-fold increase in the chance of displaying higher β_2-AR staining positivity when compared to lower fatigue patients. Fatigue is the most common and distressing symptom in cancer patients (Meneses-Echávez et al., 2015). Fatigue symptoms are linked to chronic stress and autonomic nervous system dysregulation, characterized by a prolonged sympathetic overactivation (Philipp et al., 2023; Silverman et al., 2010). Chronic adrenergic stimulation in mental fatigue are associated to reduce β-AR responsiveness (Euteneuer et al., 2014). β_2-AR dysfunction has been related to chronic fatigue symptoms, and may be caused by β_2-AR autoantibodies, polymorphism and receptor desensitization (Wirth and Scheibenbogen, 2020). The hyper activation of HPA-axis in chronic fatigue patients may induce conformational changes in β_2-AR, lead to more immunogenic epitopes and production of antibodies against the receptors (Loebel et al., 2016). Hence, our interpretation for higher β_2-ARs expression in fatigue mood patients is due receptor dysfunction. Decrease of β_2-ARs responsiveness owed to chronic fatigue may increase the receptor expression in tumor cells. This is the first study that reveals an association between the expression of β_2-AR and mental

fatigue in cancer patients; however, there is a need for a deeper evaluation of β_2-ARs responsiveness and its correlation with fatigue in cancer patients.

The univariate analysis from our study demonstrated a significant correlation between higher β_2-AR tumor expression and depression symptoms, depressive mood, and lower vigor. However, these variables do not reached significance in stepwise model. Cancer diagnosis and treatment are highly stressful and potentially traumatic (Cordova et al., 2017). Depression and anxiety can affect up to 20% and 10% of cancer patients, respectively (Pitman et al., 2018). Cancer survival patients are significantly more depressed and more anxious than the general population (Götze et al., 2020). Major depressive disease (MDD) is a psychiatry disease that highly influence individuals psychosocial functional and quality of life (Malhi and Mann, 2018). This psychiatry disorder have a positive association with anger rumination and low emotional regulation (Besharat et al., 2013). In cancer patients, depression and anxiety are related with shorter overall survival and worsen prognosis (Aarstad et al., 2005; Rieke et al., 2017; Walker et al., 2021). Our results indicate a correlation between stronger β_2-AR expression in oral cancer with higher scores of depressive and anxiety symptoms, indicating a possible association between psychiatry disorders and adrenergic pathway in tumor microenvironment. Additionally, our results indicate a positive correlation between negative mood profiles with higher β_2-AR expression. Similar results was also identified by the study conducted by Huang et al. in ovarian cancer patients (Huang et al., 2016). The use of beta-adrenoceptor agonists in human studies revealed anti-depressive effects, indicating the association between the adrenergic pathway in depression and resilience to stress (Lecrubier et al., 1981; Simon et al., 1978).

In the current study, stepwise logistic regression revealed that OSCC patients with advanced clinical staging had 86% less chance of having higher β_1-AR tumor staining positivity. However, previous studies indicate a positive correlation between advanced

clinical stage and higher β_1-AR tumor expression in OSCC patients (Dong et al., 2017; C. Zhang et al., 2020a). β-adrenergic signaling pathways may affect the cancer prognosis (Eng et al., 2014). Previous studies showed that a high tumor expression of β1-ARs and β2-ARs predict reduced survival rate in OSCC patients (Dong et al., 2017; Krishna et al., 2022; Liu et al., 2018). Otherwise, Bravo-Calderón et al. (2011) revealed that OSCC patients who showed a strong tumor expression of β_2-AR had higher overall and cancer-specific survival than those individuals with weak/negative expression of β_2-AR. Regional metastasis is a strong prognostic factor in OSCC patients, having important impact on disease staging and patient survival. Our study reported an association between β_2-AR, but not β_1-AR, with cervical metastasis in the univariate analysis. Other studies also identified the association between β_2-AR expression with lymphatic metastasis (Dong et al., 2017; Krishna et al., 2022; Shang et al., 2009; C. Zhang et al., 2020b). These findings demonstrated that the high intensity expression of β_2-AR in OSCC may play a critical role in cervical lymph node metastasis, suggesting that tumor β-adrenergic signaling may promote cancer progression and affects patient outcome. Cervical lymph node metastasis is a strong prognostic factor in OSCC patients, representing a significate impact on patient survival.

The current study has some limitations. First, the staining evaluation of the receptor in quantitative and qualitative analysis was performed separately, we did not evaluate the multiplication of both results. Therefore, our results may not indicate the whole tumor sample evaluated. For the variables sleep quality and primary tumor related pain our study only evaluated the patient report. Perineural invasion in tumor is also related to pain, and the use of sleep medications can mask the quality of the patient's sleep. However, these two data were not evaluated in our sample. Further, ARs activity and its responsiveness in tumor microenvironment was not accessed, and we did not measure the plasma and tumor levels of the receptor ligands, including Epi and NE.

Nonetheless, expression of β-ARs was positively associated with clinicopathological and psychological data from the OSCC patients. Taken together, our results reveal for the first time that psychological symptoms, such as anxiety and fatigue symptoms, are predictive factors for β_2-AR expression in OSCC cells. Our data provide additional support that patient's psychological features can influence β-adrenergic signaling in oral cancer microenvironment.

REFERENCES

Aarstad, H.J., Aarstad, A.K.H., Heimdal, J.H., Olofsson, J., 2005. Mood, anxiety and sense of humor in head and neck cancer patients in relation to disease stage, prognosis and quality of life. Acta Otolaryngol. 125, 557–565. https://doi.org/10.1080/00016480510027547

Al-Wadei, H.A.N., Plummer, H.K., Ullah, M.F., Unger, B., Brody, J.R., Schuller, H.M., 2012. Social Stress Promotes and γ-Aminobutyric Acid Inhibits Tumor Growth in Mouse Models of Non–Small Cell Lung Cancer. Cancer Prev. Res. 5, 189–196. https://doi.org/10.1158/1940-6207.CAPR-11-0177

Amin, M.B., Greene, F.L., Edge, S.B., Compton, C.C., Gershenwald, J.E., Brookland, R.K., Meyer, L., Gress, D.M., Byrd, D.R., Winchester, D.P., 2017. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA. Cancer J. Clin. 67, 93–99. https://doi.org/10.3322/caac.21388

Antoni, M.H., Lutgendorf, S.K., Cole, S.W., Dhabhar, F.S., Sephton, S.E., McDonald, P.G., Stefanek, M., Sood, A.K., 2006. The influence of bio-behavioural factors on tumour biology: pathways and mechanisms. Nat. Rev. Cancer 6, 240–248. https://doi.org/10.1038/nrc1820

Bastos, D.B., Sarafim-Silva, B.A.M., Sundefeld, M.L.M.M., Ribeiro, A.A., Brandão, J.D.P., Biasoli, É.R., Miyahara, G.I., Casarini, D.E., Bernabé, D.G., 2018. Circulating catecholamines are associated with biobehavioral factors and anxiety symptoms in head and neck cancer patients. PLoS One 13, 1–18. https://doi.org/10.1371/journal.pone.0202515

Batty, G.D., Russ, T.C., Stamatakis, E., Kivimäki, M., 2017. Psychological distress in relation to site specific cancer mortality: pooling of unpublished data from 16 prospective cohort studies. BMJ j108. https://doi.org/10.1136/bmj.j108

Beck, A., Steer, R., Ball, R., Ranieri, W., 1996. Comparison of Beck Depression Inventories -IA and -II in Psychiatric Outpatients. J. Pers. Assess. 67, 588–597. https://doi.org/10.1207/s15327752jpa6703

Beck, A.T., Epstein, N., Brown, G., Steer, R.A., 1988. An Inventory for Measuring Clinical Anxiety: Psychometric Properties. J. Consult. Clin. Psychol. 56, 4–5.

Bernabé, D.G., 2021. Catecholamines Mediate Psychologic Stress–Induced Cancer Progression. Cancer Res. 81, 5144–5146. https://doi.org/10.1158/0008-5472.can-21-3077

Bernabé, D.G., Tamae, A.C., Biasoli, É.R., Oliveira, S.H.P., 2011. Stress hormones increase cell proliferation and regulates interleukin-6 secretion in human oral squamous cell carcinoma cells. Brain. Behav. Immun. 25, 574–583. https://doi.org/10.1016/j.bbi.2010.12.012

Bernabé, D.G., Tamae, A.C., Miyahara, G.I., Sundefeld, M.L.M., Oliveira, S.P., Biasoli, É.R., 2012. Increased plasma and salivary cortisol levels in patients with oral cancer and their association with clinical stage. J. Clin. Pathol. 65, 934–939. https://doi.org/10.1136/jclinpath-2012-200695

Besharat, M.A., Nia, M.E., Farahani, H., 2013. Anger and major depressive disorder: The mediating role of emotion regulation and anger rumination. Asian J. Psychiatr. 6, 35–41.

https://doi.org/10.1016/j.ajp.2012.07.013

Bravo-Calderón, D.M., Oliveira, D.T., Marana, A.N., Nonogaki, S., Carvalho, A.L., Kowalski, L.P., 2011. Prognostic significance of beta-2 adrenergic receptor in oral squamous cell carcinoma. Cancer Biomarkers 10, 51–59. https://doi.org/10.3233/CBM-2012-0228

Cecilio, H.P., Valente, V.B., Pereira, K.M., Kayahara, G.M., Furuse, C., Biasoli, É.R., Miyahara, G.I., Oliveira, S.H.P., Bernabé, D.G., 2020. Beta - adrenergic blocker inhibits oral carcinogenesis and reduces tumor invasion. Cancer Chemother. Pharmacol. 86, 681–686. https://doi.org/10.1007/s00280-020-04149-2

Charlson, M.E., Pompei, P., Ales, K.L., MacKenzie, C.R., 1987. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. J. Chronic Dis. 40, 373–383. https://doi.org/10.1016/0021-9681(87)90171-8

Cole, S.W., Nagaraja, A.S., Lutgendorf, S.K., Green, P.A., Sood, A.K., 2015. Sympathetic nervous system regulation of the tumour microenvironment. Nat. Rev. Cancer 15, 563–572. https://doi.org/10.1038/nrc3978

Cole, S.W., Sood, A.K., 2012. Molecular pathways: Beta-adrenergic signaling in cancer. Clin. Cancer Res. 18, 1201–1206. https://doi.org/10.1158/1078-0432.CCR-11-0641

Cordova, M.J., Riba, M.B., Spiegel, D., 2017. Post-traumatic stress disorder and cancer. The Lancet Psychiatry 4, 330–338. https://doi.org/10.1016/S2215-0366(17)30014-7

Dai, S., Mo, Y., Wang, Y., Xiang, B., Liao, Q., Zhou, M., Li, X., Li, Y., Xiong, W., Li, G., Guo, C., Zeng, Z., 2020. Chronic Stress Promotes Cancer Development. Front. Oncol. 10, 1–10. https://doi.org/10.3389/fonc.2020.01492

De Giorgi, V., Gandini, S., Grazzini, M., Benemei, S., Marchionni, N., Geppetti, P., 2013. Effect of β-blockers and other antihypertensive drugs on the risk of melanoma recurrence and death. Mayo Clin. Proc. 88, 1196–1203. https://doi.org/10.1016/j.mayocp.2013.09.001

Dong, H., Liao, X.-X., Mai, H.-M., Zhou, N., Wang, D.-Y., Peng, J.-B., Yang, L.-H., 2017. Expression of beta adrenergic receptor in oral squamous cell carcinoma and its significance to the prognosis. Int J Clin Exp Pathol.

Eng, J.W.-L., Kokolus, K.M., Reed, C.B., Hylander, B.L., Ma, W.W., Repasky, E.A., 2014. A nervous tumor microenvironment: the impact of adrenergic stress on cancer cells, immunosuppression, and immunotherapeutic response. Cancer Immunol. Immunother. 63, 1115–1128. https://doi.org/10.1007/s00262-014-1617-9

Euteneuer, F., Ziegler, M.G., Mills, P.J., Rief, W., Dimsdale, J.E., 2014. In Vivo β-Adrenergic Receptor Responsiveness: Ethnic Differences in the Relationship with Symptoms of Depression and Fatigue. Int. J. Behav. Med. 21, 843–850. https://doi.org/10.1007/s12529-013-9359-1

Figueira, J.A., Sarafim-Silva, B.A.M., Gonçalves, G.M., Aranha, L.N., Lopes, F.L., Corrente, J.E., Biasoli, É.R., Miyahara, G.I., Bernabé, D.G., 2022. Predisposing factors for increased cortisol levels in oral cancer patients. Compr. Psychoneuroendocrinology 9, 100110. https://doi.org/10.1016/j.cpnec.2022.100110

Glaser, R., Kiecolt-glaser, J.K., 2005. Stress-induced immune dysfunction: implications for health. Nat. Rev. Immunol. 5. https://doi.org/10.1038/nri1571

Götze, H., Friedrich, M., Taubenheim, S., Dietz, A., Lordick, F., Mehnert, A., 2020. Depression and anxiety in long-term survivors 5 and 10 years after cancer diagnosis. Support. Care Cancer 28, 211–220. https://doi.org/10.1007/s00520-019-04805-1

Huang, T., Tworoger, S.S., Hecht, J.L., Rice, M.S., Sood, A.K., Kubzansky, L.D., Poole, E.M., 2016. Association of Ovarian Tumor β2-Adrenergic Receptor Status with Ovarian Cancer Risk Factors and Survival. Cancer Epidemiol. Biomarkers Prev. 25, 1587–1594. https://doi.org/10.1158/1055-9965.EPI-16-0534

Huang, X., Wang, H.-C., Yuan, Z., Huang, J., Zheng, Q., 2011. Norepinephrine Stimulates Pancreatic Cancer Cell Proliferation, Migration and Invasion Via ß-Adrenergic Receptor-Dependent Activation of P38/MAPK Pathway. Hepatogastroenterology 59. https://doi.org/10.5754/hge11476

IARC, I.A. for R. on C., 2022. Global Cancer Observactory [WWW Document]. URL https://gco.iarc.fr

Jeong, J.-H., Park, H.-J., Park, S.-H., Choi, Y.-H., Chi, G.-Y., 2022. β2-Adrenergic Receptor Signaling Pathway Stimulates the Migration and Invasion of Cancer Cells via Src Activation. Molecules 27, 5940. https://doi.org/10.3390/molecules27185940

Johnson, D.E., Burtness, B., Leemans, C.R., Lui, V.W.Y., Bauman, J.E., Grandis, J.R., 2021. Head and neck squamous cell carcinoma. Nat. Rev. Dis. Prim. 6. https://doi.org/10.1038/s41572-020-00224-3

Koh, M., Takahashi, T., Kurokawa, Y., Kobayashi, T., Saito, T., Ishida, T., Serada, S., Fujimoto, M., Naka, T., Wada, N., Yamashita, K., Tanaka, K., Miyazaki, Y., Makino, T., Nakajima, K., Yamasaki, M., Eguchi, H., Doki, Y., 2021. Propranolol suppresses gastric cancer cell growth by regulating proliferation and apoptosis. Gastric Cancer 24, 1037–1049. https://doi.org/10.1007/s10120-021-01184-7

Krishna, A., Singh, V., Singh, N., Singh, S., Mohanty, S.K., Singh, R., Kumar, V., Singh, U.S., Singh, R.K., 2022. Expression pattern and clinical significance of beta 2-adrenergic receptor in oral squamous cell carcinoma: an emerging prognostic indicator and future therapeutic target. Clin. Transl. Oncol. 24, 2191–2199. https://doi.org/10.1007/s12094-022-02879-8

Kwon, S., Chun, K., Kil, H., Jung, N., Shin, H.-A., Jang, J., Choi, H., Oh, K.-H., Kim, M.-S., 2021. β2-adrenergic receptor expression and the effects of norepinephrine and propranolol on various head and neck cancer subtypes. Oncol. Lett. 22, 804. https://doi.org/10.3892/ol.2021.13065

Lane, A.M., Terry, P.C., 2000. The Nature of Mood: Development of a Conceptual Model with a Focus on Depression. J. Appl. Sport Psychol. 12, 16–33. https://doi.org/10.1080/10413200008404211

Lecrubier, Y., Puech, A.J., Frances, H., Jouvent, R., Widlöcher, D., Simon, P., 1981. Beta-adrenergic stimulation and antidepressant activity. Acta Psychiatr. Scand. 63, 173–178. https://doi.org/10.1111/j.1600-0447.1981.tb00718.x

Lei, Z., Lam, Y., Li, C., Fu, Z., Ramkrishnan, A.S., Liu, S., Li, Y., 2022. β2-Adrenoceptors in the Medial Prefrontal Cortex Excitatory Neurons Regulate Anxiety-like Behavior in Mice. Int. J. Mol. Sci. 23. https://doi.org/10.3390/ijms23105578

Liu, H., Wang, C., Xie, N., Zhuang, Z., Liu, X., Hou, J., Huang, H., 2018. Activation of

adrenergic receptor β2 promotes tumor progression and epithelial mesenchymal transition in tongue squamous cell carcinoma. Int. J. Mol. Med. 41, 147–154. https://doi.org/10.3892/ijmm.2017.3248

Loebel, M., Grabowski, P., Heidecke, H., Bauer, S., Hanitsch, L.G., Wittke, K., Meisel, C., Reinke, P., Volk, H.D., Fluge, O., Mella, O., Scheibenbogen, C., 2016. Antibodies to β adrenergic and muscarinic cholinergic receptors in patients with Chronic Fatigue Syndrome. Brain. Behav. Immun. 52, 32–39. https://doi.org/10.1016/j.bbi.2015.09.013

MacCormack, J.K., Armstrong-Carter, E.L., Gaudier-Diaz, M.M., Meltzer-Brody, S., Sloan, E.K., Lindquist, K.A., Muscatell, K.A., 2021. β-Adrenergic Contributions to Emotion and Physiology During an Acute Psychosocial Stressor. Psychosom. Med. 83, 959–968. https://doi.org/10.1097/PSY.0000000000001009

Malhi, G.S., Mann, J.J., 2018. Depression. Lancet 392, 2299–2312. https://doi.org/10.1016/S0140-6736(18)31948-2

Meneses-Echávez, J.F., González-Jiménez, E., Ramírez-Vélez, R., 2015. Effects of supervised exercise on cancer-related fatigue in breast cancer survivors: a systematic review and meta-analysis. BMC Cancer 15, 77. https://doi.org/10.1186/s12885-015-1069-4

Moretti, S., Massi, D., Farini, V., Baroni, G., Parri, M., Innocenti, S., Cecchi, R., Chiarugi, P., 2013. β-adrenoceptors are upregulated in human melanoma and their activation releases pro-tumorigenic cytokines and metalloproteases in melanoma cell lines. Lab. Investig. 93, 279–290. https://doi.org/10.1038/labinvest.2012.175

Morris, L.S., McCall, J.G., Charney, D.S., Murrough, J.W., 2020. The role of the locus coeruleus in the generation of pathological anxiety. Brain Neurosci. Adv. 4, 239821282093032. https://doi.org/10.1177/2398212820930321

Pérez-Sayáns, M., Somoza-Martín, J., Barros-Angueira, F., Gayoso-Diz, P., Otero-Rey, E.M., Gándra-Rey, J.M., García-García, A., 2012. Activity of β2-adrenergic receptor in oral squamous cell carcinoma is mediated by overexpression of the ADRBK2 gene: A pilot study. Biotech. Histochem. 87, 179–186. https://doi.org/10.3109/10520295.2011.592151

Philipp, J., Baerwald, C.G., Seifert, O., 2023. Association between the Ile164 β2 Adrenergic Receptor Polymorphism and Fatigue in Patients with Rheumatoid Arthritis. Neuroimmunomodulation 93–101. https://doi.org/10.1159/000528206

Pitman, A., Suleman, S., Hyde, N., Hodgkiss, A., 2018. Depression and anxiety in patients with cancer. BMJ 361, 1–6. https://doi.org/10.1136/bmj.k1415

Powe, D.G., Voss, M.J., Habashy, H.O., Zänker, K.S., Green, A.R., Ellis, I.O., Entschladen, F., 2011. Alpha- and beta-adrenergic receptor (AR) protein expression is associated with poor clinical outcome in breast cancer: an immunohistochemical study. Breast Cancer Res. Treat. 130, 457–463. https://doi.org/10.1007/s10549-011-1371-z

Rieke, K., Schmid, K.K., Lydiatt, W., Houfek, J., Boilesen, E., Watanabe-Galloway, S., 2017. Depression and survival in head and neck cancer patients. Oral Oncol. 65, 76–82. https://doi.org/10.1016/j.oraloncology.2016.12.014

Sarafim-Silva, B.A.M., Duarte, G.D., Sundefeld, M.L.M.M., Biasoli, É.R., Miyahara, G.I., Bernabé, D.G., 2018. Childhood trauma is predictive for clinical staging, alcohol consumption, and emotional symptoms in patients with head and neck cancer. Cancer

124, 3684–3692. https://doi.org/10.1002/cncr.31597

Shaashua, L., Shabat-Simon, M., Haldar, R., Matzner, P., Zmora, O., Shabtai, M., Sharon, E., Allweis, T., Barshack, I., Hayman, L., Arevalo, J., Ma, J., Horowitz, M., Cole, S., Ben-Eliyahu, S., 2017. Perioperative COX-2 and β-Adrenergic Blockade Improves Metastatic Biomarkers in Breast Cancer Patients in a Phase-II Randomized Trial. Clin. Cancer Res. 23, 4651–4661. https://doi.org/10.1158/1078-0432.CCR-17-0152

Shang, Z.J., Liu, K., Liang, D.F., 2009. Expression of β2-adrenergic receptor in oral squamous cell carcinoma. J. Oral Pathol. Med. 38, 371–376. https://doi.org/10.1111/j.1600-0714.2008.00691.x

Shunmugasundaram, C., Rutherford, C., Butow, P.N., Sundaresan, P., Dhillon, H.M., 2020. What are the optimal measures to identify anxiety and depression in people diagnosed with head and neck cancer (HNC): a systematic review. J. Patient-Reported Outcomes 4, 26. https://doi.org/10.1186/s41687-020-00189-7

Silverman, M.N., Heim, C.M., Nater, U.M., Marques, A.H., Sternberg, E.M., 2010. Neuroendocrine and Immune Contributors to Fatigue. PM&R 2, 338–346. https://doi.org/10.1016/j.pmrj.2010.04.008

Simon, P., Lecrubier, Y., Jouvent, R., Puech, A.J., Allilaire, J.F., Widlöcher, D., 1978. Experimental and clinical evidence of the antidepressant effect of a beta-adrenergic stimulant. Psychol. Med. 8, 335–338. https://doi.org/10.1017/S0033291700014392

Smith, R., Weihs, K.L., Alkozei, A., Killgore, W.D.S., Lane, R.D., 2019. An Embodied Neurocomputational Framework for Organically Integrating Biopsychosocial Processes: An Application to the Role of Social Support in Health and Disease. Psychosom. Med. 81, 125–145. https://doi.org/10.1097/PSY.0000000000000661

Terry, P.C., Lane, A.M., Fogarty, G.J., 2003. Construct validity of the Profile of Mood States - Adolescents for use with adults. Psychol. Sport Exerc. 4, 125–139. https://doi.org/10.1016/S1469-0292(01)00035-8

Terry, P.C., Lane, A.M., Lane, H.J., Keohane, L., 1999. Development and validation of a mood measure for adolescents. J. Sports Sci. 17, 861–872. https://doi.org/10.1080/026404199365425

Thaker, P.H., Han, L.Y., Kamat, A.A., Arevalo, J.M., Takahashi, R., Lu, C., Jennings, N.B., Armaiz-Pena, G., Bankson, J.A., Ravoori, M., Merritt, W.M., Lin, Y.G., Mangala, L.S., Kim, T.J., Coleman, R.L., Landen, C.N., Li, Y., Felix, E., Sanguino, A.M., Newman, R.A., Lloyd, M., Gershenson, D.M., Kundra, V., Lopez-Berestein, G., Lutgendorf, S.K., Cole, S.W., Sood, A.K., 2006. Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. Nat. Med. 12, 939–944. https://doi.org/10.1038/nm1447

Tjioe, K.C., Cardoso, D.M., Oliveira, S.H.P., Bernabé, D.G., 2022. Stress hormone norepinephrine incites resistance of oral cancer cells to chemotherapy. Endocr. Relat. Cancer 29, 201–212. https://doi.org/10.1530/ERC-20-0460

Verza, F.A., Valente, V.B., Oliveira, L.K., Kayahara, G.M., Crivelini, M.M., Furuse, C., Biasoli, É.R., Miyahara, G.I., Oliveira, S.H.P., Bernabé, D.G., 2021. Social isolation stress facilitates chemically induced oral carcinogenesis. PLoS One 16, e0245190. https://doi.org/10.1371/journal.pone.0245190

Walker, J., Mulick, A., Magill, N., Symeonides, S., Gourley, C., Burke, K., Belot, A., Quartagno, M., van Niekerk, M., Toynbee, M., Frost, C., Sharpe, M., 2021. Major Depression and Survival in People With Cancer. Psychosom. Med. 83, 410–416. https://doi.org/10.1097/PSY.0000000000000942

Wang, C., Liu, X., Huang, H., Ma, H., Cai, W., Hou, J., Huang, L., Dai, Y., Yu, T., Zhou, X., 2012. Deregulation of Snai2 is associated with metastasis and poor prognosis in tongue squamous cell carcinoma. Int. J. Cancer 130, 2249–2258. https://doi.org/10.1002/ijc.26226

Wirth, K., Scheibenbogen, C., 2020. A Unifying Hypothesis of the Pathophysiology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): Recognitions from the finding of autoantibodies against ß2-adrenergic receptors. Autoimmun. Rev. 19, 102527. https://doi.org/10.1016/j.autrev.2020.102527

Zhang, C., Liao, X., Ma, Z., Liu, S., Fang, F., Mai, H., 2020a. Overexpression of β-Adrenergic Receptors and the Suppressive Effect of β2-Adrenergic Receptor Blockade in Oral Squamous Cell Carcinoma. J. Oral Maxillofac. Surg. 78, 1871.e1-1871.e23. https://doi.org/10.1016/j.joms.2020.05.031

Zhang, C., Liao, X., Ma, Z., Liu, S., Fang, F., Mai, H., 2020b. Overexpression of β-Adrenergic Receptors and the Suppressive Effect of β2-Adrenergic Receptor Blockade in Oral Squamous Cell Carcinoma. J. Oral Maxillofac. Surg. 78, 1871.e1-1871.e23. https://doi.org/10.1016/j.joms.2020.05.031

Zhang, H., Cui, M., Cao, J.L., Han, M.H., 2022. The Role of Beta-Adrenergic Receptors in Depression and Resilience. Biomedicines 10, 1–13. https://doi.org/10.3390/biomedicines10102378

Zhang, J., Deng, Y. tiao, Liu, J., Wang, Y. qing, Yi, T. wu, Huang, B. yan, He, S. sha, Zheng, B., Jiang, Y., 2016. Norepinephrine induced epithelial–mesenchymal transition in HT-29 and A549 cells in vitro. J. Cancer Res. Clin. Oncol. 142, 423–435. https://doi.org/10.1007/s00432-015-2044-9

Zhang, M., Wang, Q., Sun, X., Yin, Q., Chen, J., Xu, L., Xu, C., 2020. β 2 -adrenergic receptor signaling drives prostate cancer progression by targeting the Sonic hedgehog-Gli1 signaling activation. Prostate 80, 1328–1340. https://doi.org/10.1002/pros.24060

Zhang, Z.F., Feng, X.S., Chen, H., Duan, Z.J., Wang, L.X., Yang, D., Liu, P.X., Zhang, Q.P., Jin, Y.L., Sun, Z.G., Liu, H., 2016. Prognostic significance of synergistic hexokinase-2 and beta2-adrenergic receptor expression in human hepatocelluar carcinoma after curative resection. BMC Gastroenterol. 16, 1–10. https://doi.org/10.1186/s12876-016-0474-8

Zhong, S., Yu, D., Zhang, X., Chen, X., Yang, S., Tang, J., Zhao, J., Wang, S., 2016. β-blocker use and mortality in cancer patients: Systematic review and meta-analysis of observational studies. Eur. J. Cancer Prev. 25, 440–448. https://doi.org/10.1097/CEJ.0000000000000192

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

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

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

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: 1
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: 3.838.032

Apresentação do Projeto:
Desenho do estudo: Observacional
A pesquisa será realizada através da coleta de dados provenientes dos prontuários clínicos dos pacientes como também a coleta de sangue periférico para dosagens hormonais de catecolaminas e aplicação de teste psicológico realizado por uma profissional capacitada na área da psicologia. presente estudo incluirá um total de 140 pacientes e voluntários que serão divididos nos seguintes grupos: Grupo 1: 80 pacientes portadores de CEC de boca; Grupo 2: 30 pacientes portadores com leucoplasia bucal; Grupo 3: 30 voluntários saudáveis. Para todos os grupos uma ficha individual será utilizada para a coleta de variáveis sociodemográficas (idade, sexo, cor, estado civil, grau de escolaridade, renda familiar e se o paciente mora sozinho ou não), variáveis clinicopatológicas (localização do tumor, classificação TNM, estadiamento clínico, presença de comorbidade e grau histopatológico do tumor) e biocomportamentais (histórico do consumo de tabaco e álcool e qualidade e duração do sono durante os últimos 7 dias antecedentes à consulta médica). O estadiamento clínico será definido de acordo com a UICC. A intensidade do histórico de consumo e intensidade de tabaco e álcool será avaliada por meio de uma escala de escore de 1 a 4 pontos de acordo com a quantidade de consumo de cigarros ou doses de bebida alcoólica por dia.

Endereço: JOSE BONIFACIO 1193
Bairro: VILA MENDONCA **CEP:** 16.015-050
UF: SP **Município:** ARACATUBA
Telefone: (18)3636-3200 **Fax:** (18)3636-3332 **E-mail:** andrebertoz@foa.unesp.br

Objetivo da Pesquisa:

Objetivo Primário:

• Avaliar a associação das concentrações plasmáticas de mediadores relacionados ao estresse com as variações de humor em pacientes com CEC de boca e sua influência sobre o prognóstico da doença.

Objetivo Secundário:

• Avaliar os níveis plasmáticos de norepinefrina, epinefrina e IL-6 em pacientes portadores de CEC de boca, leucoplasia bucal e voluntários saudáveis sem câncer.• Comparar os níveis plasmáticos de norepinefrina, epinefrina e IL-6 dos pacientes portadores de CEC de boca entre as diferentes fases do tratamento oncológico. • Comparar as concentrações de norepinefrina, epinefrina e IL-6 no microambiente tecidual entre as amostras de CEC de boca, leucoplasia e mucosa bucal normal.• Investigar a associação entre as características emocionais relacionadas à variação de humor e as concentrações plasmáticas e teciduais de norepinefrina, epinefrina e IL-6 em pacientes com câncer de boca.• Analisar a influência dos níveis plasmáticos pré-tratamento de norepinefrina, epinefrina e IL-6 sobre o prognóstico do câncer de boca.

Avaliação dos Riscos e Benefícios:

Riscos:

A participação nesta pesquisa oferece riscos mínimos, pois sua metodologia é: coleta de sangue, cuja técnica é conhecida e aplicada em laboratórios de diagnóstico. Neste projeto, a coleta de sangue será realizada por profissionais capacitados. O teste psicológico que será utilizado é validado na população brasileira e será aplicado por profissional treinado. Nenhum dos procedimentos que serão realizados irá oferecer riscos à dignidade do paciente. Todas as informações coletadas neste estudo serão estritamente confidenciais.

Benefícios:

Esta pesquisa não irá fornecer nenhum benefício direto ao paciente. Entretanto, será oferecida ao grupo controle uma avaliação clínica intrabucal com o objetivo de realizar o diagnóstico precoce de lesões benignas e malignas. Caso for identificada alguma lesão bucal o voluntário será encaminhado para tratamento na FOA/UNESP. Além disso, o paciente portador será encaminhado a profissionais ou centros psicológicos se houver necessidade. Esperamos que os resultados do presente estudo possam trazer maiores evidências sobre a possível participação de fatores

Endereço: JOSE BONIFACIO 1193
Bairro: VILA MENDONCA **CEP:** 16.015-050
UF: SP **Município:** ARACATUBA
Telefone: (18)3636-3200 **Fax:** (18)3636-3332 **E-mail:** andrebertoz@foa.unesp.br

psicológicos ainda não investigados na etiopatogenia do câncer de boca. Além disso, os resultados poderão, em longo prazo, estimular o desenvolvimento de abordagens terapêuticas mais completas para o tratamento do câncer, que integrem a atenção aos fatores emocionais e suas manifestações clínicas.

Comentários e Considerações sobre a Pesquisa:

Nada a comentar.

Considerações sobre os Termos de apresentação obrigatória:

Incluir no TCLE risco minimo.

Recomendações:

Corrigir TCLE

Conclusões ou Pendências e Lista de Inadequações:

adequar TCLE .

Atenção especial deverá ser dada ao cronograma que será apresentado, visto que nenhum protocolo já em andamento será autorizado pelo sistema CEP/CONEP.

Considerações Finais a critério do CEP:

O CEP solicita que sejam tomadas as providências cabíveis para sanar as pendências apontadas. Informa ao(a) senhor(a) pesquisador(a) que o prazo máximo para o atendimento dessas pendências será de 30 (trinta) dias corridos a partir da emissão deste parecer. Caso o prazo não seja respeitado e/ou as pendências atendidas a contento, o protocolo será retirado do sistema e, portanto, cancelado. No momento da inclusão das alterações no sistema da Plataforma Brasil atenção especial deverá ser dada ao cronograma que será apresentado, visto que nenhum protocolo já em andamento será autorizado pelo sistema CEP/CONEP.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1491568.pdf	10/02/2020 09:56:58		Aceito
Folha de Rosto	Folha_de_rosto.pdf	10/02/2020	DANIELA BRITO	Aceito

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

Continuação do Parecer: 3.838.032

Folha de Rosto	Folha_de_rosto.pdf	09:55:25	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
Outros	QuestionarioBRUMS.pdf	29/01/2020 08:54:51	DANIELA BRITO BASTOS	Aceito

Situação do Parecer:
Pendente

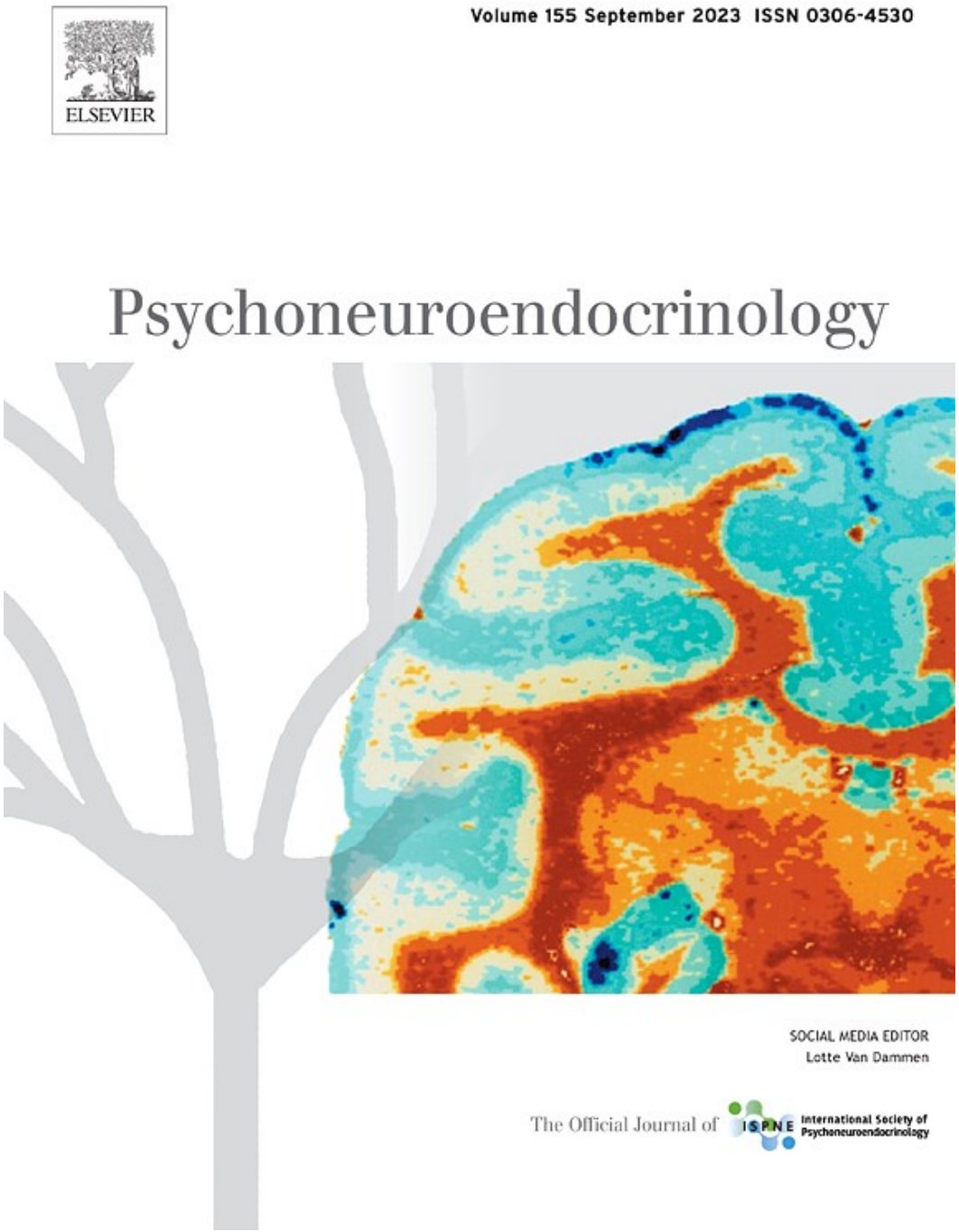
Necessita Apreciação da CONEP:
Não

ARACATUBA, 14 de Fevereiro de 2020

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

Endereço: JOSE BONIFACIO 1193
Bairro: VILA MENDONCA **CEP:** 16.015-050
UF: SP **Município:** ARACATUBA
Telefone: (18)3636-3200 **Fax:** (18)3636-3332 **E-mail:** andrebertoz@foa.unesp.br

ANEXO B – Normas de publicação da revista Psychoneuroendocrinology



Psychoneuroendocrinology Online ISSN: 1873-3360

Acesso em:

https://www.sciencedirect.com/journal/psychoneuroendocrinology