Research Paper

Might anxiety disorders promote head and neck cancer development?

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A B S T R A C T

Cancer patients present a higher risk of experiencing anxiety disorders (AD). However, it is not clear if AD might be associated with cancer development. Thus, our study aimed to evaluate if AD might be related to head and neck squamous cell carcinoma (HNSCC) development. The combination of an applied animal basic study and a retrospective diagnostic case and control study in patients was performed. As a result, we obtained that stress reduced the locomotor activity of the animals in the group stress and stress + 4NqO (p < 0.0001). The stress showed no influence on the progression of neoplasia in mice. In the same way, the case group did not present differences in anxiety scores in comparison to control. Moreover, no association between HNSCC staging and anxiety scores was observed. In conclusion, our in vivo findings in humans and animals have shown that there is no relationship between AD and oral squamous cell carcinoma.

1. Introduction

Anxiety disorders (AD) include disorders that share features of excessive fear and anxiety besides related behavioral disturbances (American Psychiatric Association, 2013). Modern life has many stress factors related to anxiety disorders (McLaughlin and Hatzenbuehler, 2009). Currently, the prevalence of AD is high in population subgroups across the globe (Remes et al., 2016). AD is not only commonly associated with other mental disorders (Disease et al., 2017) but also with head and neck diseases (Gomes et al., 2013; Mendes et al., 2013; Gomes et al., 2019). Interestingly enough, some studies lack the association of stressed people and head and neck diseases (Perdigao et al., 2007; Godinho et al., 2011; Mendes et al., 2013). The way that AD interferes with head and neck diseases still controversial.

Cancer is recognized as a threat to global development; in 2017, there were 24.5 million incident cancer cases worldwide and 9.6 million cancer deaths (Global Burden of Disease Cancer et al., 2019). In developing countries, head and neck squamous cell carcinoma (HNSCC) is a major global public health problem (De Paula et al., 2009; Pinheiro et al., 2015). Despite progress in research and therapy, patient survival has not significantly improved in recent years, posing a significant challenge for science (Fraga et al., 2012; Oliveira et al., 2014) from 15 to 61 % of HNSCC arising derived from leukoplakia (Domingos et al., 2017). Although retrospective cohort studies have shown that younger individuals are also at risk for the development of this neoplasm, HNSCC usually develops in middle-aged and older individuals (Farias et al., 2010; Marques-Silva et al., 2012; Bewley and Farwell, 2017). HNSCC is a preventable disease, in which smoking and alcohol, considered the main risk factors, is associated with 90 % of the cases (De Paula et al., 2009). Tobacco and alcohol show a synergistic effect. The risk of developing HNSCC is five to nine times higher for smokers than for non-smokers (Bewley and Farwell, 2017). Another risk factor, such as human papillomavirus, also has a role in HNSCC development (Marques-Silva et al., 2012).

The commonly used treatment for this neoplasm is surgical excision alone or with radiation therapy and chemotherapy (Solomon et al., 2018). Studies report that the patient with HNSCC most often suffers from the physical, mental, and psychosomatic impacts inherent to the diagnosis of the neoplasia and its treatment; above all, it is possible to highlight Anxiety and depression as a result of such impacts (Chen

et al., 2009). On the other hand, recently, it was demonstrated that HNSCC patients display sympathetic nervous system hyperactivity and that changes in circulating catecholamines may be associated with al-cohol consumption(Bastos et al., 2018). Moreover, chronic stress was related to oral carcinoma growth rate and progression (Xie et al., 2015). It was suggested that stress hormones might affect HNSCC behavior by influencing the tumor micro-environment through the circulating blood (Xie et al., 2014). Taken all these facts together, the purpose of the currents study is to evaluate if anxiety could interfere in the HNSCC development and staging.

2. Materials and methods

2.1. Ethical approval

2.1.1. Animal study

As it is a study with animal experimentation, this study was sub-mitted and approved by the Ethics Committee on Animal Experimentation and Welfare - CEEBEA of the Universidade Estadual de Montes Claros, Minas Gerais, Brazil through the process nº 140/2017.

2.1.2. Ethical approval of case and control study

To follow the ethical standards of international and national Research Committees throughout the procedures involving human participants, the 1964 Helsinki Declaration and its subsequent amend-ments, or comparable ethical standards, were observed. The ethical certificate approval of this study (number 1.736.940) was obtained from the Montes Claros State University Research Ethics Committee. Data were collected from October 2015 to October 2019. All patients signed an informed consent form.

2.1.3. Compliance with ethical standards

The authors declare that they have no conflicts of interest.

2.2. Experimental design

The current study combines two study designs: one on animals and the other on patients. First, we used an applied animal basic study and validate the data in a retrospective diagnostic controlled case and control study (Rohrig et al., 2009).

2.2.1. Animal basic study

2.2.1.1. Mice groups. Forty-two day-olds male Swiss mice were obtained from the Universidade Estadual de Montes Claros (UNIMONTES). The mice were randomly divided into four groups (n = ten each) and respectively subject to the following conditions: conditioned fear stress, 4NqO, CFS plus 4NqO, and control group (group without intervention, Fig. 1).

2.2.1.2. Cancer induction. Cancer induction was performed as

described before (Sobrinho Santos et al., 2017). Briefly, the mice received -nitroquinoline-1-oxide (4NqO; N8141-5 G, Sigma-Aldrich, St. Louis, USA) in drinking water to a final concentration of 50 μg/mL for 16 weeks. After 4NqO administration, the mice were followed for 13 weeks (Fig. 1). During all 29 weeks of experiments, mice had free access to water and food. Mice were housed in a 12 -h light-dark cycle, and the temperature was maintained at 23.0 ± 2.0 °C.

2.2.1.3. Conditioned fear stress (CFS)-induced freezing behavior. To simulate AD, CFS was used as previously described (Aguiar et al., 2013; Gomes et al., 2013). Briefly, in stressed groups, animals were submitted to CFS during all the experiments (29 weeks, Fig. 1). The CFS was performed in a CFS chamber (37cm × 25cm x 21 cm, Skinner Box, ELT-02, Eltrones, Joinville, SC, BR). The CFS session was lasted for 185 s with an interval of 25 s between each sound and shock. It is important to emphasize that the animals in the control group were also placed individually in the chamber and submitted to the same experimental conditions without the presence of shocks. The CFS session was performed with an isolated animal to prevent other animals from hearing noises released by the animal that was subjected to the experiment. The chamber was cleaned with 70 % ethanol before and after the entry of each mice. Freezing behavior is associated with stress during CFS (Gomes et al., 2019). To quantify the number of movements ImageJ software was used to analyze the experiment movies (Rueden et al., 2017).

2.2.1.4. Sacrifice of animals. Animals were sacrificed via decapitation through a guillotine after 29 weeks of experiments. So that the mice could not smell the blood of the animals previously sacrificed, after the sacrifice the guillotine was cleaned with 70 % alcohol. Immediately afterward, the heads of each animal were taken individually to another experimental room, where the tongue was removed. The material was placed in properly labeled containers and was fixed in a 10 % formalin solution for 48 h.

2.2.1.5. Histological preparation and analyses. The samples were included in paraffin. The specimens were submitted to complete serial sections of 5 μm each obtained using a microtome (Easy Path EP-MR10). Each section was dewaxed, rehydrated and stained with hematoxylin and eosin. The samples were covered with glass coverslips for observation and histological quantification by microscopy (Olympus FSx100, Center Valley, Palo Alto, CA) for analysis by the same pathologist, blinded for the groups. The pathologist graded the lesions of mice in absent, mild, moderate, severe dysplasia, and carcinoma as described before (Sobrinho Santos et al., 2017)

2.2.2. Case-control study

The sample size calculation was performed to achieve an alpha of 0.05, the beta of 0.05, and the study power of 0.95, and to reach a minimal between-group difference of 50 % of HNSCC incidence. A total
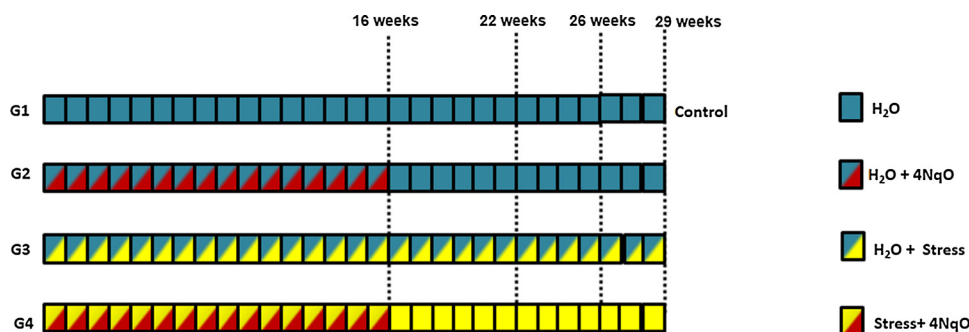

Fig. 1. Animal study design scheme.
The four groups are represented green for the water; red represents 4NqO, and yellow represents the CFS. Each square represents one week.

of 108 patients were enrolled in the study; 53 patients were cases and 55 control patients. The inclusion criteria for patients cases were with HNSCC. The exclusion criteria were patients who had cancer lesions associated with UV light, patients who had already started HNSCC treatment, or did not agree to participate in the study. The inclusion criteria for control patients' absence of HNSCC. The Beck Anxiety Inventory (BAI) questionnaire was applied to assess Anxiety in patients (Beck et al., 1988).

2.3. Statistical analysis

Statistical analysis was performed using the PASW Statistics18–SPSS software (IBM, Armonk, NY). A chi-square test was used. Statistical analysis showing confidence above 95 % (P < 0.05) was considered to be significant. Graphs were created using GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA).

3. Results

3.1. Conditioned fear stress (CFS) reduced locomotor activity

CSF has been shown to impact on the general locomotor activity of animals. In both the stress and stress + 4NqO groups, fear conditioned to stress resulted in a reduction or almost total absence of general locomotor activity. Freezing behaviors were characterized by complete immobility while breathing and reflex expression (as characterized by fear-potentiated startle) were not considered (p < 0.0001, Fig. 2 and video 1).

3.2. Conditioned fear stress does not interfere with 4NqO induced oral carcinogenesis

The morphological aspects of epithelial such as keratinization, nuclear polymorphism, tumor-stroma interaction (pattern of invasion), and the lymphocytic infiltration were used to define the worse histological grading (Pereira et al., 2012). No association with cancer progress, in terms of cell and organ morphology of the mice tongue, was observed (Table 1 and Fig. 3). CFS did not promote changes in the incidences of HNSCC in the animal model in comparison to 4NqO without CFS (Table 1).

3.3. Anxiety is not associated with HNSCC staging

Interestingly enough, HNSCC and control patients did not present
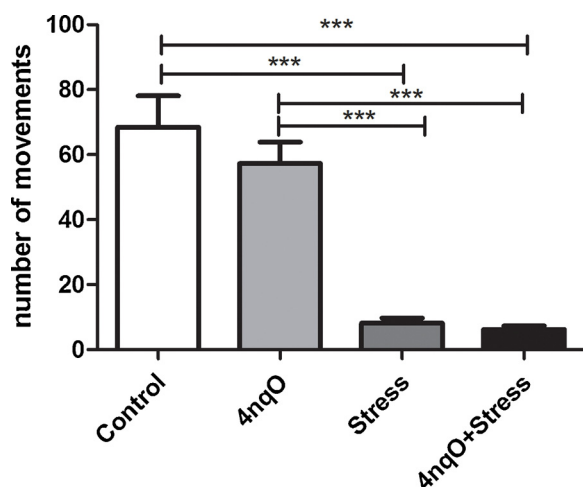

Fig. 2. Conditioned fear stress reduced locomotor activity.
Number of movements were quantified by ImageJ software, breathing and reflex expression were not considered.

Table 1
Conditioned fear stress and HNSCC progression in mice.

	Dysplasia Absent	Dysplasia Mild/ Moderate	Dysplasia Severe/ Carcinoma	p-value
Control	7(100 %)	0(0 %)	0(0 %)	0.377
4NqO	1(11,1 %)	1(11,1 %)	7(77,7 %)	
Stress	9(100 %)	0(0 %)	0(0 %)	
Stress + 4NqO	0(0 %)	3(30 %)	7(70 %)	

differences in the BAI score (Table 2). Also, BAI scores that were not associated with HNSCC grading were observed in patients (Table 3). The study included 108 patients. Their details are shown in supplementary material 2.

4. Discussion

The relation between HNSCC treatment and the increase in anxiety is very well established (Cheung et al., 2013; Curran et al., 2017; Kumar et al., 2018). Evidence demonstrated that anxiety affects 10.3 % of patients receiving treatment for neoplasms in general (Mitchell et al., 2011; Curran et al., 2017). Moreover, anxiety scores increased between diagnosis and intervention and gradually decreased (Kumar et al., 2018). Also, there is a close affinity between symptoms of stress, such as chronic arousal, tension, fatigue, worry, nervousness, discouragement, lack of patience, restlessness, interrupted sleep, avoidance, and irritability, with the HNSCC treatment (Lazarus, 1993; Kumar et al., 2018). The CFS is considered a standard animal model for the study of Anxiety or fear (Fanselow, 1980). Freezing is defined as the lack of all observable body movements, except for breathing (Fanselow, 1980; Yoshioka et al., 1996). It was observed that rats that presented freezing even 24 after the CFS induction animals in the chamber (Fanselow, 1980; Yoshioka et al., 1996). The post-shock freezing behavior is the result of a conditioned fear caused by signs associated with the shock (Yoshioka et al., 1996). Studies indicate that animals submitted to CFS show more squatting and freezing (Blanchard and Blanchard, 1969; Bolles and Collier, 1976; Fanselow, 1980) in the CFS reduced animal locomotor activity and freezing behavior. Our data are in agreement with previous studies that used the same animal model (Aguiar et al., 2013; Gomes et al., 2013, 2019).

Evidence suggested that AD might be associated with HNSCC development (Xie et al., 2014, 2015), which diverges with the current research. The divergence might be related to the cancer differences in study design. A previous animal study (Xie et al., 2015) used implanted CAL 27 cells to simulate the HNSCC while in the current study, we used chemical carcinogenesis. Ectopic models of HNSCC lack of host immunity-tumor cell interaction (Supsavhad et al., 2016) and do not consider the anatomical site (Supsavhad et al., 2016). In HNSCC, the anatomical site is an essential factor related to prognosis (Vered et al., 2011).

On the other hand, the model used in the current study also permits the development of premalignant lesions; also, our model conserved host-tumor cell interaction (Supsavhad et al., 2016). Furthermore, stressors reduce the increase in most neoplasms induced by chemical carcinogens (Strange et al., 2000). Our data demonstrated that CFS does not interfere with cancer progression. 4NqO is a carcinogen widely used in HNSCC study models (Sobrinho Santos et al., 2017).

In the current study, no differences were observed in BAI scores in the case and control group. Similar BAI results were found before (Bastos et al., 2018). Stressful events that occur in the presence of social support have less emotional consequences than if they happen in the absence of social support. Social support can help patients deal with stress so that it is no longer considered stressful and/or provides resources, thereby reducing the severity of the stressful event (Kornblith et al., 2001). In two studies with breast cancer patients and survivors, less social support, a more extended history of pre-cancer trauma, and more stressful life events directly predicted higher levels of post-
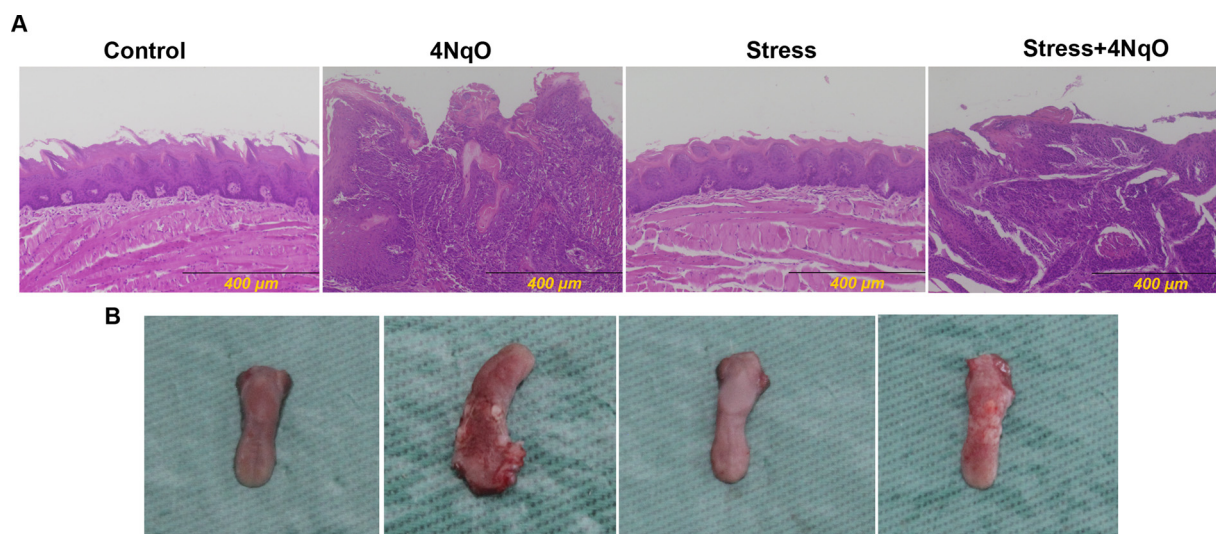
A

B

Fig. 3. Stress from conditioned fear and histological analysis.

Table 2
Beck Anxiety Inventory scores in case and control patients.

	Case	Control	p-value
Low Anxiety	35(49 %)	36(51 %)	0.386
Moderate Anxiety	17(53 %)	15(47 %)	
Potentially Concerning Levels of Anxiety	1(20 %)	4(80 %)	

Table 3
Anxiety and HNSCC staging.

TNM	Low Anxiety	Moderate Anxiety	Potentially Concerning Levels of Anxiety	p-value
TX	2(3,77 %)	1(1,88 %)	0(0%)	0.788
T1-T2	18(339 %)	10(19 %)	0(0%)	
T3-T4	15(283 %)	6(11, %)	1(1,88 %)	
N0	5(9,4 %)	2(3,77 %)	0(0 %)	0.744
N1-N2-N3	29(54;7 %)	13(245%)	1(1,88 %)	
N4	1(1,88 %)	2(3,77 %)	0 (0 %)	
M0	10 (10 %)	2 (3,77 %)	0 (0 %)	0.342
M1-M2	25 (471 %)	15(283)	1(1,88 %)	

traumatic stress disorder symptoms and general distress (Kornblith et al., 2001). Our findings are consistent with the literature that younger survivors may face more financial and social challenges, and the emotional response of elderly patients with HNSCC is more favorable since our patients are elderly (Smith et al., 2008; Rogers et al., 2015; Moschopoulou et al., 2018). Our findings, together with the results of Moschopoulou et al. (2018), demonstrate that there is no relationship between the stage of the neoplasm with Anxiety (Hahn et al., 2015; Richardson et al., 2016; Moschopoulou et al., 2018) in our results patients with low Anxiety characterize the T3 / T4, M1 / M2. Our data, together with literature, show that AD might be a consequence of the diagnose and HNSCC treatment.

It is essential to highlight that our study also presents limitations because it was a Unicenter study. Also, the animal model needs validation in multiple populations.

In conclusion, the current study did not observe the association of anxiety disorders and HNSCC development.

Author(s) contribution

Study concepts: De Souza MG,Guimaraes ALS, Gomes ESB, Santos SHS, de Paula AMB, Farias LC, Andrade, A. D. S., Costa, L. B., de Jesus SF, Paula DPS, Rocha GR.

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Manuscript review: De Souza MG,Guimaraes ALS, Santos SHS, de Paula AMB, Farias LC, Andrade, A. D. S., Costa, L. B., de Jesus SF, Paula DPS, Rocha GR, Gomes ESB.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgments

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ibror.2020.06.001.

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