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Diagnosis of oral cancer using biomarkers, a non invasive proposal

Diagnóstico de câncer oral através de biomarcadores, uma proposta não invasiva

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ABSTRACT

The present study aimed to identify, through the scientific literature, the use of biomarkers that aid in the diagnosis of cancer in the oral cavity. This is a narrative review study with a qualitative and descriptive approach. To verify the data, the databases of Periódicos Capes, PubMed and Sciencedirect were used, on the use of scientific articles, published during the time cut from 2019 to 2023, in the languages Portuguese, English and Spanish, available online and free of charge. After the application of the filters, selection of descriptors and inclusion of review studies, meta-analysis, controlled and random tests, 52 articles were identified, among which 27 were from PubMed, 14 from Capes Journals and 11 from Science Direct, in addition to the consideration of adequacy, only 17 articles were registered for review. In this important study, it was evident that despite advances in the identification of tumor markers, they still do not have sufficient capacity for the diagnosis of cancer in the oral cavity. However, it highlights its influence as an important tool for the prognosis of clinical conditions. In addition, it emerges as a less invasive alternative, with more accessible samples such as saliva and promising results.

Keywords: Oral cancer, Tumor markers, Investigation.

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INTRODUCTION

Cancer is one of the main diseases of global recurrence, among the group of 100 diseases, its main characteristic is the disordered growth of cells that later reach tissues and organs. This pathology is known to have multifactorial causes, so it is influenced by environmental factors and intrinsic mechanisms, such as immunological conditions and genetic mutations (Medeiros, 2020).

Oral neoplasia is a public health problem that has been growing constantly, being more frequent in men over 50 years of age. Risk factors for this cancer are smoking, alcoholism, poor oral hygiene, Human Papillomavirus, and sun exposure (Dias, De Almeida Coelho, 2022; Sat´ana *et al.,* 2021). In Brazil, the occurrence of cases is higher when compared to other countries, in the estimate made at each training session (2020-2022) by the National Cancer Institute (INCA), 15,190 new cases were estimated, 4,010 in women and 11,080 in men.

The most common type of malignancy of the oral cavity is squamous cell carcinoma (SCC), accounting for 90%. This cancer can affect various anatomical structures, such as the tongue, lips, gums, surface below the tongue, paranasal sinuses, salivary glands, pharynx and larynx and among others, with great potential for malignancy (Liouta *et al*., 2023)

To make the diagnosis of this cancer, a complete clinical examination of the oral cavity is usually used, in the presence of a lesion, a biopsy is performed. However, the conventional oral examination does not identify initial alterations, which prevents early diagnosis. Biopsy is a frequently used procedure, but in addition to being invasive, it is a technique of high cost and limited utility, resulting in cases of neoplasms detected late, resulting in metastasis and consequently increasing the mortality rate (Ali *et al.*, 2021; Madhura *et al.,* 2020)

Thus, there is a need for more specific and sensitive techniques that allow early diagnosis, especially of minimally invasive techniques such as liquid biopsy, optical system, oral cavity cytology, microfluidic method and artificial intelligence. Currently, in the midst of constant research in liquid biobiopsy biomarkers, non-coding RNAs and circulating tumor DNA exhibit great potential in the detection of this cancer (Amenábar; Da Silva; Punyadeera, 2020; Wang *et al.*, 2023).

On the other hand, biomarkers are a method with constant innovation, as they have a more accurate result on the situation, indicating normal and pathological processes, responding to stimuli. (Aronso; Ferner, 2017; Califf, 2018, Cardoso *et al*., 2019). Therefore, the objective is to identify, through the scientific literature, the use of biomarkers in accessible samples in the diagnosis of oral cavity cancer.

MATERIALS AND METHODS

This is a narrative review study with a qualitative and descriptive approach. The following inclusion criteria were defined: scientific articles in Portuguese, English and Spanish, available online and free of charge, expanded abstract and in full text format, published during the period from 2019 to 2023. The exclusion criteria were: articles that did not correspond to the time frame, disregard of theses, monographs, dissertations and repeated studies.

In February 2023, the search for scientific articles began in the following data libraries: Capes Journals, PubMed, and Sciencedirect. The descriptors used were extracted from the Descriptors in Health Sciences (DeCS), through the following crosses: *Biomarkers* AND *Tumor, AND Mouth neoplasms.*

After applying the time-frame filters from 2019 to 2023, selection of descriptors and inclusion of review studies, meta-analysis, controlled and random tests, 52 articles were filtered, among which 27 were from PubMed, 14 from Capes Journals and 11 from Science direct. During the search and selection of articles, the titles and abstracts of all the papers were read, excluding those that did not correlate with the theme of the present study, totaling 40 articles to be read in full.

A total of 35 articles were excluded because they did not address the central theme of the present study, leaving 17 articles to compose the study.

DEVELOPMENT

The development of Oral Cancer (OC) carcinogenesis is a multi-stage process, where one of the characteristics is molecular alterations. The level of protein expression changes in a carcinogenic environment, so the detection of a biomarker is essential to identify the risk of developing cancer. Therefore, toxic substances present in the environment produce harmful metabolites that promote genetic instability (Pillai, 2021).

OC is known to be diagnosed in advanced stages, as this neoplasm does not present signs and symptoms at the beginning of the pathology, consequently increasing the chances of treatment failure. Thus, studies have been carried out with potential biomarkers for the early diagnosis of the disease, therefore, the tumor marker identified will help to increase patient survival, in addition to being a methodology that reduces morbidity and mortality (Chiamulera *et al.*, 2021; Santos *et al.*, 2021).

It is believed that in oral cancer there may be secretion of tumor DNA through saliva, in which this molecule is found more easily, when compared to other materials such as plasma or

serum. Therefore, the collection of salivary samples from patients with suspected oral cancer may possibly serve as another tool for diagnosis, in a non-invasive way (Zhong *et al.,* 2018).

According to Patil *et al.,* (2019), the use of saliva as a parameter for research is significant for both diagnosis and response to therapy. Therefore, through its review, it describes the presence of salivary mRNA transcripts from samples of healthy individuals with SCC, with greater expression of IL8, IL1B, DUSP1, OAZ1, S100P, SAT1 genes in oral neoplasm carriers.

Paluszkiewicz and his collaborators in 2020, presented research on salivary gland cancer, and it was demonstrated through ATR-FTIR spectroscopy that there are significant changes in the secondary structure of developmental proteins, in cancer patients, compared to healthy people. Biomarkers found in salivary fluid in bands attributed to proteins, carbohydrates and inorganic phosphates have diagnostic potential, highlighting as a methodology to be used.

Protein-based detection tools are crucial as they identify post-transcriptional and post-translational changes in carcinogenesis. Tumor markers, substances in the blood that indicate cancer, are useful for monitoring post-treatment recurrences. The p53 protein plays a vital role in cell cycle regulation and apoptosis, and is a biomarker studied in the oral cavity. Mutations in this gene occur in several forms of cancer, including CECO. Its overexpression may have prognostic implications, but results vary. The expression of p53 above the basal layer is an early event in oral carcinogenesis, suggesting its importance in diagnosis (Cervino *et al*., 2019).

The AHSG and KRT6C proteins analyzed in saliva were altered in CO. KRT6C has the function of lining the stratified epithelium, being part of the keratin type 2 proteins. Overexpression of KRT6C is associated with large cell proliferation in cancer. AHSG is expressed in embryogenesis and is part of the cystatin family of proteins. In adults, this protein is limited only to the liver and osteoblasts, and is also associated with malignancy. Overexpression of AHSG in CO has been observed considerably, even though it is a poorly explored protein (Jain *et al*., 2021).

Another group of CFH proteins, FGA and SERPINA1, have been shown to have potential for early detection and prognosis assessment, through salivary proteome profiling, based on iTRAQ and MRM analyses. Salivary FGA and SERPINA1 levels were significantly elevated in patients with early-stage primary tumors without lymph node metastases. Salivary CFH levels in patients with early-stage primary tumors were higher than in healthy controls, offering an important alternative to the current method (Chu *et al.,* 2019).

In the study by Zhong *et al*., (2018), salivary samples were collected from a patient affected by oral cancer and from another healthy individual, where they were later analyzed by

immunoassay, obtaining the following biomarkers: CD59, M2BP, MRP14, catalase, and profilin, which proved to be very effective in the diagnosis of cancer.

Salivary cytokines show promise as biomarkers for early detection. Chiamulera *et al.*, (2021); Ferrari *et al.*, (2021), describe analyses of anti-inflammatory and pro-inflammatory cytokines, and it was observed that the interleukins IL-8, IL-6, TNF-a, and IL-1 beta were found in higher amounts in patients with OC, when compared to healthy patients. The cytokines with the highest concentrations were IL-8 and IL-6, therefore with greater reliability.

Another study evaluated the performance of hypoxia in oral cancer, as this marker is altered in cancer-related situations. In addition to hypoxia, the role of microRNAs was evaluated, being crucial in hypoxia responses. It was possible to observe that hypoxia markers do not increase in this neoplasm, but in present cases it may indicate radioresistance. Due to the low survival caused by cancer, this is a marker to be considered in this study (Cardoso, 2019).

Vascular endothelial growth factor (VEGF) is addressed in the development of some cancers, as tumor growth demands greater blood supply. Thus, it is related to the process of CO angiogenesis, followed by hypoxia, which is also a consequence of oral carcinogenesis. The presence of VEGF is closely linked to oral tumors, in addition to indicating low survival (Santos *et al.*, 2021).

The study by Cervino *et al.*, (2019), presents an approach of molecular techniques where the epidermal growth factor receptor (EGFR) gene was detected, being closely related to the development of CO. Thus, EGFR inhibitors are seen as one of the treatment opportunities.

In biochemical techniques, the enzyme lactate dehydrogenase (LDH) was detected in high concentrations in the serum of patients with CO, differentiating it from healthy patients, and it is interesting to consider the inclusion of LDH as an essential biomarker for CO screening, even though serum is an imprecise sample (Barbi; Purohit, 2022).

Another research target is non-coding RNAs (miRNAs), which in the study by Shaw *et al.*, 2022, a specificity and sensitivity of 91% was highlighted, while mRNAs were 90%, estimated by the Polymerase Chain Reaction (PCR) technique, which considered to be the best markers for this type of neoplasm compared to others already studied for the diagnosis of early OC in a non-invasive way.

According to Kaunein (2021), dysregulation of miRNAs has been reported in many cancers and has been shown to play a key role in the initiation, progression, invasion, and activation of disease metastasis. In Oral Squamous Cell Carcinoma (SCC), microRNAs have been implicated in tumorigenesis with the identification of different expression patterns of these

molecules, which allows the characterization of the neoplastic mass in its initial phase, although some microRNAs can differentiate independently between progressive and non-progressive lesions.

The same author highlights the importance of microRNA-21 and miRNA-31, microRNAs that can complement each other in terms of sensitivity and specificity to improve prognostic capacity. They are expressed more frequently, due to their differentiation. MicroRNA-31 expression was higher in the progressive oral leukoplakias group, these microRNAs were examined for the diagnosis of Potentially Malignant Oral Disorders (OPMD) and prediction of oral squamous cell carcinoma, with 15 miRNAs reported in at least two studies and nine microRNAs identified for oral squamous cell carcinoma shown in all studies.

According to Dioguardi *et al*., (2022) microRNA-21 stands out, as its alteration can mean carcinogenesis. In this study, microRNA-21 was identified in many articles, indicating prognosis in oral tumors, thus aiding in a less invasive treatment.

Chen *et al.,* 2021, studying miRNAs derived from gingival tumor tissue exosomes, making a comparison with squamous cells from the healthy oral epithelium and serum of these patients with SCC, were able to identify several miRNAs, especially miR-155 and miR-21 with oncogenic characteristics, capable of negatively interfering with the expression of PTEN and Bcl-6 tumor suppressor genes, while miR-126 acts by suppressing CECUM by downregulating the EGFL7 oncogene. For the author, the result of the analysis of serum exosomes allows their use as biomarkers for the diagnosis and staging of SCEC in a non-invasive way.

Aerobic glycolysis is the main energy medium for the development and proliferation of ECCs, and glucose transporters (GLUTs) are overexpressed due to the high energy demand required by these cells. In addition, GLUT expression is correlated with poor prognosis, such as the presence of GLUT-1, which is associated with chemoresistance and radioresistance in malignant neoplasms (Botha *et al*., 2021).

Elseragy (2022), describes, through hematoxylin-eosin staining, markers considered promising for the prognosis of early-stage tongue squamous cell carcinoma (SCTO). Tumor budding; invasion pattern (WPOI) and tumor stromal ratio (TSR). Tumor budding is the presence of cancer cells, (greater than five is considered high risk for poor prognosis), budding is associated with lymph and metastasis, making it a valuable prognosis for a marker. WPOI, on the other hand, is the invasion, WPOI 4 and 5 are associated with the worst overall survivals. As for the TSR, which characterizes the stroma surrounding the tumor tissue, its identification is easy and fast, it is known that the stroma acts as a barrier in tumorigenesis, and the classification

rich in stroma was associated with a higher risk of recurrence, while the stroma-poor had a better prognosis.

CONCLUSION

In this study, it was found that tumor markers are not yet capable of diagnosing OC, but it highlights the great influence on clinical and prognostic conditions. It can be analyzed in a less invasive way with more accessible samples such as saliva and serum and still obtaining promising results. Thus, techniques that use these samples provide more comfort for patients, however, more studies and investments in methodologies that guarantee specificity and sensitivity are necessary.

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