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SALIVARY METABOLOMICS AS A CANDIDATE FOR DIAGNOSIS OF ORAL CANCER. A SYSTEMATIC REVIEW

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1. LIST OF SYMBOLS AND ACRONYMS

I. OC=Oral Cancer
II. OSCC=Oral Squamous Cell Carcinoma
III. CVTE= Conventional visual and tactile examination
IV. IARC= International Agency for Research on Cancer
V. ROS= Reactive Oxygen Species
VI. HPV =Human Papillomavirus
VII. BQ=Betel Quid
VIII. WMS =Whole Mouth Saliva
IX. GCF =Gingival Crevicular fluid
X. PS =Parotid Saliva
XI. SM/SL =Submandibular/Sublingual
XII. UWMS= Unstimulated Whole-Mouth Saliva
XIII. GC/MS= Gas Chromatography-Mass Spectrometry
XIV. LC/MS =Liquid Chromatography-Mass Spectrometry
XV. NMR=Nuclear Magnetic Resonance
XVI. MS= Mass Spectrometry
XVII. HNSCC= Head and Neck Squamous Cell Carcinoma
XVIII. CE-MS= Capillary electrophoresis mass spectrometry
XIX. CE-TOF-MS =Capillary Electrophoresis Time-flight-mass Spectrometry
XX. UPLC-QTOF-MS=Ultra Performance Liquid Chromatography Quadrupole/Time-Of Flight -Mass Spectrometry
XXI. TCA Cycle =Tricarboxylic acid cycle
XXII. OLK= Oral Leukoplakia

2. ABSTRACT

Introduction: Oral cancer often known as mouth cancer, is a malignant tumor of the oral cavity and is the world's sixth most prevalent cancer. Oral squamous cell carcinoma accounts for about 90% of oral cavity cancers. The systemic study of metabolites is known as metabolomics where small molecules are generated by the process of metabolism. The aim was to evaluate how salivary metabolomics can help in the diagnosis of oral cancer and secondly review if salivary metabolomics can lead to identification of oral cancer as well as study the viability of salivary metabolomics in the context of oral cancer.

Materials and Methods: An electronic search was carried out in the databases of PubMed, Scopus, and Web of Science on salivary metabolomics for oral cancer detection until December 2022.

Results: Of 126 potentially eligible articles,7 complied with the inclusion criteria. All 7 studies evaluated the metabolites differences found in oral cancer and healthy patients. The diagnostic material in each study was unstimulated whole saliva, which was analyzed using several spectroscopic techniques. Oral squamous cell carcinoma patients varied significantly from the healthy participants in terms of metabolites. There was evidence of altered metabolic pathways, such as choline metabolism, amino acid pathways, and glycolysis, among the observed salivary metabolites.

Conclusion: Despite the limitations salivary metabolites can be used as a diagnostic tool for early diagnose as well as there is tremendous potential for the viability of salivary metabolites in relation to the earlier identification of oral cancer.

3. KEYWORDS

I. Oral Cancer

II. Salivary Metabolomics

III. Oral cancer detection

IV. Oral cancer diagnosis

V. Metabolites

4. INTRODUCTION

4.1 Oral Cancer

Oral cancer (OC), often known as mouth cancer, is a malignant tumor of the oral cavity and is the world's sixth most prevalent cancer (1). Oral squamous cell carcinoma (OSCC) accounts for about 90% of oral cavity cancers (2). OSCC is an invasive epithelial neoplasia formed histologically by squamous cells with varying degrees of differentiation (5). The disease presents as flat, scale-like structures that are seen lining the mouth and throat and are detectable due to their superficial placement (1). In comparison to breast cancer (89%) and prostate cancer (99%), it has a five-year survival rate of 62%. If identified early, the five-year survival rate for OSCC is around 85% because the disease is initially asymptomatic, mortality is high, and most people report when they acquire late-stage symptoms, at which point the disease has progressed and the survival rate drops to 15%-50% (1,2).

Carcinoma of the oral cavity is most commonly metastasized to the cervical lymph nodes while distant metastases are rare. The most affected nodes are submandibular, digastric, and upper cervical nodes located on the same side of the neck as the tumor (3). The oral cavity is an essential organ for the functions of speaking, chewing, and swallowing. Furthermore, the oral cavity is a component of the face and so when highly invasive surgery is performed for advanced-stage OC it might result in oral dysfunction and cosmetic disfigurement. Therefore, it is important to detect oral cancer at an earlier stage to the maximum extent possible because the oral cavity is a commonly examined area and many doctors assume that OC is easy to identify. However, several lesions mimic OC, such as bite wounds, periodontitis, and intractable stomatitis, because of which accurate detection of OC is still difficult leading to a delay in the detection of OC in the early stages (4).

According to recent statistics, India has the greatest incidence of oral cancer in the world, with an estimated incidence of 12.48 cases per 100,000 population in males and 5.52 cases per 100,000 population in females. The male-to-female ratio for OSCC is 4:1(1,2,3).

Asian nations account for 80% of all OSCC incidences worldwide because of the population's fast-rising usage of alcohol and cigarettes. Oral cancer is more frequent in men in all nations throughout the world. The chance of having an oral cavity tumor is 38 times higher in the population that consumes alcohol and cigarettes (1,2,3,5).

4.2 Risk Factors

Multiple risk factors have been identified for the etiopathogenesis of oral cancer and, among these, tobacco and alcohol consumption seem to have a synergistic effect. Male sex, elderly age, high-risk human papillomavirus (HPV), dietary habits, oral bacteria, UV radiation, and betel quid chewing (popular in South-East Asia) have all been shown to play a significant role in the appearance of the disease. Furthermore, several premalignant lesions, such as erythroplakia, leukoplakia, oral submucous fibrosis, and oral lichen planus, have been associated with the onset of neoplasia (5).

The International Agency for Research on Cancer (IARC) has determined that smoking different types of tobacco (such as bidis, pipes, cigars, and cigarettes) is harmful to humans. Furthermore, reactive oxygen species (ROS), which have been linked to multistage carcinogenesis, are produced in large quantities in the oral cavity when chewing. Pro-carcinogens in tobacco smoke, such as benzo-pyrene, are degraded by oxidizing enzymes, mainly cytochrome p450, with some culminating in the generation of reactive carcinogenic intermediates (6).

Alcohol has been linked to oral carcinogenesis, both separately and in conjunction with smoking. More crucially, alcohol may function as a solvent, enhancing carcinogen penetration into target tissues. Acetaldehyde, an alcohol metabolite, has recently been discovered as a tumor promoter (6).

Human papillomavirus (HPV) is also linked to both benign and malignant mouth lesions. This virus has been found in condylomas, localized epithelial hyperplasia, squamous cell papilloma, and malignant oral lesions. HPV positive is greater in malignancies of the oral cavity (59%), pharynx (43%), and larynx (33%). Only a tiny proportion of HPV-infected lesions, particularly those with HPV subtypes 16,18, progress to malignant transformation .Fruits and vegetables (rich in vitamins A and C) are said to be preventive against oral neoplasia, but red chili

powder and meat are regarded to be risk factors. Vegetables and fruits with antioxidant characteristics that protect against mouth cancer and precancer are high in b-carotene, vitamin C, and vitamin E (6).

The most major etiological cause of oral submucous fibrosis is betel chewing. The usage of betel quid containing both areca nut and tobacco is associated with a substantially increased relative risk of oral cancer, ranging from 8 to 15 times that of using the quid without tobacco. Chewing betel quid generates ROS (Reactive Oxygen Species) that is harmful to the oral mucosa and can be directly implicated in tumor generation by promoting mutation or rendering the mucosa vulnerable to BQ components and environmental toxins (6).

ROS are produced and released in the saliva of BQ chewers in alkaline circumstances during the autooxidation of areca nut (AN) polyphenols. ROS can directly contribute to tumor development by promoting genotoxicity and gene mutation, or by damaging salivary proteins and oral mucosa, causing structural changes that may enhance the entry of other BQ components and environmental toxins. In the saliva of BQ chewers, areca alkaloids are nitrosated to AN-specific nitrosamines. These AN-specific nitrosamines are mutagenic, genotoxic, and carcinogenic (6).

Early indications of oral cancer are persistent red or white mucous membrane lesions, a non-healing ulcer, an island or thickening of the structure of the oral cavity, atypical changes in the mucous membrane, a toothache without a clear cause, and unexplained bleeding from the gingiva or nose. Late symptoms include induction of the afflicted area, paraesthesia of the tongue and lips, stiffness of the jaw, dysphagia, dyspnoea, vision impairment, earache, and swollen lymph nodes on the neck (3).

Even though the oral cavity is easily accessible for examination, research suggests that a quarter of patients are late with the onset of treatment. At least three variables impact the late detection of oral cancer: low incidence, a lack of knowledge and awareness of oral cavity cancer in the general population and among healthcare personnel, and challenges in the health system that make patients and dentists difficult to approach. Older age, male gender, lower education, and lower socioeconomic level are all unfavorable factors (3).

4.3 Diagnostic Techniques

Conventional visual and tactile examination (CVTE) is still the most often used method for detecting OC (7). Early detection and treatment of premalignant oral lesions are vital for lowering mortality. A comprehensive clinical oral examination and surgical biopsy are now regarded as the gold standards for detecting pre-malignant lesions and oral cancer. More precisely, the World Health Organization and the National Institute of Dental and Craniofacial Research recommend that mucosal lesions that persist for two weeks or more after eliminating potential local irritants be biopsied (8).

Biopsies are classified into two types: incisional and excisional, with incisional biopsies being the more common. To reach more visible lesions, core-needle aspiration, fine-needle aspiration, or punch forceps can be used, but posteriorly placed lesions may necessitate general anesthesia. Although biopsy is the gold standard, it has numerous drawbacks: the procedure can be exceedingly stressful, uncomfortable, and invasive for the patients; additionally, it involves high costs, a high degree of complexity that requires clinician training, and can cause infection or damage to nearby tissues (8).

On the contrary, cytological analysis of a smear is less intrusive but also less sensitive and specific than a biopsy. Modern cytological procedures, such as brush biopsy and micro-biopsy, could be used to avoid repeated biopsies in the follow-up of precancerous lesions (8). OSCC prognosis is determined by several variables, including the patient's lifestyle (smoking, alcohol usage, etc.), the existence of comorbidities, and the tumor stage. In this context, the TNM system allows for the determination of the main tumor’s features, lymph node involvement, and the existence of distant metastases to better define the prognosis. Despite advancements in therapeutic options, the five-year survival rate remains extremely poor, being < 50% (8).

4.4 Salivary Metabolomics

The systemic study of metabolites is known as metabolomics where small molecules are generated by the process of metabolism (9). Metabolomics is part of the ‘omic’ group that also includes proteomics, transcriptomics, and genomics which are associated with the discovery and quantification of small molecules

involved in metabolic processes. The identification of a wide range of metabolites in bodily fluids is considered an important diagnostic tool for the assessment of disease biomarkers (10).

Saliva is a complex biological fluid produced in the oral cavity (9). It plays a vital role in oral homeostasis. Other functions of saliva include lubrication, digestion, taste, tooth protection, buffering, and immune defense by protecting against bacteria, viruses, and fungi. Saliva contains a variety of biological and molecular components, including oral mucosa transudate, desquamated oral epithelial cells, blood cells, oral bacteria, proteins, metabolites, and inorganic ions (13).

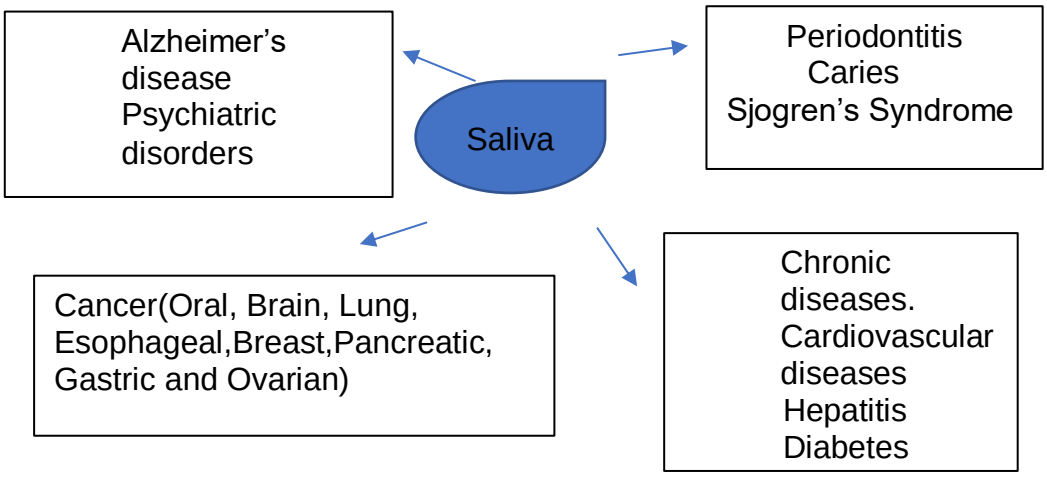

Fig. 1. Systemic diseases with saliva as an etiopathogenic factor (13).

Furthermore, it is secreted by three major glands and up to one thousand minor glands, Major glands such as the parotid, submandibular, and sublingual, secrete up to 90% of the total salivary volume. The remaining amount is contributed by minor glands (9). It is crucial to make the distinction between the many fluids that may be classified under the umbrella term "saliva". Whole-mouth saliva (WMS) is a more accurate word for the fluid generated by spitting or drooling. WMS is primarily the net output of major and minor glands, eukaryotic cells (epithelial and leukocytic), bacteria, and gingival-crevicular fluid (GCF) this is summarised in Table 1 (11).

GCF is a filtrate generated from serum that enters the mouth through the gingival edges of the teeth. While non-glandular contributions to WMS account for just a small portion of the net fluid volume, they have a major impact on the net composition. Descriptors are used to identify the gland from which saliva is

generated when referring to the saliva produced by certain glands. The fluid generated by the parotid glands is known as parotid saliva (PS), while the fluid produced by the submandibular and sublingual glands is known as submandibular/sublingual (SM/SL) saliva (11).

This is because the submandibular and sublingual ducts share an entrance to the oral cavity, making a collection of either fluid in isolation exceedingly impracticable, if not impossible. Minor gland saliva is the common name for secretions from minor glands, while anatomical characteristics are occasionally used to distinguish the place (e.g., labial or palatal) (11).

Table 1: A summary of the net contributions to whole-mouth saliva (11).

Glandular Fluid	**Gingival Crevicular Fluid**	**Cellular Components**
Major glands Minor glands	Serum transudate Immune cell infiltrate	Shed epithelial cells. Oral bacteria Immune cell infiltrate (tonsillar)

Saliva is a clear, slightly acidic (pH 6.0–7.0) composed of water (99%), proteins (0.3%), inorganic (0.2%), and organic substances. Amylase, cystatins, lactoferrins, mucins, lysozymes, transferrins, and a variety of secretary immunoglobulins are among the most important proteins. The most common inorganic species found in saliva are sodium, potassium, calcium, magnesium, and chloride, as well as carbonates and bicarbonate (9).

The components contained in the saliva might be either the saliva's natural components or metabolites transferred from the plasma. Ultrafiltration through gap junctions between cells of secretory units, transudation of plasma compounds into the oral cavity, from crevicular fluid or directly from the oral mucosa, and selective transport through cellular membranes by passive diffusion of lipophilic molecules or active transport through protein channels are all processes involved in the passage of plasma components into saliva (10).

The diversity of DNA, RNA, and proteins found in saliva is the primary source of information in the saliva. Salivary DNA contains the genetic information of the host human body, the oral microbiota, and the DNA viruses that infect it.

Salivary RNA contains information on the transcription rates of host genes as well as those of oral bacteria (10).

Salivary proteins include genetic information and aid in the understanding of the host body's and oral microbiota's translational control. Furthermore, saliva can be used to detect a variety of potential markers such as non-organic compounds, proteins, cell cycle markers (p16, p53, and so on), growth factors (epidermal growth factor, transforming growth factor, and so on), cell surface markers, DNA, mRNA, microRNA, oxidants, and antioxidants, among others (10).

Saliva offers many advantages compared to other biofluids, as a diagnostic fluid it is a readily accessible and informative fluid making it ideal for the early detection of various diseases. It can be collected safely and non – invasively with minimal training, it is produced continually on demand, and it is rich in biological information and how close it is to the oral lesions (9,10,11).

There are numerous methods for acquiring saliva, including using proprietary goods, such as salivette kits, which are chewable cotton swabs that absorb spit. Chewing and taste stimulation are both standard methods for increasing salivary flow rates, however many researchers choose to collect saliva passively, by spitting or drooling into a container while the fluid is generated in the mouth. This is known as unstimulated whole-mouth saliva (UWMS) in the literature (11).

Saliva collection from certain glands might be more difficult. Parotid gland saliva is normally collected using a Lashley cup, whereas submandibular and sublingual gland saliva is typically collected using pipetting techniques, and minor gland saliva can be collected using filter papers or capillary tubes (11).

Despite benefits like as simplicity of collection and preparation, saliva is far less researched by metabolic profiling than fluids such as urine and plasma, as indicated by the number of research outputs on the respective fluids. It is unknown why this is the case; nevertheless, it is possible that a perceived lack of an established literature base may inhibit researchers inexperienced with salivary collection (11).

The identified principle salivary metabolites can be used as promising biomarkers for accurately predicting the probability of disease understanding the mechanisms underlying different diseases or making them ideal for the early diagnosis of oral cancer (10). The salivary metabolic profile has been called the

"mirror of the body" because it can provide a general perspective on significantly altered metabolites due to aberrant enzymatic regulation, capture oncometabolites resulting from metabolic rewiring, and highlight those altered pathways during metabolic reprogramming. All of these can serve as appropriate metabolite markers for continuing OSCC-related alterations that do not manifest as evident clinical symptoms (10).

More than 100 metabolites have been linked to OSCC malignant growth, including choline, carnitine, lactate, glutamate, sialic acid, histidine, polyamines, pipecolic acid, and trimethylamine N-oxide (10). Metabolomics offers several advantages. Two important characteristics enable this technique to be a highly sensitive and rapid measure of the system phenotype. One is that the metabolome is the final downstream product of transcription and translation thereby being closest to the phenotype and secondly the dynamics of primary metabolism operate in timescales of second (10).

4.5 Metabolomic Analytic Techniques

The metabolic analysis is often divided into two distinct methods: targeted and untargeted. The targeted method focuses on identifying and measuring specific metabolites such as an enzyme substrate, direct products of a protein, a specific type of molecule, or members of a specific pathway while the targeted technique is often hypothesis-driven, untargeted metabolites might provide new hypotheses for future testing by measuring all the different metabolites in a biological system (11).

Nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC/MS), and liquid chromatography-mass spectrometry (LC/MS) are the most frequent analytical methods used in metabolomics. The method consists of two steps: an experimental approach based on MS or NMR spectroscopy tailored to profile low molecular weight molecules, followed by multivariate data processing (10,11,12).

The metabolic profiling experimental technique analyses the metabolome holistically and from a position of limited biological knowledge. To identify the various metabolites in a single metabolite, powerful analytical methods combined with suitable sample preparation are necessary, and the use of LC/MS provides the benefit of simultaneous detection of several metabolites (10,11,12).

The term LC/MS refers to the combination of liquid chromatography and mass spectrometry because mass spectrometry is more sensitive and specific than other chromatographic detectors, it is useful to combine it with chromatographic procedures. Liquid chromatography in LC/MS is modified and distinct from traditional HPLC. The purpose of liquid chromatography is to separate the components of a mixture so that they may be recognised and measured (10,11,12).

Mass spectrometers work by ionising the analyte molecules and then analysing the ions and any fragment ions that are formed during the ionisation process depending on their mass-to-charge ratio (11,12).

Due to its comprehensive coverage of diverse molecular species, mass spectrometry (MS) has become a popular platform for proteomics/metabolomics research (10). Mass spectrometry is an analytical method that produces a mass spectrum by ionising chemical species and sorting the ions based on their mass-to-charge ratio. To strengthen its mass-resolving and mass-determining capabilities, mass spectrometry is routinely used with chromatography and other separation technologies (12).

MS is normally made up of three basic components: An ion source, a mass analyzer, and a detector. The ion source changes sample molecules into ions and the mass analyser resolves the ions before they are measured by the detector. Since metabolites have various chemical characteristics, it is frequently necessary to analyse the biological material in both positive and negative modes under a scan range of m/z 50 to 1000 to maximize metabolome coverage. The raw LC-MS data is then pre-processed into a peak list for simple interpretation and comparison. Following pre-processing, the LC-MS raw data are summarised by a peak list, to which statistical analysis may be done to find those peaks whose intensity levels are significantly different between different biological groups (10,12).

Nuclear magnetic resonance identifies hydrogen atoms in metabolites of a biological sample, and all hydrogen-containing molecules will produce a 1H NMR spectrum. The preparation of an NMR sample is straightforward, and some bio-fluids, such as serum, may not require any preparation. Methanol can be used to eliminate lipoproteins from plasma-containing proteins and lipids that interfere

with NMR spectrum quality. Nuclear magnetic resonance gives exceptional analytical precision in the identification of cancer metabolomic markers. (12).

4.6 Metabolomics study of oral cancer tissues and cells

The study of metabolomics in oral cancer or head and neck squamous cell carcinoma (HNSCC) is beneficial for understanding the pathophysiological processes and underlying mechanisms of these tumors. Certain amino acids, glutathione, and polyamine were shown to be more likely to be discovered in HNSCC than in normal tissues in early metabolomics investigations, while taurine, choline, glutamate, lactate, and lipid were discovered to have diagnostic potential. When the metabolic profiles of matched HNSCC, adjacent normal tissues, and associated lymph-node metastatic tissues were examined, it was discovered that both primary and metastatic HNSCC tissues had higher levels of amino acids, choline-containing compounds, creatine, taurine, and glutathione, and lower levels of triglycerides (12).

Moreover, there is an increase in glucose and glutamine consumption in OSCC tissues. The most abundant amino acid in oral squamous cell carcinoma tissues is glutamate whereas in normal tissues the most prominent tissue is glutamine. Metabolomics analysis of tumor tissues or cells has also been used to detect the metabolites associated with metastatic potential, stemness, and precancerous lesions of oral cancer (12).

Metabolomics approach is also applied to analyse the effect of key metabolic genes on the metabolism in oral cancer. The knockdown of the metabolic enzyme adenylate kinase 2 or phosphorylate glycerol kinase 1 gene has led to distinct changes in metabolic phenotypes in oral cancer cells. Multiple metabolic pathway alterations have been observed in oral cancer, which include highly active glycolysis, increased influx of amino acids, glutaminolysis, lipolysis, TCA cycle, choline ,phospholipid metabolism, and antioxidant mechanism .Increased levels of choline-containing molecules and alteration of membrane biogenesis were observed in OSCC tissues in numerous studies. Choline is an important constituent of the cellular membrane phospholipid, and abnormal choline metabolism is regarded as a metabolic hallmark for tumor development and progression (12).

5. JUSTIFICATION AND HYPOTHESIS

Justification

The use of saliva as a screening medium offers a quick, low-cost, secure, and non-invasive method. It has recently been considered to as "the mirror of the body" since it is the most important biological medium for evaluating health and illness. It is increasingly seen as a means to test for diseases. The metabolic profile of saliva is sometimes referred to as the "mirror of the body." This is because it has the ability to collect onco-metabolites that result from metabolic rewiring. It also offers an overview on metabolites with notable aberrant enzymatic regulation and focuses on the changed pathways during metabolic reprogramming (15).

Oral cancer is the sixth-most common head-and-neck cancer in the world. Oral squamous cell carcinoma is a multifactorial condition marked by a complex interaction of environmental variables and molecular changes that lead to unrestricted cell growth. With a low five-year survival rate, the condition continues to be difficult for medical personnel to diagnose and treat. The low survival rate of oral squamous cell carcinoma is attributed to a number of factors, including inadequate screening and diagnostic tools, a lack of knowledge of the precursor lesions of oral cancer, and delayed diagnosis (16).

Striving for the best and most conservative diagnosis methods is a key objective in medicine. Therefore, the integration of salivary metabolomics in oral cancer research, seems legitimate.

This review provides more up-to-date information on how salivary metabolomics helps in the identification of oral cancer. It is necessary to know the role of salivary metabolomics involved in the diagnosis of oral cancer as this will provide oncologists and the scientific community a better understanding on how to use this diagnostic tool for more accurate and quicker detection of oral cancer**.** Moreover, this review provides more depth into how the different metabolomic analytic techniques lead to the identification of metabolites present in oral cancer and which metabolites are prominent in oral cancer tissues and cells.

Hypothesis

The working hypothesis of our study considers that the analysis of salivary metabolomic biomarkers will constitute an interesting resource for the detection of oral cancer processes.

6. OBJECTIVES

General Objectives

This systematic review aims to provide an overview on how salivary metabolomics can help in the diagnosis of oral cancer.

Specific Objectives

I. Review if salivary metabolomics leads to earlier identification of oral cancer.

II. Study the viability of salivary metabolomics in the context of oral cancer.

7. MATERIALS AND METHODS

This systematic review was conducted according to the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analysis)(14).

7.1 Identification of the PIO question

The biomedical online databases PubMed, Scopus, and Web of Science were used to search for articles with scientific relevance indexed about the application of salivary metabolomics to help in the diagnosis of oral cancer published from January 2011 until December 2022 to answer the following question: *In adult patients does salivary metabolomics improve the diagnosis in oral cancer?*

The study question was formed according to the PIO question (Population, Intervention, Outcome).The format of the question was established as the following:

- **P** (Population): Adult patients
- **I** (Intervention): Salivary metabolomics
- **O**(Outcome): Improve the diagnosis of oral cancer

7.2 Criteria of eligibility

The inclusion criteria were:

- **Type of study**: Randomized controlled clinical trials, studies of prospective and retrospective cohorts and case studies; Publications available in full text and in English and Spanish; published in the last 10 years (January 2011-December 2022).
- **Type of patients:** Patients of both genders with oral cancer aged between 18-99.
- **Type of Intervention:** Salivary metabolomics in identification and diagnosis of oral cancer.
- **Type of Outcome Variables:** Studies that provided data related to salivary metabolomics improving the diagnosis of oral cancer.

The exclusion criteria included were systematic reviews and meta-analysis, bibliographic reviews, animal studies, editorial material, and letters as well as a sample size with fewer than 5 patients.

7.3 Source of information and database

An individual search on each selected platform (PubMed, Scopus and Web of Science) was performed to obtain the articles to answer the PIO question and the objectives. On all databases, the filter for the following languages was applied: English and Spanish. Furthermore, the filter of articles between January 2011 to December 2022 was applied. The keywords were: ‘’Adult Patient;'' Oral cancer’’ and ‘’Salivary metabolomics”. The keywords were combined with the Boolean operators ‘AND’ and ‘OR’ as well as the controlled terms (“MeSH” for Pubmed) in an attempt to obtain the best search results. A summary of the search carried out on in all the databases is shown in Table 2.

The search on Pubmed-Medline was:("adult"[MeSH Terms] OR "adult"[All Fields] OR "adults"[All Fields] OR "adult s"[All Fields]) AND ("patient s"[All Fields] OR "patients"[MeSH Terms] OR "patients"[All Fields] OR "patient"[All Fields] OR "patients [All Fields]) AND "mouth neoplasms"[MeSH Terms] AND ("salivary"[All Fields] AND "metabolomics"[MeSH Terms])

On Scopus, the search was: (ALL (adult AND patient) AND (‘’Oral cancer’’) AND ALL (Salivary metabolomics’’))

On the Web of Science search was as follows: adult patient (All Fields) AND "oral cancer" (All Fields) AND "salivary metabolomics" (All Fields).

Furthermore, a cross search of potentially relevant articles was carried out interesting for analysis. Duplicate studies were removed from the review.

Table 2: Search Summary in each database.

Database	Search	Number of articles
Pubmed	("adult"[MeSH Terms] OR "adult"[All Fields] OR "adults"[All Fields] OR "adult s"[All Fields]) AND ("patient s"[All Fields] OR "patients"[MeSH Terms] OR "patients"[All Fields] OR "patient"[All Fields] OR	8

	"patients s"[All Fields]) AND "mouth neoplasms"[MeSH Terms] AND ("salivary"[All Fields] AND "metabolomics"[MeSH Terms])	
Scopus	(ALL (adult AND patient) AND (''Oral cancer'') AND ALL (Salivary metabolomics''))	117
Web of Science	adult patient (All Fields) AND "oral cancer" (All Fields) AND "salivary metabolomics" (All Fields)	1

7. 4 Study selection process

A three-stage selection process was carried out. The selection of studies was carried out by two reviewers (SI & JA). The first stage reviewed the titles to eliminate irrelevant articles. In the second stage, the title and abstracts were filtered according to the type of studies, language, a sample size. In the third stage, each article was read completely, and the data was taken according to the eligibility to be included in the systematic review.Any disagreement in the eligibility of the study was resolved by discussion between both reviewers until a consensus was reached.

The degree of agreement regarding the inclusion of potential studies was calculated by k-statistics (Cohen kappa test) for the second and third stages of selection.

7.5 Data Extraction

To obtain the results from the search the following criteria were included: Authors, Type of study, Year of publication, Language, Patient's Age and Gender, Number of patients in the study, Patients with Oral Cancer and Oral Squamous Cell Carcinoma, and Inclusion and Exclusion criteria.

Principle variable

Salivary metabolomics: By discovering metabolites present in saliva can help distinguish which metabolites are more prominent in those who have oral cancer and hence lead oral cancer diagnosis earlier and better prognosis for patients.

Secondary Variables

Earlier identification of oral cancer: This will be beneficial in the medical and dental field as it led to detection and diagnosis of the sixth most common cancer in the world. This will allow for better treatment plans and prognosis of the patient.

Viability of salivary metabolomics: The capacity to make use of salivary metabolomics in the detection of oral cancer can save lives because it is painless for the patient and results are obtained faster than biopsies.

7.6 Quality and risk of bias assessment

The articles selected for this systematic review were evaluated through the CASPe, Critical Appraisal Skills Program.(31).It includes tools for the evaluation of seven different types of designs. (Diagnostic test studies, systematic reviews, clinical trials, cohort studies, case-control studies, economic evaluation studies, and case series) and depending on the type of study, a series of questions were answered about the type of study design, study objectives inclusion/exclusion criteria, results, and conclusions, as well as conflicts of interest.

After answering the questions, it was subsequently determined whether the study had high, medium, or low quality to proceed with its inclusion or exclusion. Depending on whether it presented a yes, partially, or no in the method section, it was classified according to the quality it presented, as well as in the rest of the areas (research questions results, conclusions, conflicts of interest and external validity) in order to obtain the quality of each article.

The degree of inter-examiner agreement of the quality of assessment methodological was obtained with Cohen's Kappa test, following the scale proposed by Landis and Koch (32).

7.7 Synthesis of data

To summarise and compare the variables various characteristics of the studies were compared. The characteristics were: Country, type of study, sample size , age (range/mean), gender and diagnosis, the type of saliva, as well as centrifuging and storing of the sample collected along with the method of analysis of saliva collected.

Factors such as age where the mean age range was between 57 to 66 years old plays a role in oral cancer as oral cancer is mainly diagnosed over 55 years old. The type of saliva, along with centrifuging and storing of the sample collected along with the method of analysis of saliva collected all are important factors to help find which metabolites are prominent in the saliva of patients with oral cancer.

A meta-analysis was not possible due to a lack of studies on salivary metabolomics in oral cancer. As a result, the data given here should be carefully considered and were presented in every study group descriptively.

8. RESULTS

8.1 Selection of Studies. Flow chart

A total of 126 articles were obtained from the initial search process: PubMed(n=8), Scopus (n=117) and Web of Science(n=1). Of these publications 14 were identified as potentially eligible articles by screening the titles and abstracts. Full text articles were subsequently obtained and thoroughly evaluated. As a result, 7 articles met the criteria for inclusion and were included in this systematic review (Fig.2). The information related to the excluded articles (and the reasons for their exclusion) is presented in Table 3.

The k value for inter-examiner agreement on the inclusion of the studies was 1.0 (titles and abstracts) and 1.0 (full texts), which indicates a “good” and “complete” agreement, respectively, according to the Landis and Koch criteria (32).

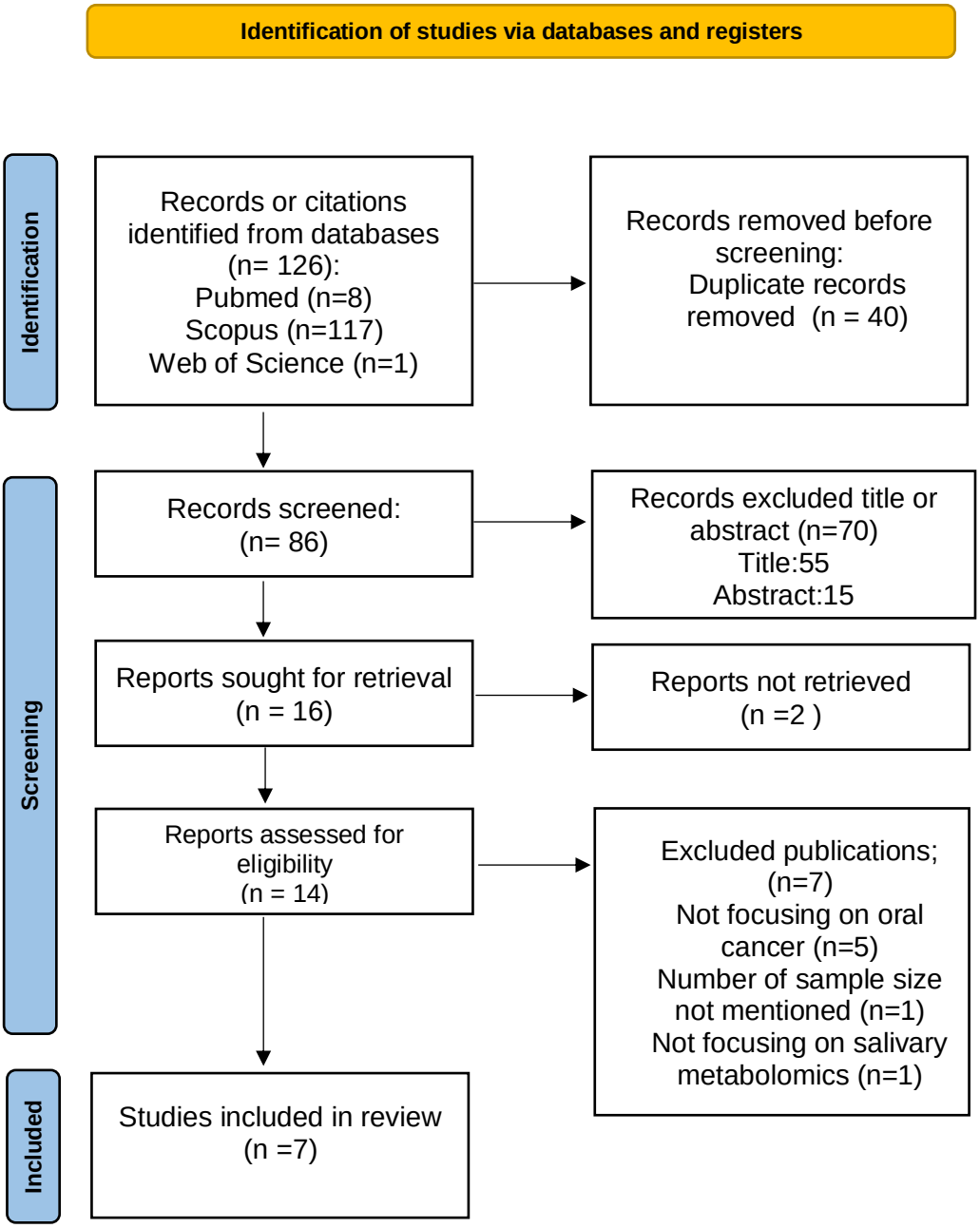

Fig. 2. PRISMA 2020 flow diagram for searches of databases, registers, and other sources.

Table 3: Articles excluded (and their reason for exclusion) from this systematic review.

Author. Year	Publication	Reason for exclusion
Wang.2016 (17)	Med Oncol	No focus on oral cancer
Kaczor Urbanowicz.2016(18)	Journal of Cellular and Medical Medicine	No focus on oral cancer
Ai.2012(19)	International Journal of Oral Science	No focus on salivary metabolomics
Wang.2014 (20)	Scientific Reports	Number of sample size was not mentioned
Meleti.2020 (21)	Metabolites Journal	No focus on oral cancer
Gonzalez-Covarrubias.2022(22)	Metabolites Journal	No focus on oral cancer
Hyvarinen.2021(23)	Metabolites Journal	No focus on oral cancer

8.2 Analysis of the characteristics of the revised studies

A total of 7 studies were analysed in this systematic review. All the studies chosen for this systematic review are case control studies(24-30).The studies were conducted in five different countries. Three of the studies were carried out in Japan (24,28,30),one in China(25), one in Brazil(26),one in Thailand(27) and one in United States of America(29).All these studies evaluated the metabolites differences found in Oral cancer and healthy patients.

A total of 447 participants were analysed approximately half of which served as the control groups. The age of the participants was mostly middle age between 57 to 66 years old and there were slightly more females than males. Six out of the seven studies included participants diagnoses with OSCC (24,25,26,28-30).

All seven studies collected unstimulated whole saliva. Six out of seven studies stored the collected sample at a temperature of -80(24,25,26,28-30) and the one which didn't store the sample at -80 stored it at a temperature of -20 (27).The general characteristics are summarised in Table 4 and detailed characteristics are summarised in Table 5.

Table 4:General characteristics of the included articles.

Author/Year	Country	Type of Study	Sample	Age(range/mean)	Gender M/F Ratio	Diagnosis
Ohshima et al.2021	Japan	Case control	43	65	21:22	OSCC
Wei et al.2011	China	Case control	71	58	39:32	OSCC
De Sa Alves et al.2021	Brazil	Case control	68	57	40 :28	OSCC
Supawat et al.2021	Thailand	Case control	25	57	15 :10	OC
Ishikawa et al.2016	Japan	Case control	68	65	30 :38	OSCC
Lohavanichbur et al.2018	United States of America	Case control	100	56	55 :45	OSCC
Ishikawa et al.2021	Japan	Case Control	72	66	38 :34	OSCC

Table 5: Detailed characteristics of included studies.

Author/Year	Type of Saliva	Centrifugation and storing	Method of Analysis
Ohshima et al.2021	Unstimulated whole saliva	Centrifuged at 2600× g for 15 min at 4 ◦C,	CE-TOF-MS
Wei et al.2011	Unstimulated whole saliva	Centrifuged at 3500× g for 20 min at 4 ◦C immediately stored at −80 ◦C until analysis	UPLC-QTOF-MS
De Sa Alves et al.2021	Unstimulated whole saliva	Stored at −80 ◦C until analysis	GC-MS
Supawat et al.2021	Unstimulated whole saliva	Immediately stored at −20 ◦C until analysis	NMR spectroscopy
Ishikawa et al.2016	Unstimulated whole saliva	Immediately stored at −80 ◦C	CE-TOF-MS
Lohavanichbur et al.2018	Unstimulated whole saliva	Centrifuged at 1300× g at 4 ◦C stored at −80 ◦C	NMR and LC-MS
Ishikawa et al.2021	Unstimulated whole saliva	Stored at −80 ◦C	CE-TOF-MS

8.3 Assessment of methodological quality and risk of bias

The articles selected for this systematic review were evaluated through the CASP, (Critical Appraisal Skills Program) Case Control Study Standard Checklist (Table 6).After answering the questions with either Yes, No or Can't tell, it was subsequently determined whether the study had a high, medium, or low risk of bias. Three of studies had a low risk of bias(25,26,30) and the remaining four studies overall results were medium risk of bias (24,27,28,29).

The k value (Cohen kappa test) on the agreement between the two reviewers of the methodological quality was 1.0 according to the Landis and Koch scale (32).

Table 6: CASP Case Control Study Standard Checklist (31).

Author/Year	Ohshima et al.2021	Wei et al.2011	De Sa Alves et al.2021	Supawat et al.2021	Ishikawa et al.2016	Lohavanichbur et al.2018	Ishikawa et al.2021
Did the study address a clearly focused issue?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Did the authors use an appropriate method to answer their question?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were the cases recruited in an acceptable way?	Can't tell	Yes	Yes	Yes	Yes	Yes	Can't tell
Were the controls selected in an acceptable way?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the exposure accurately measured to minimise bias?	Can't tell	Yes	Can't tell	Can't tell	Can't tell	No	Yes
Aside from the experimental intervention, were the groups treated equally?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Have the authors taken account of the potential	No	Yes	Can't tell	Can't tell	No	Yes	Yes

confounding factors in the design and/or in their analysis?							
How large was the treatment effect?	Can tell	Can't tell	Can tell	Can't tell	Can tell	Can't tell	No
How precise was the estimate of the treatment effect?	Can't tell	Can't tell	Can't tell	Can't tell	Can't tell	Can't tell	No
Do you believe the results?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Can the results be applied to the local population?	Yes	Yes	Can't tell	Yes	Yes	Can't tell	Yes
Do the results of this study fit with other available evidence?	Yes	Yes	Yes	Yes	Yes	Yes	Yes

8.4 Synthesis of Results

8.4.1 Diagnosis of oral cancer by salivary metabolomics

A total of 5 studies (24,26,27-29) included the following metabolites found common between them: Choline, Creatine, Lactate, Polyamines and Trimethylamine N -oxide . All of these metabolites mentioned showed significant differences in OSCC patients compared to healthy patients.

In the following studies (24,26) Choline was significantly high and in the other studies (25,27,28) it was found significantly low. Creatine was found significantly high in the following studies (25,26,27,28) and low in the rest of studies in this review. Lactate was substantially high in these studies (25,27,28).Polyamines was distinctly high in these studies (24,26,27) and Trimethylamine N- oxide in these studies (24.27) all of these findings are summarised in Table 7.

Table 7: Metabolites found significantly high or low in the following studies.

Author/Year	Choline	Creatine	Lactate	Polyamines	Trimethylamine N -oxide
Ohshima et al.2021	↑	↓	↓	↑	↑
De Sa Alves et al.2021	↓	↑	↑	↓	↓
Supawat et al.2021	↑	↓	↓	↑	↓
Ishikawa et al.2016	↓	↑	↓	↑	↑
Lohavanichbur et al.2018	↓	↑	↑	↓	↓

Significantly high: ↑ **Significantly low:** ↓

8.4.2. Earlier identification of oral cancer by metabolomics

De Sá Alves et al. (26) conducted the first investigation on a group of Latin Americans with OSCC. The findings revealed altered metabolic pathways, such as the malate-aspartate shuttle and beta-alanine metabolism, as well as the Warburg effect. There were 10 salivary metabolites identified as possible OSCC indicators above the AUC = 0.9 threshold: malic acid, lactate, catechol, 2-ketoadipic acid, creatine , methionine, urea, leucine, inosine, and protocatechuic acid. Metabolites lactate and creatine were also detected in the study of Lohavanichbutr et al. (29).

Supawat et al. (27) detected 11 metabolites (isobutyrate, fucose ,propionate, choline, , trimethylamine N-oxide ,cisaconitate acetoacetate, methanol, glycine , taurine and aspartate were found in saliva samples from normal subjects and oral cancer patients. Trimethylamine N-oxide (TMAO) and glycine levels were substantially greater in oral cancer patients' saliva than in normal participants' saliva. This finding implies that the metabolic profiles of saliva samples from healthy people and oral cancer patients are not the same.

8.4.3.Viability of salivary metabolomics in the context of oral cancer

Lohavanichbutr et al. (29) compared T1/T2 vs. controls and T3/T4 vs. T1/T2 to see whether of the 80 metabolites were linked with early or late-stage malignancy. Using FDR < 0.1 as a criterion, 60 metabolites were found to be distinguishable between early-stage tumor and controls. One of the 80 metabolites (cystathionine ketimine) was found in significant high amounts in early-stage tumor from late-stage tumor (p-value 0.003, FDR 0.079).

Additionally, several studies (24,28,30) used MetaboAnalyst to examine the salivary metabolomics data this took into consideration the number of metabolites detected in the individual metabolic pathways and any alterations found between the cases and controls.

Among these three studies the following pathways were found alanine, glutamate and aspartate along with TCA Cycle and citric acid pathway. The pathways which were significantly greater OSCC patients were glutamate and aspartate.

9. DISCUSSION

This systematic review provides information based on the scientific evidence on the results of salivary metabolomics for oral cancer detection. The objective of this was to provide an overview of on how salivary metabolomics can help in the diagnosis of oral cancer and secondly review if salivary metabolomics can lead to identification of oral cancer as well as study the viability of salivary metabolomics in the context of oral cancer.

9.1 Diagnosis of oral cancer by salivary metabolomics

From the studies present in this review it can be said that salivary metabolomics can help in the diagnosis of oral cancer. The use of saliva as a sample for metabolite detection benefits both the patient and the dentist, because saliva can be accumulated frequently in a non- invasive manner for oral cancer screening (28).

Ishikawa et al. (28) examine the metabolomic profiles in saliva samples obtained from patients with oral cancer, as well as in their oral tissues and adjacent matched control tissues and find that 17 metabolites are consistently enhanced in the saliva and tumor tissues. These metabolites include amino acids such as valine, tryptophan, and threonine, as well as others. Spermidine in the polyamine route and many metabolites, including choline, methionine, and S-adenosyl methionine, in a pathway upstream of the polyamine production pathway, were also enhanced (28).

The salivary metabolites of control patients and patients with oral cancer might be significantly differentiated. These modifications to metabolism may aid in the development of salivary metabolites of oral cancer and generate interest in larger-scale research to confirm the findings (28).

Added to that, Wei et al.(25) chose a panel of five salivary metabolites: valine, n- eicosanoic acid, lactic acid Y- aminobutyric acid and phenylalanine, Reviver operating curves for oral squamous cell cancer were used to assess the prediction potential of each of the five salivary metabolites. Combining valine, lactic acid, and phenylalanine produced acceptable accuracy, specificity, and potential predictive value in discriminating oral squamous cell cancer from control groups (25).

This work effectively confirmed the efficacy of salivary metabolome diagnostics for cancer, and the findings imply that the saliva metabolomics technique complements clinical detection of oral squamous cell carcinoma, leading to a better disease diagnosis and prognosis (25)

9.2 Earlier identification of oral cancer by metabolomics

Oral cancers come into close contact with saliva, and tumor cell by-products are released into the saliva. As a result, the presence of hydrolysed products from local tissue damage or tumor cells in the saliva of oral cancer patients allows for salivary metabolite identification. All of the studies demonstrate that salivary metabolomics can help identify oral cancer. Each study examined the saliva sample acquired using various procedures.

Ohshima et al.(24) analyses metabolic changes in saliva samples of Japanese patients with OSCC using capillary electrophoresis-mass spectrometry metabolome analysis. Choline is the metabolite with the largest statistically significant difference between OSCC patients and healthy persons out of the twenty-five potential metabolites (24).

Choline is exceptionally important for prognosis and treatment outcome prediction. Its aberrant metabolism is a typical feature linked to progression. It promotes membrane production and breakdown, which aids metastasis. If the levels rise substantially, the prognosis will be poor, as will the response to treatment (24).

Significant changes in other metabolic pathways, including the branched-chain amino acid and aromatic amino acid pathways, polyamine metabolism, urea cycle, creatine metabolism, and 3-hydroxybutyric acid metabolism, were discovered in addition to reduced choline metabolism. Almost all identified changes in the salivary metabolome are related to synthesis and degradation processes, which are reflected in cancer cell uncontrolled growth (28).

Furthermore, Ishikawa et al. (28) examine salivary metabolites for oral cancer screening by analysing both saliva and tumor tissue samples. There were 85 metabolites reported to be elevated in tumor tissue and 43 in saliva, with seventeen indicators shared by both types of data. According to multivariate logistic regression modelling, the combination of S-adenosylmethionine and pipecolate has a strong power to differentiate oral cancer patients from controls.

There were no significant differences in the salivary metabolome depending on OSCC stage. Salivary trimethylamine N-oxide and glycine levels are considerably greater in oral cancer patients, according to Supawat et al.(27).

Ishikawa et al. (30) studies the prognostic significance of certain saliva metabolites. Two metabolites (5-hydroxylysine and 3-methylhistidine) were shown to be crucial to overall survival based on the OSCC group. Only 3-methylhistidine is shown to be a statistically significant prognostic predictor for OS in the validation group's investigation. N-acetylglucosamine was shown to be a potentially relevant predictive marker for disease-free survival by the training group (30).

According to this concept, OSCC patients with salivary 3-methylhistidine concentrations above the median had significantly lower OS rates than those with lower levels. The authors speculate that salivary 3-methylhistidine could serve as an important metabolite for identifying and diagnosis oral cancer (30).

Moreover, the use of saliva samples from non-treated oral cancer patients is a strength of all of the studies considered in the present review. As a result, the data obtained is particularly important to understanding biochemical alterations in patients with oral cancer. The findings should aid in distinguishing between individuals with oral cancer and healthy individuals (30).

9.3 Viability of salivary metabolomics in the context of oral cancer

OSCC is a complex illness caused by a sequence of interconnected biochemical changes rather than a single disruptive event. As a result, a panel of metabolite markers will increase sensitivity and specificity for OSCC detection. Several studies have examined salivary metabolome changes to identify OSCC from other disorders of the oral mucosa, which may be considered oral potentially malignant conditions. This is very important for cancer prevention and early detection of lesions (25,26).

Wei et al. (25) examined the stratification value of five salivary metabolites (-aminobutyric acid, phenylalanine, valine, n-eicosanoic acid, and lactic acid) using ROC curves. Based on this, 2-3-item combinations were constructed, and the predictive power for OSCC in patients with oral leukoplakia (OLK) and no changes in the oral mucosa was good (25).

For OLK, the combination of valine, lactic acid, and phenylalanine outperformed OSCC in terms of accuracy, sensitivity, specificity, and positive predictive value. Increased lactic acid levels and decreased amino acid levels have been linked to increased glycolysis and an inefficient tricarboxylic acid cycle in cancer tissues during cell development. These findings, according to the authors, may help supplement clinical differentiation of OSCC from OLK, enhancing prognosis along with earlier identification (25).

Because of the multifactorial nature of oncogenesis and the variability of oncogenic pathways, it is improbable that all cancers of a certain organ can be diagnosed by a single metabolite with high specificity and sensitivity. As a result, several statistical methodologies were applied into a prediction model to uncover metabolite combinations that may identify OSCC patients (25).

Although encouraging, the sensitivity (86.5%) and specificity (82.4%) are insufficient to qualify as a clinical tool for illness screening. Efforts should be undertaken to research and verify other possible metabolites as well as to combine them to increase the power of oral cancer prediction (25).

Added to that, the study conducted by De sa Alves et al.(26) presents that salivary metabolic screening in individuals exposed to risk factors such as smoking, and alcohol intake can reveal potential salivary biomarkers of oral cancer and enhance the early identification of carcinoma. As a consequence, additional research with bigger populations may yield comparable results and new insights, allowing these metabolites to be employed as a non-invasive tool in oral cancer screening (26).

9.4 Limitations

Many metabolic indicators continue to have unclear physiological and pathological implications. Saliva is undeniably a diagnostic resource that is simple to collect and use for analytical diagnostics. Changes in saliva composition, particularly metabolites, may be quite dynamic and are influenced by a variety of factors such as dental health, current microbiome activity, and dietary patterns.

External variables such as collection or processing temperature and duration could potentially impact the stability of the salivary metabolome. As a result, there is a possibility of compromised diagnostic accuracy when assessing

such dynamic components. More standardised diagnostic test accuracy studies that take external factors into consideration through rigorous design, conduct, and reporting are required.

Although not all articles clearly mentioned the histological diagnosis of OSCC, it may be suggested that most of these individuals had the most prevalent type of oral cancer. Additionally, despite the diversity of identified metabolites that disrupt various metabolic pathways, only a few researchers presented statistical criteria to quantify the diagnostic accuracy of these metabolites, allowing only qualitative examination.

9.5 Future prospectives

Despite the fact that metabolomics is a relatively new concept compared to genomes, transcriptomics, and proteomics, it has had a considerable influence in identifying metabolites connected to a variety of disorders, including cancer.

It gives critical information on various metabolic alterations that occur throughout cancer, revealing specific pathways involved in its development. Furthermore, it identifies many onco-metabolites, from which countless new potential metabolites might be developed for early diagnosis and therapy monitoring.

The application of salivary metabolites is the future of an exciting new approach to treatment and prevention. It is non-invasive, which benefits both the patient and the healthcare professional, and it is very easy to use because no training is required. The sampling is straightforward, quick, and painless for the patient. It is simple to execute on young children and can be collected in the dental clinic.

The research of salivary metabolomics is advancing, and by increasing continuous scientific advancement, new metabolites that can help prevent several malignancies and considerably enhance quality of life can be discovered. This will help the general population by making prevention more accessible, quick to reach, and affordable, enhancing efficiency and lowering costs.

On a larger scale, the vast amount of information gathered from a single saliva sample can lead to pharmacological breakthroughs and new medical horizons for a more personalized approach.

10. CONCLUSIONS

Primary Conclusions

The purpose of this systematic review was to provide an overview on how salivary metabolomics can help in the diagnosis of oral cancer. Based on the primary objective salivary metabolites can be used as a diagnostic tool for early diagnose and monitor staging in patients with oral cancer.

Secondary conclusions

In relation to the secondary aims there is tremendous potential for the viability of salivary metabolites in relation to the earlier identification of oral cancer.

Future research using various types of technology, a universal collection technique, and medical personnel education will make salivary metabolomic diagnostics one of the most widely used tools for preventing and monitoring oral cancer.

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12. ANNEX

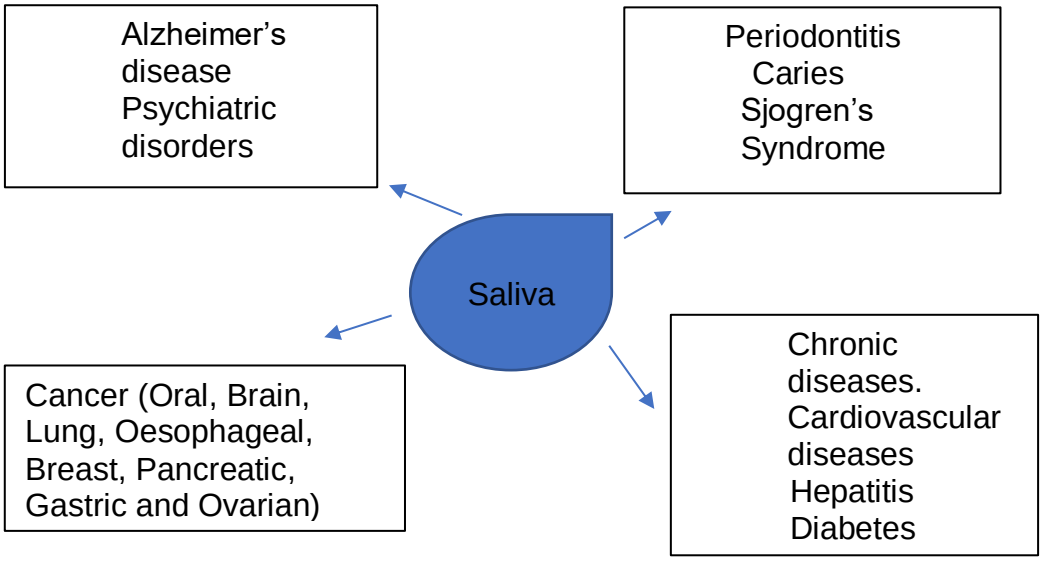

Fig. 1. Systemic diseases with saliva as an etiopathogenic factor (13).

Table 1*:* A summary of the net contributions to whole-mouth saliva (11).

Glandular Fluid	Gingival Crevicular Fluid	Cellular Components
Major glands	Serum transudate	Shed epithelial cells.
Minor glands	Immune cell infiltrate	Oral bacteria
		Immune cell infiltrate (tonsillar)

Table 2 : Search Summary in each database.

Database	Search	Number of articles
Pubmed	("adult"[MeSH Terms] OR "adult"[All Fields] OR "adults"[All Fields] OR "adult s"[All Fields]) AND ("patient s"[All Fields] OR "patients"[MeSH Terms] OR "patients"[All Fields] OR "patient"[All Fields] OR "patients s"[All Fields]) AND "mouth neoplasms"[MeSH Terms] AND ("salivary"[All Fields] AND "metabolomics"[MeSH Terms])	8
Scopus	(ALL (adult AND patient) AND (‘’Oral cancer’’) AND ALL (Salivary metabolomics’’))	117
Web of Science	adult patient (All Fields) AND "oral cancer" (All Fields) AND "salivary metabolomics" (All Fields)	1

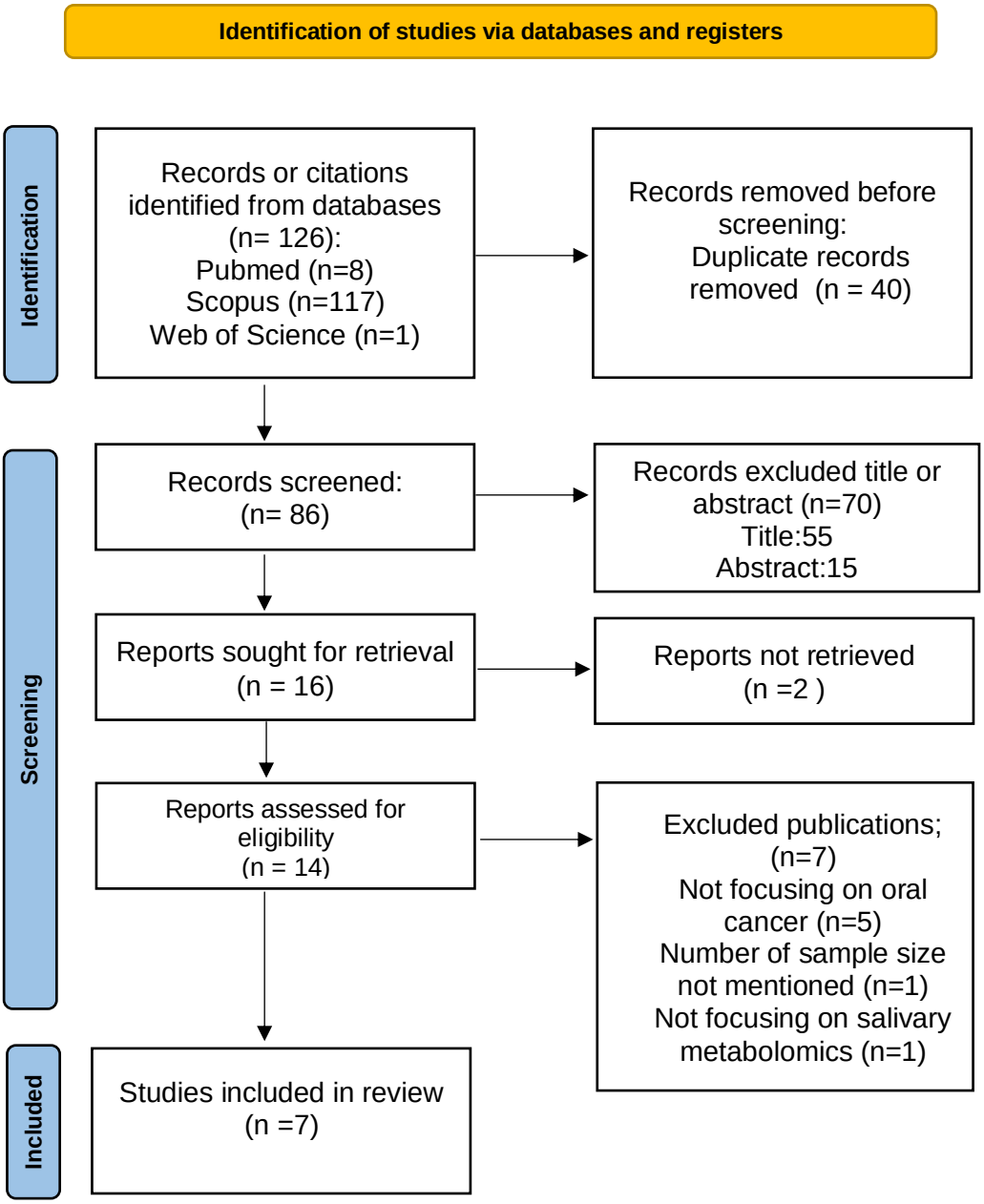

Fig. 2. PRISMA 2020 flow diagram for searches of databases, registers, and other sources.

Table 3: Articles excluded (and their reason for exclusion) from this systematic review.

Author. Year	Publication	Reason for exclusion
Wang.2016 (17)	Med Oncol	No focus on oral cancer
Kaczor Urbanowicz.2016(18)	Journal of Cellular and Medical Medicine	No focus on oral cancer
Ai.2012(19)	International Journal of Oral Science	No focus on salivary metabolomics
Wang.2014 (20)	Scientific Reports	Number of sample size was not mentioned
Meleti.2020 (21)	Metabolites Journal	No focus on oral cancer
Gonzalez-Covarrubias.2022(22)	Metabolites Journal	No focus on oral cancer
Hyvarinen.2021(23)	Metabolites Journal	No focus on oral cancer

Table 4 :General characteristics of the included articles.

Author/Year	Country	Type of Study	Sample	Age(range/mean)	Gender M/F Ratio	Diagnosis
Ohshima et al.2021	Japan	Case control	43	65	21:22	OSCC
Wei et al.2011	China	Case control	71	58	39:32	OSCC
De Sa Alves et al.2021	Brazil	Case control	68	57	40 :28	OSCC
Supawat et al.2021	Thailand	Case control	25	57	15 :10	OC
Ishikawa et al.2016	Japan	Case control	68	65	30 :38	OSCC
Lohavanichbur et al.2018	United States of America	Case control	100	56	55 :45	OSCC
Ishikawa et al.2021	Japan	Case control	72	66	38 :34	OSCC

Table 5: Detailed characteristics of included studies.

Author/Year	Type of Saliva	Centrifugation and storing	Method of Analysis
Ohshima et al.2021	Unstimulated whole saliva	Centrifuged at 2600× g for 15 min at 4 ◦C	CE-TOF-MS
Wei et al.2011	Unstimulated whole saliva;	Centrifuged at 3500× g for 20 min at 4 ◦C stored at −80 ◦C until analysis	UPLC-QTOF-MS
De Sa Alves et al.2021	Unstimulated whole saliva	Stored at −80 ◦C	GC-MS
Supawat et al.2021	Unstimulated whole saliva	Immediately stored at −20	NMR spectroscopy
Ishikawa et al.2016	Unstimulated whole saliva	Immediately stored at −80 ◦C	CE-TOF-MS
Lohavanichbur et al.2018	Unstimulated whole saliva	Centrifuged at 1300× g at 4 ◦C for 10 min; stored at −80 ◦C	NMR and LC-MS
Ishikawa et al.2021	Unstimulated whole saliva	Stored at −80 ◦C	CE-TOF-MS

Table 6: CASP Case Control Study Standard Checklist (31).

Author/Year	Ohshima et al.2021	Wei et al.2011	De Sa Alves et al.2021	Supawat et al.2021	Ishikawa et al.2016	Lohavanichbur et al.2018	Ishikawa et al.2021
Did the study address a clearly focused issue?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Did the authors use an appropriate method to answer their question?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were the cases recruited in an acceptable way?	Can't tell	Yes	Yes	Yes	Yes	Yes	Can't tell
Were the controls selected in an acceptable way?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the exposure accurately measured to minimise bias?	Can't tell	Yes	Can't tell	Can't tell	Can't tell	No	Yes
Aside from the experimental intervention, were the groups treated equally?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Have the authors taken account of the potential confounding factors in the	No	Yes	Can't tell	Can't tell	No	Yes	Yes

design and/or in their analysis?							
How large was the treatment effect?	Can tell	Can't tell	Can tell	Can't tell	Can tell	Can't tell	No
How precise was the estimate of the treatment effect?	Can't tell	Can't tell	Can't tell	Can't tell	Can't tell	Can't tell	No
Do you believe the results?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Can the results be applied to the local population?	Yes	Yes	Can't tell	Yes	Yes	Can't tell	Yes
Do the results of this study fit with other available evidence?	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 7: Metabolites found significantly high or low in the following studies.

Author/Year	Choline	Creatine	Lactate	Polyamines	Trimethylamine N -oxide
Ohshima et al.2021	↑	↓	↓	↑	↑
De Sa Alves et al.2021	↓	↑	↑	↓	↓
Supawat et al.2021	↑	↓	↓	↑	↓
Ishikawa et al.2016	↓	↑	↓	↑	↑
Lohavanichbur et al.2018	↓	↑	↑	↓	↓

Significantly high: ↑ **Significantly low:** ↓

PRISMA 2020 Checklist for Systematic Reviews

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Title Page
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	4-14
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	15
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	16
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	
Search strategy	7	Present the full search strategies for all databases, registers, and websites, including any filters and limits used.	17-18
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	18
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	18
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	19
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	

Section and Topic	Item #	Checklist item	Location where item is reported
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	24-26
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	21
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	22
Study characteristics	17	Cite each included study and present its characteristics.	23-24
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	24-26
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimates and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	29-31

Section and Topic	Item #	Checklist item	Location where item is reported
	23b	Discuss any limitations of the evidence included in the review.	32-33
	23c	Discuss any limitations of the review processes used.	32-33
	23d	Discuss implications of the results for practice, policy, and future research.	33
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code, and other materials	27	Report which of the following are publicly available and where they can be found template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

SALIVARY METABOLOMICS AS A CANDIDATE FOR DIAGNOSIS OF ORAL CANCER : SYSTEMATIC REVIEW

Running title: Salivary Metabolomics as a Candidate for Diagnosis of Oral Cancer

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Abstract

Introduction: Oral cancer often known as mouth cancer, is a malignant tumor of the oral cavity and is the world's sixth most prevalent cancer. Oral squamous cell carcinoma accounts for about 90% of oral cavity cancers. The systemic study of metabolites is known as metabolomics where small molecules are generated by the process of metabolism. A systematic review was carried to see how salivary metabolomics can help in the diagnosis of oral cancer.

Materials and Methods: An electronic search was carried out in the databases of PubMed, Scopus, and Web of Science on salivary metabolomics for oral cancer detection until December 2022.

Results: Of 126 potentially eligible articles,7 complied with the inclusion criteria. All 7 studies evaluated the metabolites differences found in oral cancer and healthy patients. The diagnostic material in each study was unstimulated whole saliva, which was analyzed using several spectroscopic techniques. Oral squamous cell carcinoma. patients varied significantly from the healthy participants in terms of metabolites. There was evidence of altered metabolic pathways, such as choline metabolism, amino acid pathways, and glycolysis, among the observed salivary metabolites.

Conclusion: Despite the limitations salivary metabolites can be used as a diagnostic tool for early diagnose as well as there is tremendous potential for the viability of salivary metabolites in relation to the earlier identification of oral cancer.

Keywords: *Oral cancer, Salivary metabolomics, Oral cancer detection, Oral cancer diagnosis, Metabolites*

Introduction

Oral cancer is the sixth-most common head-and-neck cancer in the world. Oral squamous cell carcinoma is a multifactorial condition marked by a complex interaction of environmental variables and molecular changes that lead to unrestricted cell growth. The low survival rate of . Oral squamous cell carcinoma is attributed to a number of factors, including inadequate screening and diagnostic tools, a lack of knowledge of the precursor lesions of oral cancer, and delayed diagnosis (1).

The use of saliva as a screening medium offers a quick, low-cost, secure, and non-invasive method. It is increasingly seen as a means to test for diseases. The metabolic profile of saliva is sometimes referred to as the "mirror of the body." This is because it has the ability to collect onco-metabolites that result from metabolic rewiring. It also offers an overview on metabolites with notable aberrant enzymatic regulation and focuses on the changed pathways during metabolic reprogramming (9,10).

Several articles have published systematic reviews on salivary metabolomics in oral cancer however in these reviews there was no focus on whether salivary metabolites can be used in the detection of oral cancer

Therefore, this review provides more depth into how the different metabolomic analytic techniques lead to the identification of metabolites present in oral cancer and which metabolites are prominent in oral cancer tissues and cells.

It is necessary to know the role of salivary metabolomics involved in the diagnosis of oral cancer as this will provide oncologists and the scientific community a better understanding on how to use this diagnostic tool for more accurate and quicker detection of oral cancer.

The aim of the present systematic review was to systematically review the following question: In adult patients does salivary metabolomics improve the diagnosis in oral cancer?

Material and Methods

This systematic review complies with the PRISMA statement (Preferred Reporting Items for Systematic reviews and Meta-Analyses) (14).

- Focus question:

The focus question was established according to the PIO structured question:

P (Population): Adult patients.

I (Intervention): Salivary metabolomics.

O (Outcome): Improve the diagnosis of oral cancer.

- Eligibility criteria:

The inclusion criteria were:

- Type of study: Randomized controlled clinical trials, studies of prospective and retrospective cohorts and case studies; Publications available in full text and in English and Spanish; published in the last 10 years (January 2011-December 2022).
- Type of patients: Patients of both genders with oral cancer aged between 18- 99. 17
- Type of Intervention: Salivary metabolomics in identification and diagnosis of oral cancer.
- Type of Outcome Variables: Studies that provided data related to salivary metabolomics improving the diagnosis of oral cancer.

  The exclusion criteria included were systematic reviews and meta-Analysis bibliographic reviews, animal studies, editorial material, and Letters as well as a sample size with fewer than 5 patients.

- Information sources and data search:

An individual search on each selected platform mentioned above (PubMed, Scopus and Web of Science) was performed to obtain the articles to answer the PIO question and the objectives. On all databases, the filter for the following languages was applied: English and Spanish. Furthermore, the filter of articles between January 2011 to December 2022 was applied. The keywords were: ‘’Adult Patient;'' Oral cancer’’ and ‘’Salivary metabolomics”. The keywords were

combined with the Boolean operators ‘AND’ and ‘OR’ as well as the controlled terms (“MeSH” for Pubmed) in an attempt to obtain the best search results.
The search on Pubmed-Medline was:("adult"[MeSH Terms] OR "adult"[All Fields] OR "adults"[All Fields] OR "adult s"[All Fields]) AND ("patient s"[All Fields] OR "patients"[MeSH Terms] OR "patients"[All Fields] OR "patient"[All Fields] OR "patients [All Fields]) AND "mouth neoplasms"[MeSH Terms] AND ("salivary"[All Fields] AND "metabolomics"[MeSH Terms]). No filters were applied during the search.

- Search strategy:

A three-stage selection process was carried out. The selection of studies was carried out by two reviewers (SI & JA). The first stage reviewed the titles to eliminate irrelevant articles. In the second stage, the title and abstracts were filtered according to the type of studies, language, a sample size. In the third stage, each article was read completely, and the data was taken according to the eligibility to be included in the systematic review.Any disagreement in the eligibility of the study was resolved by discussion between both reviewers until a consensus was reached.The degree of agreement regarding the inclusion of potential studies was calculated by k-statistics (Cohen kappa test) for the second and third stages of selection.

- Data Extraction

The following information was extracted from the studies and arranged in table according to Authors, Type of study, Year of publication, Language, Patient’s Age and Gender, Number of patients in the study, Patients with Oral Cancer and Oral Squamous Cell Carcinoma, and Inclusion and Exclusion criteria.

- Quality and risk of bias assessment:

The articles selected for this systematic review were evaluated through the CASPe, Critical Appraisal Skills Program. It includes tools for the evaluation of seven different types of design and depending on the type of study, a series of questions were answered about the type of study design, study objectives inclusion/exclusion criteria, results, and conclusions, as well as conflicts of interest. After answering the questions, it was subsequently determined whether

the study had high, medium, or low quality to proceed with its inclusion or exclusion. Depending on whether it presented a yes, partially, or no in the method section, it was classified according to the quality it presented, as well as in the rest of the areas (research questions results, conclusions, conflicts of interest and external validity) in order to obtain the quality of each article. The degree of inter-examiner agreement of the methodological quality assessment was obtained with the Cohen kappa test, following the scale proposed by Landis and Koch (32).

- Data synthesis:

To summarise and compare the variables various characteristics of the studies were compared. The main characteristics were Country, type of study, sample size , age (range/mean), gender and diagnosis, the type of saliva, as well as centrifuging and storing of the sample collected along with the method of analysis of saliva collected were all placed in a table in order to analyse the data and obtain feasible outcomes. A meta-analysis was not able to be performed due to the lack of on salivary metabolomics in oral cancer.

Results:

- Study selection:

A total of 126 articles were obtained from the initial search process: PubMed(n=8), Scopus (n=117) and Web of Science(n=1). Of these publications 14 were identified as potentially eligible articles by screening the titles and abstracts. Full text articles were subsequently obtained and thoroughly evaluated. As a result, 7 articles met the criteria for inclusion and were included in this systematic review (Fig.1). The k value for inter-examiner agreement on the inclusion of the studies was 1.0 (titles and abstracts) and 1.0 (full texts), which indicates a “good” and “complete” agreement, respectively, according to the Landis and Koch criteria (32).

- Study characteristics:

A total of 7 studies were analysed in this systematic review. All the studies chosen for this systematic review are case control studies(24-30).The studies were conducted in five different countries. Three of the studies were carried out in Japan (24,28,30),one in China(25), one in Brazil(26),one in Thailand(27) and one

in United States of America(29).All these studies evaluated the metabolites differences found in Oral cancer and healthy patients. Six out of the seven studies included participants diagnoses with OSCC (24,25,26,28-30).
A total of 447 participants were analysed approximately half of which served as the control groups(Table 1).

- Risk of bias:

Three of studies had a low risk of bias(25,26,30) and the remaining four studies overall results were medium risk of bias (24,27,28,29).The k value (Cohen kappa test) regarding to the agreement between the reviewers of the methodological quality was 0.78 according to the scale of Landis & Koch (32).

- Synthesis of results:

Diagnosis of oral cancer by salivary metabolomics:

A total of 5 studies (24,26,27-29) included the following metabolites found common between them: Choline, Creatine, Lactate, Polyamines and Trimethylamine N -oxide . All of these metabolites mentioned showed significant differences in OSCC patients compared to healthy patients (Table 2).

Earlier identification of oral cancer by metabolomics:

One study (26) conducted the first investigation on a group of Latin Americans with oral squamous cell carcinoma the findings revealed altered metabolic pathways, such as the malate-aspartate shuttle and beta-alanine metabolism, as well as the Warburg effect. There were 10 salivary metabolites identified as possible OSCC indicators above the AUC = 0.9 threshold: malic acid, lactate, catechol, 2-ketoadipic acid, creatine , methionine, urea, leucine, inosine, and protocatechuic acid. Metabolites lactate and creatine were also detected in the study of Lohavanichbutr et al. (29). Another study (27) reported that Trimethylamine N-oxide and glycine levels were substantially greater in oral cancer patients' saliva than in normal participants' saliva. This finding implies that the metabolic profiles of saliva samples from healthy people and oral cancer patients are not the same.

Viability of salivary metabolomics in the context of oral cancer:

Several studies (24,28,30) used MetaboAnalyst to examine the salivary metabolomics data this took into consideration the number of metabolites detected in the individual metabolic pathways and any alterations found between the cases and controls. Among these three studies the following pathways were found alanine, glutamate and aspartate along with TCA Cycle and citric acid pathway. The pathways which were significantly greater OSCC patients were glutamate and aspartate.

Discussion:

A meta-analysis was not possible due to a lack of studies on salivary metabolomics in oral cancer. As a result, the data given here should be regarded with carefully and were presented in every study group descriptively.

Diagnosis of oral cancer by salivary metabolomics:

The results of this systematic review revealed salivary metabolomics can help in the diagnosis of oral cancer. The use of saliva as a sample for metabolite detection benefits both the patient and the dentist, because saliva can be accumulated frequently in a non- invasive manner for oral cancer screening.

Ishikawa et al. (28) examine the metabolomic profiles in saliva samples obtained from patients with oral cancer, as well as in their oral tissues and adjacent matched control tissues and find that 17 metabolites are consistently enhanced in the saliva and tumor tissues. These metabolites include amino acids such as valine, tryptophan, and threonine, as well as others. Spermidine in the polyamine route and many metabolites, including choline, methionine, and S-adenosyl methionine, in a pathway upstream of the polyamine production pathway, were also enhanced.

Earlier identification of oral cancer by metabolomics:

Oral cancers come into close contact with saliva, and tumor cell by-products are released into the saliva. As a result, the presence of hydrolysed products from local tissue damage or tumor cells in the saliva of oral cancer patients allows for salivary metabolite identification. All of the studies demonstrate that salivary metabolomics can help identify oral cancer. Each study examined the saliva

sample acquired using various procedures Ohshima et al.(24) analyses metabolic changes in saliva samples of Japanese patients with OSCC using capillary electrophoresis-mass spectrometry metabolome analysis. Choline is the metabolite with the largest statistically significant difference between OSCC patients and healthy persons out of the twenty-five potential metabolites. Choline is exceptionally important for prognosis and treatment outcome prediction. processes, which are reflected in cancer cell uncontrolled growth. Furthermore, Ishikawa et al. (28) examine salivary metabolites for oral cancer screening by analysing both saliva and tumor tissue samples. There were no significant differences in the salivary metabolome depending on OSCC stage.

Viability of salivary metabolomics in the context of oral cancer:

Several studies have examined salivary metabolome changes to identify OSCC from other disorders of the oral mucosa, which may be considered oral potentially malignant conditions. This is very important for cancer prevention and early detection of lesions. Wei et al. (25) examined the stratification value of five salivary metabolites (-aminobutyric acid, phenylalanine, valine, n-eicosanoic acid, and lactic acid) using ROC curves. Based on this, 2-3-item combinations were constructed, and the predictive power for OSCC in patients with oral leukoplakia (OLK) and no changes in the oral mucosa was good. For OLK, the combination of valine, lactic acid, and phenylalanine outperformed OSCC in terms of accuracy, sensitivity, specificity, and positive predictive value. These findings, according to the authors, may help supplement clinical differentiation of OSCC from OLK, enhancing prognosis along with earlier identification.

Despite the limitations salivary metabolites can be used as a diagnostic tool for early diagnose as well as there is tremendous potential for the viability of salivary metabolites in relation to the earlier identification of oral
cancer.

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Table 1 :General characteristics of the included articles.

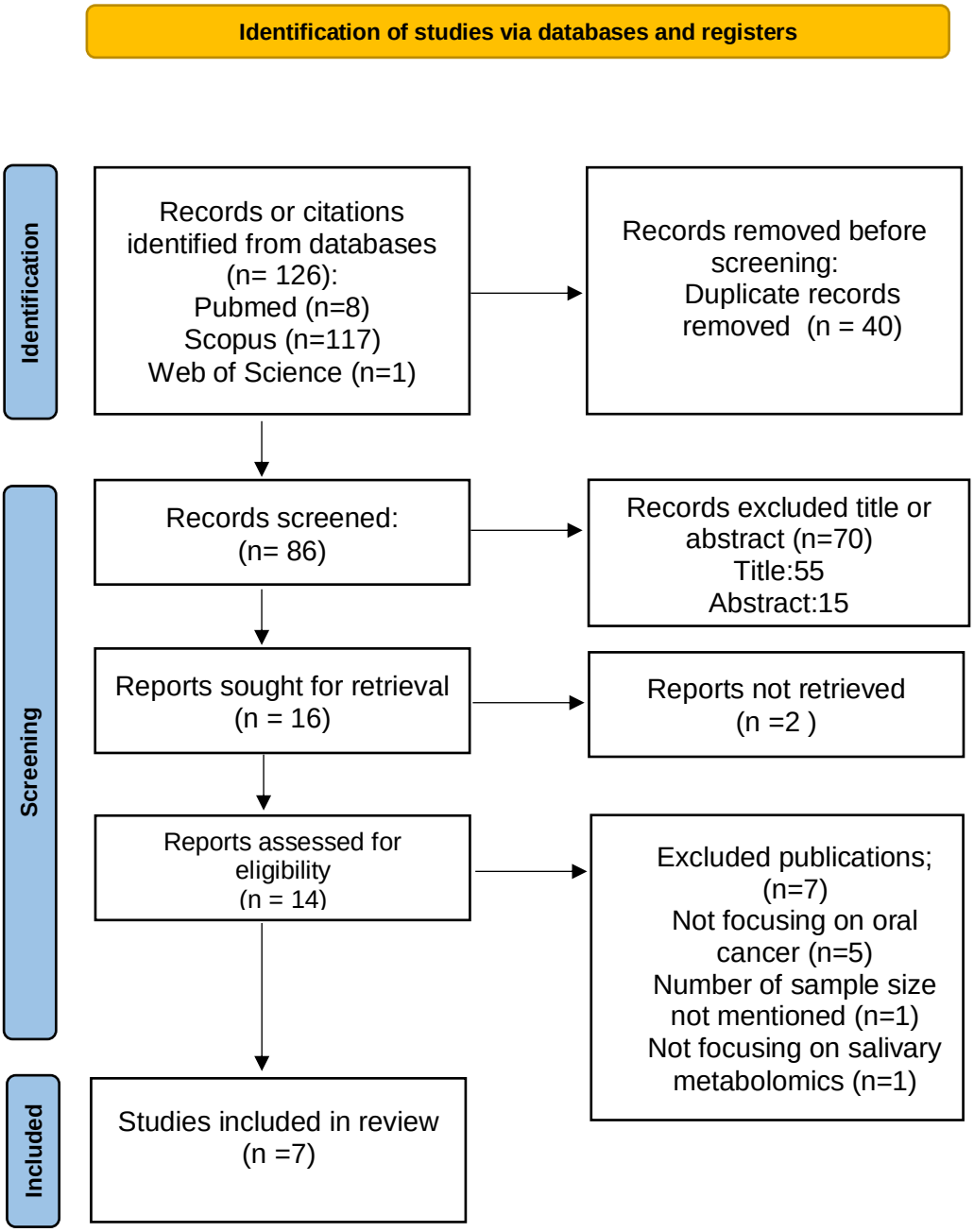
Author/Year	Country	Type of Study	Sample	Age(range/mean)	Gender M/F Ratio	Diagnosis
Ohshima et al.2021	Japan	Case control	43	65	21:22	OSCC
Wei et al.2011	China	Case control	71	58	39:32	OSCC
De Sa Alves et al.2021	Brazil	Case control	68	57	40 :28	OSCC
Supawat et al.2021	Thailand	Case control	25	57	15 :10	OC
Ishikawa et al.2016	Japan	Case control	68	65	30 :38	OSCC
Lohavanichbur et al.2018	United States of America	Case control	100	56	55 :45	OSCC
Ishikawa et al.2021	Japan	Case control	72	66	38 :34	OSCC

Table 2: Metabolites found significantly high or low in the following studies.

Author/Year	Choline	Creatine	Lactate	Polyamines	Trimethylamine N -oxide
Ohshima et al.2021	↑	↓	↓	↑	↑
De Sa Alves et al.2021	↓	↑	↑	↓	↓
Supawat et al.2021	↑	↓	↓	↑	↓
Ishikawa et al.2016	↓	↑	↓	↑	↑
Lohavanichbur et al.2018	↓	↑	↑	↓	↓

Significantly high: ↑ **Significantly low:** ↓

Fig.1. PRISMA 2020 flow diagram for searches of databases, registers and other sources.



LA METABOLÓMICA SALIVA COMO CANDIDATA PARA EL DIAGNÓSTICO DE CÁNCER ORAL: REVISIÓN SISTEMÁTICA

Titulo corto: Metabolómica salival como candidata para el diagnóstico de cáncer oral

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Resumen

Introducción: El cáncer oral, comúnmente conocido como cáncer de boca, es un tumor maligno de la cavidad oral y es el sexto cáncer más prevalente en el mundo. El carcinoma oral de células escamosas representa alrededor del 90% de los cánceres de la cavidad oral. El estudio sistémico de los metabolitos se conoce como metabolómica, donde se generan pequeñas moléculas por el proceso del metabolismo. Se realizó una revisión sistemática para ver cómo la metabolómica salival puede ayudar en el diagnóstico del cáncer oral.

Materiales y Métodos : Se realizó una búsqueda electrónica en las bases de datos de PubMed, Scopus y Web of Science sobre metabolómica salival para la detección del cáncer oral hasta diciembre de 2022.

Resultados: De 126 artículos potencialmente elegibles, 7 cumplieron con los criterios de inclusión. Los 7 estudios evaluaron las diferencias de metabolitos encontradas en cáncer oral y pacientes sanos. El material de diagnóstico en cada estudio fue saliva total no estimulada, que se analizó utilizando varias técnicas espectroscópicas. Los pacientes con carcinoma oral de células escamosas variaron significativamente de los participantes sanos en términos de metabolitos. Hubo evidencia de rutas metabólicas alteradas, como el metabolismo de la colina, las rutas de aminoácidos y la glucólisis, entre los metabolitos salivales observados.

Conclusión: A pesar de las limitaciones, los metabolitos salivales se pueden utilizar como herramienta de diagnóstico para el diagnóstico temprano, así como existe un enorme potencial para la viabilidad de los metabolitos salivales en relación con la identificación más temprana de oral cáncer.

Palabras claves: *Oral cancer, Salivary metabolomics, Oral cancer detection, Oral cancer diagnosis, Metabolites*

Introducción

El cáncer oral es el sexto cáncer de cabeza y cuello más común en el mundo. El carcinoma oral de células escamosas es una condición multifactorial marcada por una interacción compleja de variables ambientales y cambios moleculares que conducen a un crecimiento celular sin restricciones. La baja tasa de supervivencia del carcinoma oral de células escamosas se atribuye a una serie de factores, que incluyen herramientas de detección y diagnóstico inadecuadas, falta de conocimiento de las lesiones precursoras del cáncer oral y diagnóstico tardío (1).

El uso de la saliva como medio de detección ofrece un método rápido, económico, seguro y no invasivo. Se ve cada vez más como un medio para detectar enfermedades. El perfil metabólico de la saliva a veces se denomina el "espejo del cuerpo". Esto se debe a que tiene la capacidad de recolectar oncometabolitos que resultan del recableado metabólico. También ofrece una descripción general de los metabolitos con una regulación enzimática aberrante notable y se centra en las vías modificadas durante la reprogramación metabólica (9,10).Varios artículos han publicado revisiones sistemáticas sobre la metabolómica salival en el cáncer oral; sin embargo, en estas revisiones no se centró en si los metabolitos salivales se pueden usar en la detección del cáncer oral. Por lo tanto, esta revisión proporciona más profundidad sobre cómo las diferentes técnicas analíticas metabolómicas conducen a la identificación de metabolitos presentes en el cáncer oral y qué metabolitos son prominentes en los tejidos y células del cáncer oral.

Es necesario conocer el papel de la metabolómica salival involucrada en el diagnóstico del cáncer oral, ya que esto brindará a los oncólogos y a la comunidad científica una mejor comprensión sobre cómo utilizar esta herramienta de diagnóstico para una detección más precisa y rápida del cáncer oral.

El objetivo de la presente revisión sistemática fue revisar sistemáticamente la siguiente pregunta: ¿En pacientes adultos, la metabolómica salival mejora el diagnóstico del cáncer oral?

Material y métodos

Esta revisión sistemática cumple con la declaración PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) (14).

- Pregunta PIO:

La pregunta de enfoque se estableció de acuerdo con la pregunta estructurada PIO:

P (Población): Pacientes adultos.

I (Intervención): Metabolómica salival.

O (Resultado): Mejorar el diagnóstico del cáncer oral.

- Criterios de elegibilidad:

Los criterios de inclusión fueron:

- Tipo de estudio: Ensayos clínicos controlados aleatorizados, estudios de cohortes prospectivos y retrospectivos y estudios de casos; Publicaciones disponibles en texto completo y en inglés y español; publicados en los últimos 10 años (enero 2011-diciembre 2022).
- Tipo de pacientes: Pacientes de ambos sexos con cáncer oral con edades entre 18-99 años. 17
- Tipo de Intervención: Metabolómica salival en la identificación y diagnóstico del cáncer bucal.
- Tipo de variables de resultado: estudios que proporcionaron datos relacionados con la metabolómica salival que mejoran el diagnóstico del cáncer oral.

Los criterios de exclusión incluidos fueron revisiones sistemáticas y revisiones bibliográficas de metanálisis, estudios en animales, material editorial y cartas, así como un tamaño de muestra con menos de 5 pacientes.

- Fuentes de información y estrategia de búsqueda:

Se realizó una búsqueda individual en cada una de las plataformas seleccionadas mencionadas anteriormente (PubMed, Scopus y Web of Science) para obtener los artículos que respondieran la pregunta PIO y los objetivos. En todas las bases de datos se aplicó el filtro para los siguientes idiomas: inglés y español. Además, se aplicó el filtro de artículos entre enero de 2011 a diciembre de 2022. Las palabras clave fueron: ‘’Adult Patient;'' Oral cancer’’ and ‘’Salivary metabolomics”. Las palabras clave se combinaron con los operadores booleanos 'AND' y 'OR', así como con los términos controlados ("MeSH" para Pubmed) en un intento de obtener los mejores resultados de búsqueda.

La búsqueda en Pubmed-Medline fue: :("adult"[MeSH Terms] OR "adult"[All Fields] OR "adults"[All Fields] OR "adult s"[All Fields]) AND ("patient s"[All Fields] OR "patients"[MeSH Terms] OR "patients"[All Fields] OR "patient"[All Fields] OR "patients [All Fields]) AND "mouth neoplasms"[MeSH Terms] AND ("salivary"[All Fields] AND "metabolomics"[MeSH Terms]). No se aplicaron filtros durante la búsqueda.

- Proceso de selección de los estudios

Se llevó a cabo un proceso de selección en tres etapas. La selección de estudios estuvo a cargo de dos revisores (SI y JA). La primera etapa revisó los títulos para eliminar artículos irrelevantes. En la segunda etapa, el título y los resúmenes se filtraron según el tipo de estudios, el idioma y el tamaño de la muestra. En la tercera etapa, cada artículo fue leído en su totalidad y los datos fueron tomados de acuerdo con la elegibilidad para ser incluidos en la revisión sistemática. Cualquier desacuerdo en la elegibilidad del estudio se resolvió mediante discusión entre ambos revisores hasta llegar a un consenso. El grado de acuerdo con respecto a la inclusión de estudios potenciales se calculó mediante el estadístico k (prueba kappa de Cohen) para la segunda y tercera etapa de selección.

- Extracción de datos:

La siguiente información se extrajo de los estudios y se organizó en una tabla según Autores, Tipo de estudio, Año de publicación, Idioma, Edad y sexo del paciente, Número de pacientes en el estudio, Pacientes con cáncer bucal y carcinoma de células escamosas bucales e Inclusión y Criterios de exclusión.

- Valoración de la calidad:

Los artículos seleccionados para esta revisión sistemática fueron evaluados a través del CASPe, Critical Appraisal Skills Program. Incluye herramientas para la evaluación de siete tipos diferentes de diseño y, dependiendo del tipo de estudio, se respondieron una serie de preguntas sobre el tipo de diseño del estudio, los objetivos del estudio, los criterios de inclusión/exclusión, los resultados y las conclusiones, así como los conflictos de interés. Luego de responder las preguntas, posteriormente se determinó si el estudio tenía calidad alta, media o baja para proceder a su inclusión o exclusión. En función de si presentaba un sí, parcialmente o un no en el apartado de método, se clasificaba según la calidad que presentaba, así como en el resto de las áreas (preguntas de investigación resultados, conclusiones, conflictos de interés y validez externa) para obtener la calidad de cada artículo. El grado de concordancia Inter examinador de la evaluación de la calidad metodológica se obtuvo con la prueba kappa de Cohen, siguiendo la escala propuesta por Landis y Koch (32).

- Síntesis de datos:

Resumir y comparar las variables diversas características del se compararon los estudios. Las principales características fueron el país, el tipo de estudio, el tamaño de la muestra, la edad (rango/media), el género y el diagnóstico, el tipo de saliva, así como el centrifugado y almacenamiento de la muestra recolectada junto con el método de análisis de saliva. Recopilados se colocaron en una tabla para analizar los datos y obtener resultados factibles. No se pudo realizar un metanálisis debido a la falta de sobre la metabolómica salival en el cáncer oral.

Resultados:

- Selección de estudios:

Del proceso de búsqueda inicial se obtuvieron un total de 126 artículos: PubMed(n=8), Scopus (n=117) y Web of Science(n=1). De estas publicaciones, 14 se identificaron como artículos potencialmente elegibles mediante la selección de títulos y resúmenes. Los artículos de texto completo se obtuvieron posteriormente y se evaluaron minuciosamente. Como resultado, 7 artículos cumplieron con los criterios de inclusión y fueron incluidos en esta revisión sistemática (Fig. 1). El valor k para el acuerdo entre examinadores sobre la inclusión de los estudios fue de 1,0 (títulos y resúmenes) y 1,0 (textos completos), lo que indica un acuerdo "bueno" y "completo", respectivamente, según los criterios de Landis y Koch (32).

- Análisis de las características de los estudios revisados:

En esta revisión sistemática se analizaron un total de 7 estudios. Todos los estudios elegidos para esta revisión sistemática son estudios de casos y controles(24-30). Los estudios se realizaron en cinco países diferentes. Tres de los estudios se realizaron en Japón (24, 28, 30), uno en China (25), uno en Brasil (26), uno en Tailandia (27) y uno en los Estados Unidos de América (29). Todos estos Los estudios evaluaron las diferencias de metabolitos encontradas en cáncer oral y pacientes sanos. Seis de los siete estudios incluyeron diagnósticos de OSCC en los participantes (24,25,26,28-30).Se analizó un total de 447 participantes, aproximadamente la mitad de los cuales sirvieron como grupos de control (Tabla 1).

- Evaluación de la calidad metodológica:

Tres de los estudios tenían un riesgo de sesgo bajo (25,26,30) y los resultados generales de los cuatro estudios restantes tenían un riesgo de sesgo medio (24,27,28,29). El valor k (prueba kappa de Cohen) con respecto a la concordancia entre los revisores de la calidad metodológica fue de 0,78 según la escala de Landis & Koch (32).

- Síntesis de resultados:

Diagnóstico del cáncer oral por metabolómica salival:

Un total de 5 estudios (24,26,27-29) incluyeron los siguientes metabolitos comunes entre ellos: Colina, Creatina, Lactato, Poliaminas y N-óxido de Trimetilamina. Todos estos metabolitos mencionados mostraron diferencias significativas en pacientes con carcinoma oral de células escamosas en comparación con pacientes sanos (Tabla 2).

Identificación más temprana del cáncer oral por metabolómica:

Un estudio (26) realizó la primera investigación en un grupo de latinoamericanos con carcinoma oral de células escamosas y los hallazgos revelaron vías metabólicas alteradas, como la lanzadera de malato-aspartato y el metabolismo de la beta-alanina, así como el efecto Warburg. Se identificaron 10 metabolitos salivales como posibles indicadores de OSCC por encima del umbral de AUC = 0,9: ácido málico, lactato, catecol, ácido 2-cetoadípico, creatina, metionina, urea, leucina, inosina y ácido protocatecúico. Los metabolitos lactato y creatina también se detectaron en el estudio de Lohavanichbutr y cols. (29). Otro estudio (27) informó que Los niveles de N-óxido de trimetilamina y glicina fueron sustancialmente mayores en la saliva de los pacientes con cáncer oral que en la saliva de los participantes normales. Este hallazgo implica que los perfiles metabólicos de muestras de saliva de personas sanas y pacientes con cáncer oral no son los mismos.

Viabilidad de la metabolómica salival en el contexto del cáncer oral:

Varios estudios (24,28,30) utilizaron MetaboAnalyst para examinar los datos de la metabolómica salival, lo que tuvo en cuenta el número de metabolitos detectados en las vías metabólicas individuales y cualquier alteración encontrada entre los casos y los controles. Entre estos tres estudios se encontraron las siguientes vías de alanina, glutamato y aspartato junto con el ciclo TCA y la vía del ácido cítrico. Las vías que fueron significativamente mayores en pacientes con OSCC fueron glutamato y aspartato.

Discusión:

No fue posible realizar un metanálisis debido a la falta de estudios sobre la metabolómica salival en el cáncer oral. Como resultado, los datos proporcionados aquí deben considerarse con cuidado y se presentaron en cada grupo de estudio de forma descriptiva.

Diagnóstico del cáncer oral por metabolómica salival:

Los resultados de esta revisión sistemática revelaron que la metabolómica salival puede ayudar en el diagnóstico del cáncer oral. El uso de saliva como muestra para la detección de metabolitos beneficia tanto al paciente como al odontólogo, ya que la saliva se puede acumular con frecuencia de forma no invasiva para la detección del cáncer oral.

Ishikawa y cols. (28) examinan los perfiles metabolómicos en muestras de saliva obtenidas de pacientes con cáncer oral, así como en sus tejidos orales y tejidos de control adyacentes y encuentran que 17 metabolitos aumentan constantemente en la saliva y los tejidos tumorales. Estos metabolitos incluyen aminoácidos como valina, triptófano y treonina, entre otros. También se mejoraron la espermidina en la vía de la poliamina y muchos metabolitos, incluidos la colina, la metionina y la S-adenosil metionina, en una vía corriente arriba de la vía de producción de la poliamina.

Identificación más temprana del cáncer oral por metabolómica:

Los cánceres orales entran en estrecho contacto con la saliva y los subproductos de las células tumorales se liberan en la saliva. Como resultado, la presencia de productos hidrolizados de daño tisular local o células tumorales en la saliva de pacientes con cáncer oral permite la identificación de metabolitos salivales. Todos los estudios demuestran que la metabolómica salival puede ayudar a identificar el cáncer oral. Cada estudio examinó la muestra de saliva adquirida usando varios procedimientos. Ohshima y cols. (24) analizan los cambios metabólicos en muestras de saliva de pacientes japoneses con OSCC usando análisis de metaboloma por electroforesis capilar-espectrometría de masas. La colina es el metabolito con la mayor diferencia estadísticamente significativa entre los pacientes con OSCC y las personas sanas de los veinticinco metabolitos potenciales. La colina es excepcionalmente importante para el

pronóstico y la predicción del resultado del tratamiento. procesos, que se reflejan en el crecimiento descontrolado de células cancerosas. Además, Ishikawa et al. (28) examinan los metabolitos salivales para la detección del cáncer oral mediante el análisis de muestras de tejido tumoral y de saliva. No hubo diferencias significativas en el metaboloma salival según el estadio del OSCC.

Viabilidad de la metabolómica salival en el contexto del cáncer bucal:

Varios estudios han examinado los cambios en el metaboloma salival para identificar el OSCC de otros trastornos de la mucosa oral, que pueden considerarse afecciones orales potencialmente malignas. Esto es muy importante para la prevención del cáncer y la detección temprana de lesiones. Wei y cols. (25) examinaron el valor de estratificación de cinco metabolitos salivales (-ácido aminobutírico, fenilalanina, valina, ácido n-eicosanoico y ácido láctico) usando curvas ROC. En base a esto, se construyeron combinaciones de 2-3 ítems, y el poder predictivo para OSCC en pacientes con leucoplasia oral (OLK) y sin cambios en la mucosa oral fue bueno. Para OLK, la combinación de valina, ácido láctico y fenilalanina superó a OSCC en términos de precisión, sensibilidad, especificidad y valor predictivo positivo. Estos hallazgos, según los autores, pueden ayudar a complementar la diferenciación clínica de OSCC de OLK, mejorando el pronóstico junto con una identificación más temprana.

A pesar de las limitaciones, los metabolitos salivales se pueden usar como una herramienta de diagnóstico para el diagnóstico temprano, así como también existe un tremendo potencial para la viabilidad de los metabolitos salivales en relación con la identificación temprana de infecciones cancer orales.

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Tabla 1 : Características generales de los artículos incluidos.

Autor/Año	País	Tipo de estudio	Muestra	Edad (rango/media)	Género Ratio M/F	Diagnóstico
Ohshima et al.2021	Japón	Control de caso	43	65	21:22	OSCC
Wei et al.2011	China	Control de caso	71	58	39:32	OSCC
De Sa Alves et al.2021	Brasil	Control de caso	68	57	40 :28	OSCC
Supawat et al.2021	Tailandia	Control de caso	25	57	15 :10	OSCC
Ishikawa et al.2016	Japón	Control de caso	68	65	30 :38	OSCC
Lohavanichbur et al.2018	Estados Unidos de América	Control de caso	100	56	55 :45	OSCC
Ishikawa et al.2021	Japón	Caso de control	72	66	38 :34	OSCC

Tabla 2: Metabolitos encontrados significativamente altos o bajos en los siguientes estudios.

Autor/Año	Colina	Creatina	lactato	Poliaminas	N-óxido de trimetilamina
Ohshima et al.2021	↑	↓	↓	↑	↑
De Sa Alves et al.2021	↓	↑	↑	↓	↓
Supawat et al.2021	↑	↓	↓	↑	↓
Ishikawa et al.2016	↓	↑	↓	↑	↑
Lohavanichbur et al.2018	↓	↑	↑	↓	↓

Significativamente alto ↑ **Significativamente bajo** ↓

Fig. 1. Diagrama de flujo de PRISMA 2020 para búsquedas en bases de datos, registros y otras fuentes.