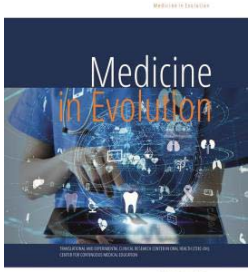
Systemic correlations of the oral microbiome and salivary parameters in smoking patients



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Abstract

The oral microbiome is intricately linked to human health and systemic diseases, with smoking being a prevalent risk factor. This meta-analysis aimed to investigate the impact of smoking on salivary parameters in individuals aged 20-55 without systemic diseases who exclusively smoked traditional cigarettes. Ethical approvals were not necessary as existing data from English-language publications between 2021-2022 were used. The analysis included 33 studies with 2813 participants (1392 smokers and 1421 non-smokers). The primary focus was on salivary pH changes in smokers compared to controls, with varied collection methods and restrictions. Additionally, 10 studies assessed total salivary antioxidant capacity in both groups. The results revealed lower salivary flow in smokers, while salivary antioxidant capacity varied. Overall, smoking emerged as a significant public health concern in the EU, contributing to high mortality rates. The findings demonstrate that smoking is associated with decreased salivary pH, reduced salivary flow, and lower total salivary antioxidant capacity, potentially leading to oral mucosal and dental problems and an increased risk of periodontal disease and premalignant lesions.

Keywords: Oral microbiome, saliva, smoking, dysbiosis, microenvironment, salivomics

INTRODUCTION

The oral cavity is one of the most important windows of interaction between the human body and the environment. The microenvironment in different parts of the oral cavity has different microbial compositions and is influenced by complex signals between the host and external environmental factors. These processes may affect or reflect overall human health, as certain health conditions are influenced by the composition of oral bacteria, and disruption of the microbial community is correlated with certain systemic diseases. The oral microbiome cooperates with the host to reflect information about the state of immunity and metabolism through bidirectional communication throughout the oral cavity and systemic organs [1]. The oral microbiome is a complex environment consisting of more than 1,000 species of bacteria, viruses, fungi and protozoa [2]. While bacteria dominate the microbiome in the oral cavity, the importance of viruses, fungi and protozoa should not be ignored. The balance of commensal bacteria together with the other natural microorganisms inhabiting the oral cavity coexist to maintain the health of the oral microbiome. Research is ongoing to explore the link between oral microbiome health and systemic health. Specifically, this review updates the importance of the link between oral dysbiosis and low-grade inflammatory diseases. Several key conditions characterize dysbiosis, such as loss of diversity in the microbial population, loss of benefits of “healthy” microbes, and expansion of pathogenic microbes [2]. Relationships between species, classified as synergistic, signaling, or antagonistic, are disrupted when diversity loss occurs, contributing to dysbiosis in the oral microbiome. Loss of the benefits of a healthy oral microflora can decrease the host's immune response and increase susceptibility to external diseases as well as opportunistic infections, which occur as commensal microbes transform into pathogenic microbes in response to ecosystem change.

With advances in microbiome research, the association between the oral microbiome and a variety of human chronic diseases has been studied, including inflammatory bowel disease [3], cancers [4], cardiovascular disease [5], Alzheimer's disease [6], diabetes [7], rheumatoid arthritis [8], and premature birth [9]. Furthermore, changes in the oral microbiome in systemic disease states are gradual and repeatable. Therefore, oral microbes can reflect human health and disease status in real time and have important value in early warning of disease risk and prediction of curative effect. More than 700 types of microorganisms are colonized in the human oral cavity [10]. The oral microbiome stands as a crucial and intricate microbial community within the human body, earning its place among the top five research priorities (oral cavity, nasal cavity, vagina, gut, skin) of the Human Microbiome Project (HMP), which it is not limited to understanding the role of oral microorganisms in caries, periodontal disease and other oral diseases. The evidence is increasingly leaning towards Miller's theory of oral lesions. The inflammation of periodontitis leads to the loss of connective tissues and bone [11]. Extensive infiltration of inflammatory cells occurs in the connective tissue near the periodontal epithelium [12]. It is generally believed that this low-grade inflammation will disrupt the health of the whole body or worsen the health of the whole body or may worsen another chronic source of the population causing peripheral inflammation. Thus, periodontitis is also called "low-grade systemic disease" interfering with a variety of systemic diseases. Numerous evidences in the last two decades have shown that bacteria and viruses are closely related to the development of tumors [13]. For example, the role of human papillomavirus in oral cancer [14], Helicobacter pylori in gastric cancer [15], Chlamydia pneumoniae in lung cancer [16], Salmonella typhi in gallbladder cancer [17], Streptococcus bovis [18], Bacteroides fragilis [19] and especially the periodontal pathogen Fusobacterium nucleatum in colon cancer [20]. These studies

demonstrated a possible role for bacteria in the development of tumors, and the results of subsequent research provide some evidence to support it. There is much evidence that oral microorganisms can induce cancer through direct or indirect factors [21]. For example, oral microorganisms can secrete polysaccharides or use their flagella to accumulate on the surface of tumor cells in large numbers, which induces chronic inflammation and cytokine secretion directly promotes tumor cell growth. Along the same lines, there is ample evidence supporting the association between the oral microbiome and human systemic diseases [22]. This correlation could be linked to the capacity of numerous oral microbes to impact the inflammatory microenvironment.

Smoking as a risk factor

Despite the growing knowledge of the adverse effects of smoking on general health, smoking remains one of the most widespread addictions worldwide [23]. Globally, approximately 1.1 billion smokers and more than 8 million people die each year from smoking [24]. Smoking acts as a source for a variety of diseases, including cardiovascular disease (CVD), chronic obstructive pulmonary disease (COPD), cancer and periodontal disease (POD), as one of the top five risk factors for the global burden of disease [25–27]. According to the Alcohol and Drug Survey, 15% of people currently smoke cigarettes, 17% of men and 13% of women. Adolescents between the ages of 15 and 19 were found to smoke at an estimated rate of 8%, with 10% of males and 6% of females being current smokers. The frequency was 16% among people aged 20–24 years and 25 years and older [28]. Tobacco smoking has numerous and well-documented negative consequences. The oral cavity is the first exposed to cigarette smoke, where soft and hard tissues come into direct contact, making it the first area of confrontation [29]. Tobacco smoking, especially in the form of cigarettes, has been shown to be a significant risk factor for periodontitis [30]. Apart from plaque, smoking has been identified as an important risk factor for marginal periodontitis. It also affects periodontitis prevalence, severity, progression and response to treatment. According to epidemiological research, smokers have a much higher risk for marginal periodontitis than nonsmokers, and the increased risk is proportional to the duration and rate of smoking [31,32]. Various gingival and periodontal problems such as gingivitis, alveolar bone loss, tooth mobility, oral lesions, ulcerations, halitosis, and stained teeth are more common among smokers [33]. According to a meta-analysis, exposure to environmental cigarette smoke is associated with a significantly increased risk of lung cancer [34,35]. Kumar et all (2014) predict that there is a high chance of developing oral cancer regardless of how you use tobacco (smoked, chewed, etc.).

Effects of smoking on salivary parameters

Smoking is considered to be one of the risk factors that reduces salivation. The oral mucosa is "bathed" by saliva; therefore, saliva is the first to interact with cigarette smoke. Cigarette smoke contains 4000 bioactive carcinogenic chemical compounds that lead to structural and functional changes in saliva [36]. Harmful substances specific to tobacco such as nitrosamines and benzopyrenes, along with nicotine (an addictive component), are absorbed by the covering mucosa and enter the systemic circulation [37]. Smoking is associated with multiple adverse effects in the oral cavity, one of which is periodontal disease [38].

Salivomics

Salivomics deals with the integrative study of saliva, as well as its constituents and functions. Saliva not being a homogeneous fluid has been studied from several directions.

Salivomics includes the following key components: genomics, epigenomics, transcriptomics, proteomics, metabolomics and microbiomics. The 3 major "–omics" groups include circulating DNA (genomics), RNA (transcriptomics), and proteins (proteomics). Saliva contains acellular DNA, 70% being from the host and 30% from the oral microbiota. Salivary DNA is stable and the quality of the molecules relatively high, which creates the possibility that salivary DNA can be a useful source for identifying biomarkers [39].

Saliva is of crucial importance for quality of life and oral health in particular. It is secreted by the salivary glands and contains a wide variety of biomolecules, such as proteins, peptides and other substances involved in the protection of the oral tissue.

Among the inhibitory factors in maintaining a normal salivary secretion are mentioned: stress, pain and negative emotions. The importance of saliva in the oral cavity becomes apparent when its production is chronically inhibited or permanently stopped. Adverse effects in patients with head and neck radiotherapy or in those suffering from Sjogren's syndrome is cited as examples. In these situations, the loss of the protective effect of saliva makes the teeth much more vulnerable to various disorders [40]. Saliva plays an essential role in maintaining the integrity of oral structures, even in personal relationships, also in digestion or in the control of oral infections. Saliva also plays an extremely important role in the protection of teeth against carious processes. The action of saliva in this direction can be extended to 4 aspects: the dilution and elimination of sugars as well as other substances; buffer capacity of saliva; the balance between the demineralization and remineralization processes as well as the antimicrobial action.

Another important role attributed to saliva is to contribute to the elucidation of the diagnosis in the case of certain disorders and to participate in the monitoring of the evolution of some diseases or in the dosage of medicines or drugs. The advantages of saliva as a diagnostic tool are: the ease of obtaining saliva samples and also the positive correlation between the level of several parameters in serum and their value in saliva [41]. The secretion of the minor salivary glands is continuous and aims to ensure the permanent moistening of the oral mucosa; unlike the secretion of the major salivary glands, which is produced discontinuously, reflexively, being correlated with a series of stimuli such as gustatory or olfactory ones [42].

Chemical composition of saliva

Saliva is made up of 99.5% water and 0.5% dry residue consisting of 0.2% inorganic substances and 0.3% organic substances. Among the inorganic substances we mention: sodium, chlorine, potassium, bicarbonate, calcium, fluorine, thiocyanate. In certain pathological situations saliva may contain lead or mercury salts.

The organic substances present in the composition of saliva can be classified into two categories: nitrogenous (protein and non-protein) and non-nitrogenous substances.

Proteinaceous organic substances include: serum proteins: immunoglobulins, lactoferrin, coagulation factors, proteins of glandular origin: enzymes, mucins, blood group substances, bacterial aggregation factors, proline-rich proteins, salivary hormones.

Non-protein nitrogenous organic substances are those originating from the catabolism of proteins, namely: urea, creatinine, uric acid, ammonia.

Non-nitrogenous organic substances are carbohydrates and lipids [43].

The antioxidant capacity of saliva

Total antioxidant capacity (TAC) is the number of free radicals scavenged by a test solution, and is used to assess the antioxidant capacity of biological samples, including saliva. An antioxidant is any substance that, when found in low concentration compared to the

concentration of an oxidizable substrate (proteins, carbohydrates, lipids, DNA), prevents the oxidation of that substrate.

In the body, the main function of antioxidants is to protect the body against the destructive effects of free radicals. Free radicals can be produced in cells and tissues from endogenous causes: inflammatory, metabolic diseases, decreased immunity or exogenous: food, pollution, irradiation [44].

The roles of saliva

In addition to the digestive role, saliva is also attributed a protective, excretory, endocrine role and also contributes to maintaining hydro electrolytic homeostasis, maintaining body temperature within normal limits and speech [45].

The presence of food in the oral cavity stimulates mechanical and chemical, gustatory and olfactory receptors. Subsequently, following the development of autonomic nervous reflexes, there is an increase in salivary secretion sufficient for food processing [46].

Saliva protects the oral mucosa and the esophagus, thus the volume of saliva present in the oral cavity before swallowing is 1.1 ml, and after swallowing it decreases to 0.8 ml. The esophagus contains mucous glands, which secrete bicarbonate and mucus to form a protective film. This film has a thickness similar to the salivary film in the mouth.

The dental protection provided by saliva is against abrasion, attrition, erosion and tooth decay. The so-called acquired salivary film, (PSD), which is a protein layer covering all enamel surfaces, has been described. It covers the underlying dentin or cementum in the absence of enamel. The presence of certain lipids was also detected.

Cellular proteins appear to be derived from desquamated oral epithelial cells in saliva. The thickness of the film is estimated to be between 0.3-1.1 μm in different areas of the oral cavity, being dependent on the susceptibility to abrasive forces. The film starts to regenerate/reform a few seconds after the sanitized enamel surface comes into contact with saliva. The salivary film has a lubricating role. The main inorganic salivary components involved in tooth protection are calcium, phosphate and bicarbonate. Although salivary phosphate acts as a buffer against acids, the phosphate concentration falls when the flow is stimulated by acid and is too low to have a buffering effect. Tissue factor in salivary exosomes accelerates hemostasis, and most salvage antibacterial factors are assumed to be sufficient to prevent infection of an oral wound [47].

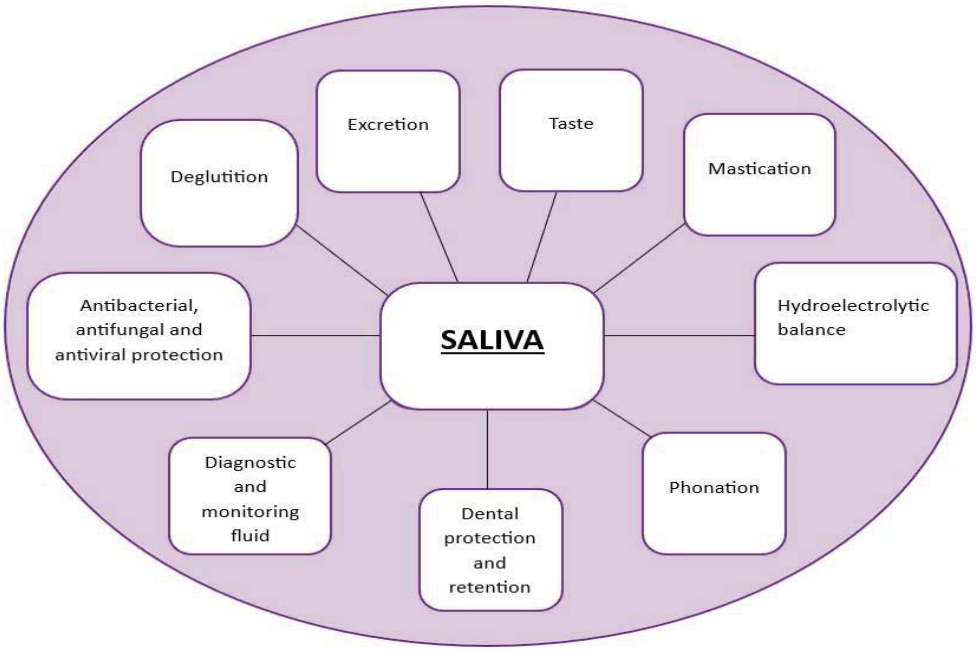

Figure 1. The saliva roles in the body

Aim and objectives

The present paper aims to use the meta-analysis method to carry out a comparative study between several publications published in specialized medical journals and which had as their objective the analysis of the influence of smoking on several parameters characterizing saliva.

MATERIAL AND METHODS

1.1 Ethical notices

As all analyzes were published in previous studies, no ethical approvals or express patient consent were required.

1.2 Search strategy

- Finding the results was possible by using a systemic review using the databases: PubMed, Google Scholar, jiaphd, ncbi, ersjournals, semanticscholar, scielo, bds;

Key words used in the search were: tobacco, saliva, salivary pH, salivary flow, salivary total antioxidant capacity.

The search was limited to studies published between the years 2021-2022. We only selected publications in English-language journals.

1.3 Inclusion criteria in the study

- Patients without systemic diseases
- Patients who showed signs of periodontopathic
- The age of the subjects was between 20 and 55 years
- Studies conducted on subjects belonging to all geographical regions were chosen
- Only subjects smoking classic cigarettes were chosen

1.4 Exclusion criteria from the study

- Studies that included subjects smoking electronic cigarettes
- Studies that included hookah smoking subjects
- Studies that focused on the consumption of tobacco in other forms (chewable)

- Studies performed without saliva stimulation, both in smokers and in control groups
- Studies older than 2012

1.5 Data collection methods

In order to obtain the necessary data for this study, we sought the following information:

- The name of the main author
- The year of publication of the article
- Country of origin of the population study
- The age of the people studied
- Smoking subjects and control groups
- Quantification of salivary pH
- Measurement of salivary flow
- Determination of total salivary antioxidant capacity

1.6 Definition of a smoker

- A smoker is considered to be a person who has smoked more than 100 cigarettes, (including roll-your-own cigarettes, cigars), during their lifetime and smoked in the last 28 days before the examination.
- A former smoker was defined as someone who had smoked more than 100 cigarettes in their lifetime but had not smoked in the last 28 days before the study.
- A non-smoker is defined as a person who has not smoked more than 100 cigarettes and was not smoking at the time of the study [48].

RESULTS

In the initial search we identified a number of 56 studies. After removing duplicates, we retained 48 articles that we subjected to a more thorough evaluation. After analyzing the title of the articles and the related abstracts, we excluded 8 studies that we considered unsuitable for the present research. After applying the exclusion criteria from the study (e-cigarette smokers, hookah smokers, tobacco users in other forms, studies performed without saliva stimulation, both in smokers and in control groups, studies older than 2012), we kept 33 published articles that will be the object of study of this paper.

The 33 studies analyzed in the present work totaled 2813 participants. Of these, 1392 subjects entered the smoking groups and the remaining 1421 constituted the control and non-smoking groups.

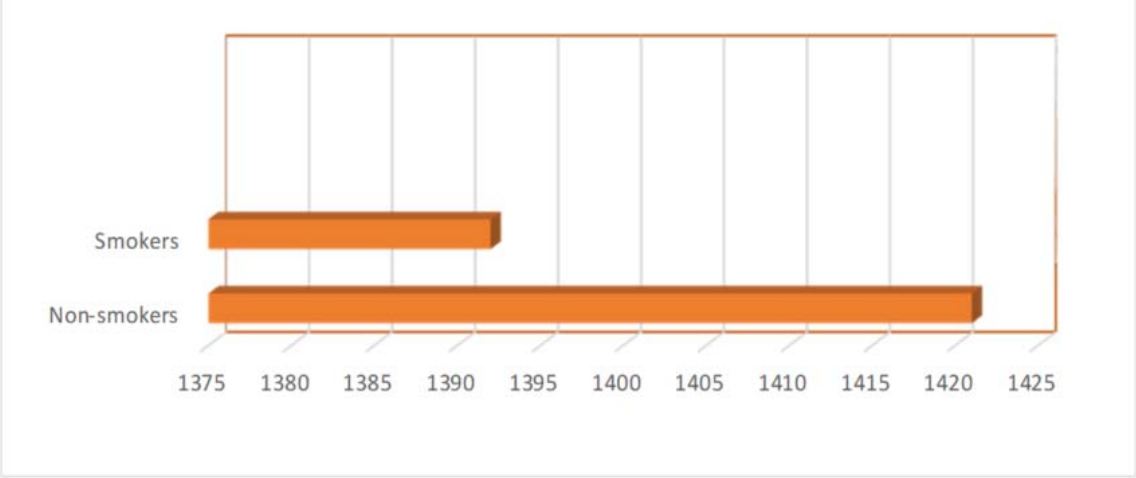

Figure 2. Control groups and case groups

In the 33 articles included in the present work, after applying the inclusion and exclusion criteria, we analyzed the following parameters: salivary pH, salivary flow and total salivary antioxidant capacity (TAC). The chart below illustrates the distribution of the total number of subjects participating based on various parameters.

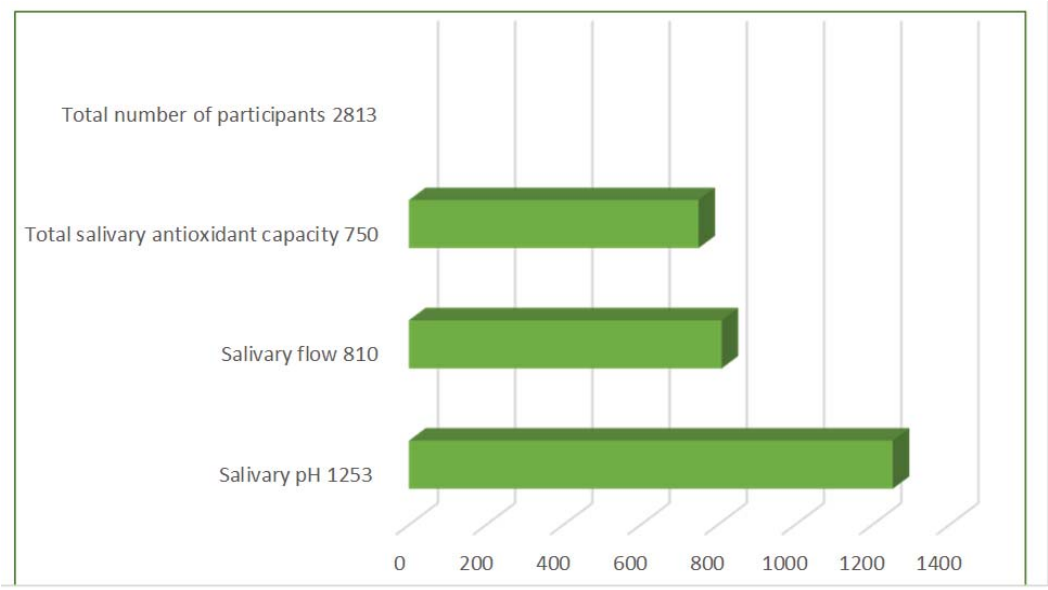

Figure 3. Distribution of study participants according to the monitored parameter

Salivary pH analysis

A number of 12 studies analyzed in the present paper determined salivary pH in groups of smokers and also in control groups. The total number of people included in this study is 1253, of which 660 are smokers and 593 included in the control groups.

Methods for determining pH varied from one study to another. Saliva collection was done in sterile test-tube containers [49,50], also sterile graduated containers [51–55], or Falcon tubes [56].

Measurement of pH was also done differently, with some studies using digital pH meters [49,50,52,55–57] and others using pH indicator saliva swabs [51,53,54].

Saliva collection was performed in all studies in the morning between 9 am and 12 pm to avoid diurnal variations.

Certain restrictions were imposed on the subjects before collection and also during saliva collection. These varied slightly from one study to another. In some of the studies patients were not allowed to smoke, drink or eat two hours before the saliva collection [50]. In another study [49], subjects were not allowed to eat or drink anything other than water, also for two hours before collection.

In another situation [56], they avoided any oral stimulation for 90 minutes before and did not smoke for an hour before the saliva was collected.

In the case of the study [57], the method of analysis was a more complex one, the measurements being made three times in a total time of 30 minutes, the first two determinations after coffee consumption and the third one after water ingestion.

In other situations, the subjects also avoided eating and drinking but also rinsing the oral cavity [54].

In addition to the restrictions mentioned in most studies, some research also required subjects not to speak or swallow during the procedure [55].

In other studies [51,55], subjects were asked not to eat, drink, smoke, or brush their teeth one hour before or during saliva sample collection.

Since in some studies the subjects included in the research were diagnosed with various pathologies associated with such as periodontal disease [52] the instructions for collecting saliva in this sample were different. Thus, these patients were asked not to forcefully spit into the collection tube to avoid contaminating the sample with blood from the inflamed gingival tissue or with a possible ulcerative lesion.

In some studies [49], subjects were asked why they started smoking cigarettes. The answers were among the most diverse. Most of the respondents cited the pleasure of the gesture, others the entourage, dissatisfaction with personal life, stress and anxiety, boredom. Others motivated the practice of this habit to lose weight or invoked fear and shame, problems at work, reducing anger.

According to other authors [57], the most common reasons why the subjects of those studies started smoking were: stress followed by curiosity and the desire for affirmation.

Some of the studies included in the present analysis had as their objective, in addition to the evaluation of salivary pH changes in smokers and the determination of the oral hygiene score, the value of the DMFT index, the assessment of tartar load on the teeth as well as the gingival bleeding associated with smokers and the control group [57].

According to a 2019 study published by Moradi et al., DMFT represents:

- DT = decayed teeth
- MT = missing teeth
- FT = teeth with fillings

The DMFT is an index used globally to assess oral status and dental health. This is the most important index used in epidemiological studies, in community health.

It seems that poor oral hygiene was identified more frequently in smokers and the DMFT index was higher in the smoking group. Also, in this group, a greater loss of the number of teeth was found compared to the group of non-smokers. The level of tartar was also higher in the group of smokers.

On the other hand, gingival bleeding was more common in the non-smoking group. A possible explanation for this result is the necrosis of peripheral capillary vessels induced by the action of cigarette smoke on the oral mucosa [57].

All the results regarding the research of pH variations collected from the analysis of the 33 articles in the present study, we included them in the form of tables in which we followed: the country of origin, the number of participants (smokers and non-smokers), we also have extracted the method of determining this parameter and of course the pH values in both the smoking and control groups.

We will be able to compare the obtained values later depending on the country in which each study was carried out and depending on the measurement method used for each individualresearch.

Table 1. Determination of pH

Name of author, year	*Country*	*pH determination method*	*Participants Non-smokers/Smokers*		*pH value Non-smokers / Smokers*	
1.Alpana Kanwar, 2013	India	Strips	20	20	7.03±0.14	6.8±0.11
2.Mala Singh, 2015	India	Strips	35	35	7.10±0.24	6.30±0.36
3. Ramesh G, 2015	India	pH-metre	15	15	8.03±0.36	7.66±0.5
4.Saraswathi Gopal K, 2016	India	Strips	20	20	6.97±0.11	6.12±0.5
5. Grover N, 2016	India	pH-metre	20	20	7.00±0.28	6.75±0.11
6.Ahmadi- Motamayel Fatemeh, 2016	Iran	pH-metre	251	259	7.52±0.43	7.42±0.48
7.Mohamad Reza Golomohamadi, 2018	Iran	pH-metre	37	92	7.04±0.06	6.57±0.06

8.Iqbal Dohan Challap, 2019	Irak	pH-metre	50	50	7.168	7.058
9.Gökçen Ömeroğlu Şimşek, 2019	Turkey	pH-metre	39	43	6.84±0.37	6.67±0.41
10. Sri Jaya Ranjitha, 2019	India	pH-metre	60	60	8.6±0.61	7.9±0.75
11. Abha Rani, 2020	India	pH-metre	20	20	7.18±0.17	6.81±0.25
12.Senthilkumaran M, 2021	India	pH-metre	26	26	6.254	6.062
Total/Average pH			593	660	7.19±0.23	6.84±0.29

The average pH value from all the studies analyzed and divided into the two categories, smokers/non-smokers, can be found in chart number 3.

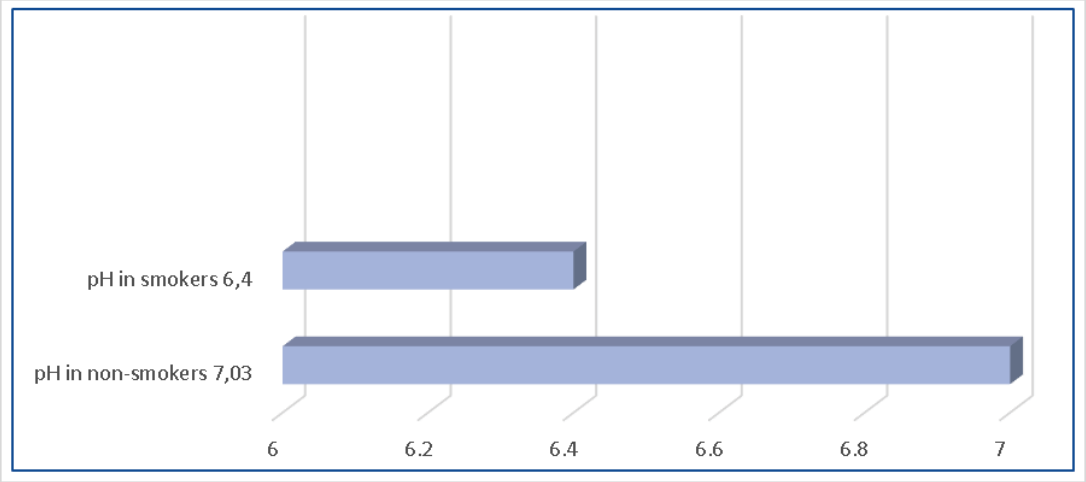

Figure 4. Average pH value in smokers/non-smokers

In table number 2 we have presented the results of the pH in both smokers and non-smokers, but measured only by the strip method. We identified three studies, all conducted in India and totaling 150 subjects, 75 smokers, 75 non-smokers. The average pH values in smokers were 6.4±0.32, compared to those of non-smokers of 7.03±0.16.

Table 2. Determination of pH with strips

Article	*Country*	*Non-smokers*	*Smokers*	*pH non-smokers*	*pH smokers*
1.Saraswathi Gopal K, 2016	India	20	20	6.97±0.11	6.12±0.5
2.Mala Singh, 2015	India	35	35	7.10±0.24	6.30±0.36
3.Alpana Kanwar, 2013	India	20	20	7.03±0.14	6.8±0.11
Total/Average pH		75	75	7.03±0.16	6.4±0.32

Table 3. Determination of pH with pH meters

Article	*Country*	*Non-smokers*	*Smokers*	*pH non-smokers*	*pH smokers*
1. Ramesh G, 2015	India	15	15	8.03±0.36	7.66±0.5
2.Grover N, 2016	India	20	20	7.00±0.28	6.75±0.11
3.Ahmadi- Motamayel Fatemeh, 2016	Iran	251	259	7.52±0.43	7.42±0.48
4.Mohamad Reza Golomohamadi, 2018	Iran	37	92	7.04±0.06	6.57±0.06
5.Iqbal Dohan Challap, 2019	Irak	50	50	7.168	7.058
6.Gökçen Ömeroğl u Şimşek, 2019	Turkey	39	43	6.84±0.37	6.67±0.41
7. Sri Jaya Ranjitha, 2019	India	60	60	8.6±0.61	7.9±0.75
8. Abha Rani, 2020	India	20	20	7.18±0.17	6.81±0.25
9.Senthilkumaran M, 2021	India	26	26	6.254	6.062
Total/Average pH		518	635	7.29±0.32	6.98±0.36

In the table above, we have grouped the researches in which the pH measurement was carried out with pH meters. We identified a total of 9 studies. The calculated mean values were: for smokers, 6.98±0.36 and for non-smokers, 7.29±0.32.

The comparison between the pH values obtained by the two measurement methods can be found in the following chart.

Figure 5. Difference between pH measured with strips and pH meter

It is found that pH values measured with strips, in both smokers and non-smokers, are lower than those obtained using pH meters.

Measurement of salivary flow

11 articles from the specialized literature studied by the method of meta-analysis in the present work quantified the changes in salivary flow in smoking subjects and in control groups (non-smokers).

The results analyzed in the present study can be found in table number 4.

Table 4. Determination of salivary flow

	Name of author, year	*Country*	*Salivary flow determination method*	*Participants Non-smokers*	*Smokers*	*The value of salivary flow Non-smokers*	*Smokers*
1	Alpana Kanwar, 2013	India	Volumetric	20	20	5.65±0.45 ml/10min	4.34±0.35 ml/10min
2	Mala Singh, 2015	India	Volumetric	35	35	0.36±0.06 ml/min	0.20±0.05 ml/min
3	Sabarni Chakrabarty, 2015	India	Volumetric	30	30	1.08 ml/min	0.77 ml/min
4	Saraswathi Gopal K, 2016	India	Volumetric	20	20	0.42 ml/min	0.22 ml/min
5	Arezoo Alaee, 2017	Iran	Schirmer Test	50	50	24.8±2.4 Mm	15.8±2.1 Mm
6	Fateme Arbabi Kalati, 2017	Iran	Volumetric	28	25	3.2±0.75 ml/5min	3.2±0.4 ml/5min

7	Sankepalli Shwetha, 2018	India	Volumetric	15	15	3.09±0.48 ml/5min	2.31±0.65 ml/5min
8	Endang W Bachtiar, 2018	Indonesia	Volumetric	25	25	0.33 ml/min	0.3 ml/min
9	Sri Jaya Ranjitha, 2019	India	Volumetric	60	60	9.02±1.06 ml/10min	6.97±1.35 ml/10min
10	Abha Rani, 2020	India	Schirmer Test	20	20	33.22±2.30 mm/3min	22.6±3.57 mm/3min
11	Seeme Nigar, 2022	Pakistan	Volumetric	163	54	0.42 ml/min	0.37 ml/min
	Total			456	354		

A total of 810 subjects were included in the study, of which 354 were smokers and 456 belonged to the control groups.

In all analyzed studies, unstimulated saliva was collected. In general, the time interval in which saliva samples were collected was, as in the case of pH determination, in the morning, between 9-12. There were small differences regarding the restrictions imposed on subjects before and during harvesting.

For example, in one study [53], in order to collect saliva, subjects were not allowed to eat, drink, perform oral hygiene, chew, or smoke tobacco one hour before and during the study. In another situation [58], subjects were not even allowed to talk or swallow during saliva collection.

The method of saliva collection was by spitting into a sterile graduated tube, the subjects using a glass funnel to perform this maneuver. After collection, salivary flow was measured and expressed in milliliters/5 minutes in some studies [53] and in milliliters/minute in others [51]. In other studies [59], saliva collection took 3 minutes using Falcon tubes, subject restrictions being similar to the other studies already mentioned.

In another study [60], the collection was performed at the same time interval, the difference to other studies was that food, fluid and smoking restrictions were imposed two hours before the start of the study. The saliva collection method in this case was a special one because a modified Schirmer test was used, applied to the oral cavity.

For the correct performance of saliva collection, the subjects were instructed to swallow once at the beginning of the maneuver to empty the oral cavity and not to swallow during the test. During the procedure, subjects were asked to raise and retract their tongue slightly to avoid wetting the strip used in the Schirmer test.

In the study coordinated by Seeme Nigar [61] and carried out recently, in 2022, the collection of saliva was also done in the morning, between 8 and 12, the authors motivating the choice of this time period, so that the salivary secretion is not influenced by the rhythm circadian.

Similar to other studies, the participants were instructed not to eat, drink liquids, not smoke, not to perform oral hygiene an hour before the start of the collection, as well as during it. In addition to other researches, after collecting the saliva samples, the presence of the number of unrestored active caries was also analyzed.

To avoid erroneous results, patients were instructed to limit lip and tongue movements and also not to swallow.

The two methods of saliva collection in the case of determining salivary flow were the volumetric method, applied in most studies, 9 and the Schirmer test in two situations.

Regardless of the method of saliva collection, in the case of smokers, salivary flow was found to be lower than in non-smokers.

Determination of total salivary antioxidant capacity

In order to evaluate this salivary parameter and to be able to appreciate the influence of smoking on the antioxidant capacity of saliva, we compared a number of 10 scientific works published in the specialized literature that quantified, among other things, the antioxidant capacity of saliva in smokers and in control groups (people non-smokers).

The total number of subjects analyzed in the respective studies was 750, of which 378 were smokers and in the control group 372 were non-smokers. The collected results are presented in table number 5.

Table 5. Determination of total salivary antioxidant capacity

1. Sedigheh Bakhtiari, 2015	Iran	Complete antioxidant capacity kit	30	30	0.529±0.167 U/ml	0.741±0.123 U/ml
2. Parisa Falsafi, 2016	Iran	Complete antioxidant capacity kit	50	50	4.10±0.73 mol/dL	1.87±0.49 mol/dL
3. Masoomeh Shirzaiy, 2017	Iran	FRAP	37	27	698.3±231.86 µmol	378.43±207.3 µmol
4. Obi Ugochukwu L, 2017	Nigeria	FRAP	58	58	1310.41±230.86 µmol/L	1148.07±171,98 µmol/L
5. Shahba'a Munther, 2018	Irak	Complete antioxidant capacity kit	60	60	0.52±0.03 mm/L	0.48±0.03 mm/L
6.Sankepalli Shwetha, 2018	India	FRAP	15	15	800±67 µmol/L	573±60 µmol/L
7. Maciej Wrzol, 2019	Poland	Trolox	20	10	0.81±0.48 mmol/g	0.79±0.39 mmol/g
8. Hamed Mortazavi, 2020	Iran	ELISA kit	40	40	0.39±0.18 U/L	0.29±0.17 U/L
9. Ala Ghazi, 2020	Iran	Complete antioxidant capacity kit	34	32	0.63±1.11	0.17±0.16
10. Pourya Pashaei, 2020	Iran	Complete antioxidant capacity kit	28	56	0.059±0.8 µmol/L	0.0439±0.037 µmol/L
Total			372	378		

In most studies, the collection was performed by spitting saliva into sterile containers, Falcon tubes [62–65], over a period of several minutes.

The imposed restrictions were similar to those in the cases of determining changes in other salivary parameters influenced by smoking, pH, respectively, salivary flow. The most frequent restrictive measures were: abstinence from eating, drinking, smoking 90 minutes before the procedure [62,66–71].

A special requirement addressed to the participants was not to carry out intense physical activities 48 hours before the saliva collection. Sample collection was carried out as in other situations, in the morning, between 8 and 12.

In the case of determining the antioxidant capacity of saliva, after collection it was centrifuged [62,63] to remove squamous cells, debris and germs. After this process, a clear and homogeneous saliva sample is obtained, optimal for analysis.

After harvesting, the antioxidant capacity of the saliva was measured in vitro using the FRAP (Ferric Reducing Antioxidant Power) method, using an antioxidant capacity kit [62,63,66].

According to the European Commission for Public Health, tobacco consumption represents the greatest health risk that can be avoided and at the same time the most

important cause of premature death in the EU, causing the death of almost 700,000 people annually. According to statistics, about 50% of smokers die prematurely (on average, 14 years earlier).

Despite significant progress in recent years, the number of smokers in the EU remains high (26 % of the total population and 29 % of 15–24-year-old) [72].

CONCLUSIONS

After analyzing the results obtained in the selected studies, we came to the conclusion that smoking through its components induces in the case of smokers a decrease in salivary pH, producing acidification of the oral environment and this can lead to the appearance of lesions on the oral mucosa, as well as on the dental tissues.

Regarding salivary flow, we conclude that it decreases in smoking groups compared to non-smoking groups and the vast majority of studies link a low salivary volume to the development of cariogenic bacteria in the oral cavity.

The vast majority of results published in the articles analyzed in the present study identified a decrease in total salivary antioxidant capacity in smoking subjects followed by an increased incidence of periodontal disease as well as premalignant lesions.

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