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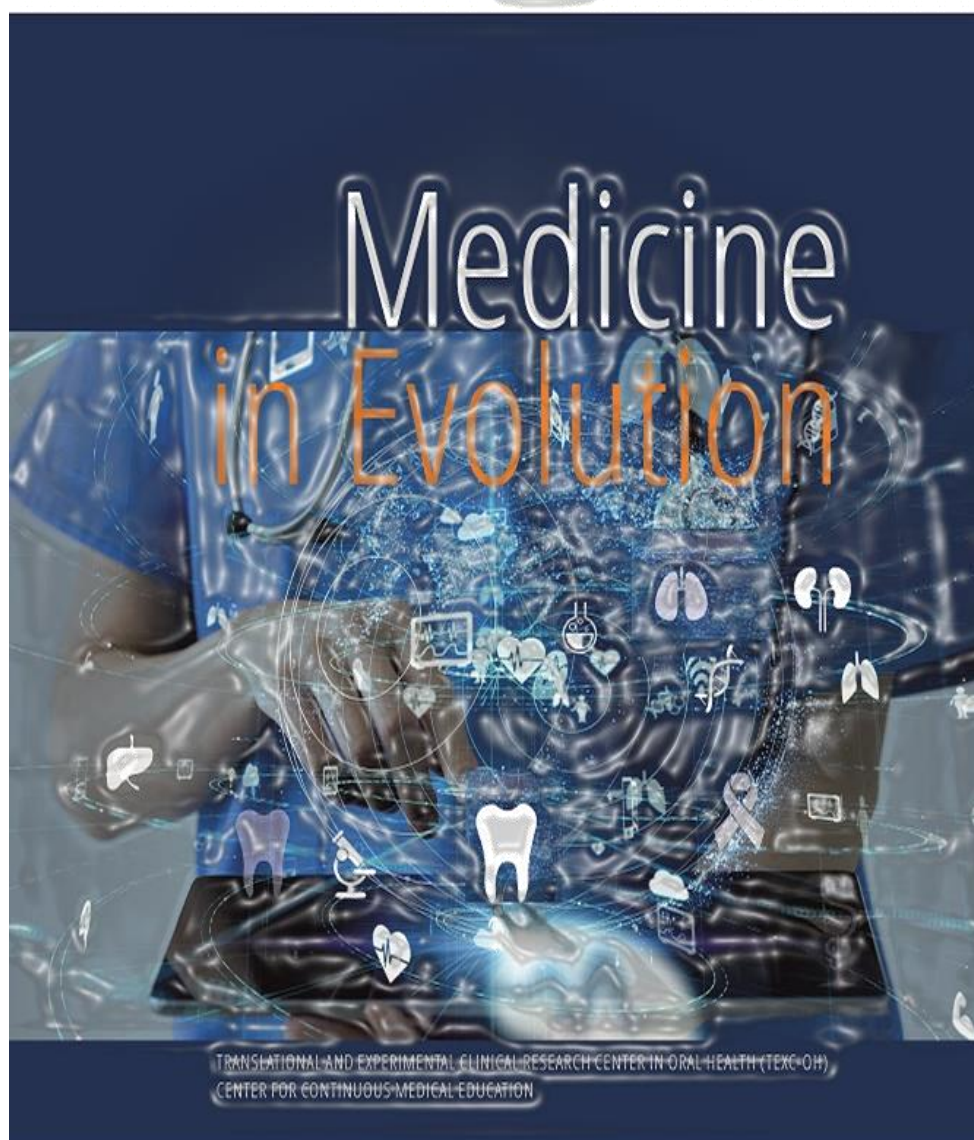
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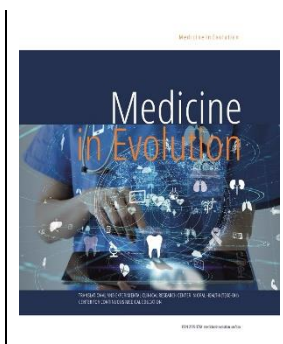
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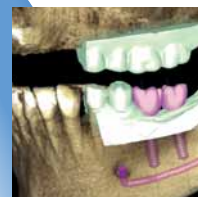
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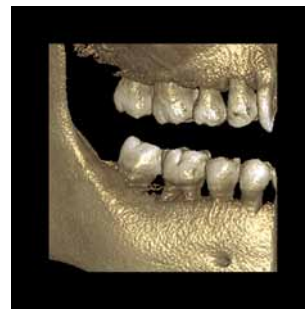
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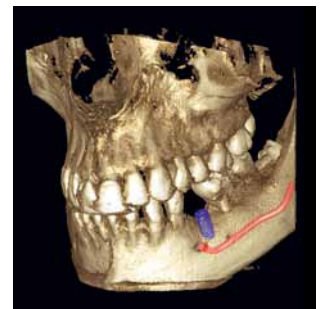
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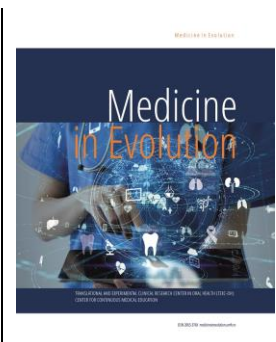
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Oral health of hemodialysis patients compared to healthy individuals



Slușanschi O.¹, Țandără A.¹, Gârneață L.², Giura A.C.¹, Preoteasa C.³, Oancea R.⁴, Funieru C.¹

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Abstract

Aim and objectives: The aim was to evaluate the oral health status of hemodialysis patients and compare it to individuals with no kidney disease. **Materials and Methods:** A cross-sectional study was conducted on 217 hemodialysis patients and 50 controls, in Bucharest. Demographic data, DMFT index, Silness and Løe plaque index, clinical attachment level and periodontal pocket depth were recorded. The data was analyzed using SPSS (16.0). **Results:** There were significant differences between the two groups regarding missing teeth - 11.7 ± 8.7 for dialysis patients and 9.2 ± 8.0 for controls and for the Silness and Løe plaque index - 1.4 ± 1.3 in dialysis patients and 0.8 ± 0.6 in controls. Severe periodontal disease was more prevalent in the hemodialysis group, 33.5% compared to 11.4% in controls. **Conclusion:** Oral health related to missing teeth, severe periodontal disease and oral hygiene is significantly more precarious for patients undergoing hemodialysis.

Keywords: Renal insufficiency, haemodialysis, oral health, periodontal disease

INTRODUCTION

A large number of studies performed worldwide during the last 25 years on chronic kidney disease (CKD) and end stage renal disease (ESRD) patients reported a diminished oral health status for this population [1-7]. These studies found that periodontal disease, especially in severe forms, is more frequent in ESRD patients [2,4], leading to increased tooth loss and diminished oral health related quality of life.

Malnutrition and inflammation, which are frequently present in hemodialysis (HD) patients, are considered indicators of poor prognostic for survival [6, 8]. Oral infections, especially periodontal disease, can contribute to malnutrition and inflammation in these patients [3], having a potentially negative influence on the metabolic disorders and survival of these patients [5, 6].

Aim and objectives

The aim of the study was to evaluate the oral health status of HD patients and compare it to individuals with no kidney disease.

MATERIALS AND METHODS

A cross-sectional study was conducted on patients with end-stage renal insufficiency from two hemodialysis centers in Bucharest – Fresenius Nephrocare Dialysis Centre and IHS Sf. Pantelimon Dialysis Centre - in 2011 and 2012, respectively. All patients treated in the two centers, who gave their informed consent, were included in the study. The study was approved by the Ethics Committee of the Carol Davila University of Medicine and Pharmacy in Bucharest.

The data was gathered by a single person, through an interview and a dental examination, during the dialysis session.

Demographical data and data regarding the educational level and the smoking habit were obtained through an interview of the patient. Clinical data included: the DMFT index, the plaque index according to Silness and Loe, the total number of functional teeth, the clinical attachment level (CAL), the periodontal pocket depth (PPD). Periodontal measurements were done using the periodontal probe recommended by the World Health Organisation (WHO). The severity of the periodontal disease was considered in accordance to the evaluation system proposed by the Centre for Disease control (CDC) and the American Academy of Periodontology in 2003 [9]: severe periodontal disease - 2 or more approximal sites with CAL \geq 6 mm and 1 or more approximal sites with PPD \geq 5 mm, on different teeth; moderate periodontal disease - 2 or more approximal sites with CAL \geq 4 mm or 2 or more approximal sites with PPD \geq 5, on different teeth.

Statistical analysis was performed using the 16.0 version of the SPSS. A p value of 0.5 or less was considered statistically significant.

RESULTS

Dialysis group

The total number of patients was 217 of which 55.3% (120) were men. The mean age of the group was 59.2 \pm 12.4 years. There were 82.5 % (179) subjects living in an urban environment. Among the subjects, 23% (50) had higher education and 46.5% (101) had secondary education. Regarding the smoking habit, 81% (177) didn't smoke, 1.4% (3) smoke 2 packets a day and 6% (13) smoke 1 packet per day. The primary renal disease was represented by chronic glomerulopathies for 29% of the subjects and diabetes for only 12%.

Control group

There were 50 individuals in the control group, of which 42% (21) were men. The mean age of the group was 60 ± 15 years. There were 86% (43) controls living in an urban area. Within this group, 36% (18) had higher education and 42% (21) had secondary education. Among the subjects 36% (18) smoked, with 10% (5) smoking 1 packet a day.

Analysis showed no statistically significant differences between the dialysis and control groups regarding mean age, gender distribution, living environment, education or smoking habit.

Comparison between dialysis patients and controls

Data regarding the dental and oral hygiene status is available in Table 1. Dialysis patients had statistically significant more missing teeth, less fillings and functional remaining teeth and poorer oral hygiene than controls.

Edentulous mandibular ridge class I and II Kennedy and edentulous maxillary ridge class I Kennedy are statistically significant ($p < 0.01$ for Pearson χ^2) more frequent in dialysis patients.

Table 1. Dental and oral hygiene status: comparison between dialysis patients and controls (means \pm SD)

	Dialysis group	Control group	Test Mann-Whitney
Caries - D	1.8 \pm 2.4	1.5 \pm 2.2	p=0.294
Missing teeth - M	11.7 \pm 8.7	9.2 \pm 8.0	p=0.050
Fillings - F	3.4 \pm 4.6	6.0 \pm 4.8	p<0.001
DMFT index	16.9 \pm 8.4	16.7 \pm 7.5	p=0.766
Functional remaining teeth	11.7 \pm 9.4	19.6 \pm 8.6	p=0.050
Silness and Loe plaque index	1.4 \pm 1.3	0.8 \pm 0.6	p<0.000

Periodontal markers and disease were assessed only for individuals with more than 4 remaining teeth, namely on 86.6% (188) of the dialysis patients and on 88% (44) of the controls. Table 2 contains data related to the periodontal status.

Table 2. Periodontal status: comparison between dialysis patients and controls

	Dialysis group	Control group	p
Mean PPD	2.6 \pm 0.9	2.2 \pm 0.8	p=0.018*
Max PPD	4.6 \pm 1.5	4.0 \pm 1.4	p=0.014*
Mean CAL	3.5 \pm 1.4	2.8 \pm 1.1	p=0.001*
Max CAL	6.6 \pm 2.3	5.2 \pm 1.7	p=0.001*
No/mild periodontal disease	13.8%	31.8%	p<0.001**
Severe periodontal disease	33.5%	11.4%	p<0.001**
Moderate/severe periodontal disease (<51 years old)	68%	31%	p=0.018***
Moderate/severe periodontal disease (>65 years old)	96%	77%	p= 0.048***

* Test Mann-Whitney; **Test t Student for percentage comparison; *** Test χ^2 Pearson.

The severity of periodontal disease is also different between the two groups. Severe periodontal disease is statistically significant more frequent in dialysis patients ($p < 0.001$ fort Mann-Whitney test). To further investigate the distribution of periodontal disease between the groups we performed an analysis on 3 age intervals: younger than 51 years, between 51-65 years and older than 65 years. The difference between dialysis patients and controls is statistically significant for the age segments younger than 51 ($p = 0.018$, for Pearson χ^2) and older than 65 ($p = 0.048$, for Pearson χ^2).

The relative risk (odds ratio) regarding periodontal disease was performed: the risk of having moderate/severe periodontal disease is approx. 3 time higher (odds ratio=2.9, with

1.4-6.2 95% confidence interval) for the dialysis group when compared to controls. Also, a loglinear model was performed to check whether having periodontal disease is influenced by factors (gender, age, place of residence) other than being on haemodialysis. The analysis showed that the only significant interaction was between the variables „periodontal disease” and „being on haemodialysis”, emphasising that the higher prevalence of periodontal disease among haemodialysis patients is not significantly influenced by age, gender or place of residence but only by having terminal renal insufficiency.

DISCUSSIONS

This study found severe periodontal disease is prevalent in 33.5% of the dialysis patients which is higher than the approx. 18% prevalence reported by the World Health Organisation for the European region 2022 [10] and higher than in the individuals in the control group. These findings are in accordance with most of the studies performed on the dialysis population around the world which concluded that periodontal disease is more prevalent in HD patients than in the general population [1, 2, 5]. Moreover, oral hygiene is also more precarious within the ESRD individuals which could lead to the further aggravation of the periodontal disease and increased tooth loss.

The percentage of dialysis patients with total edentulism or fewer than 4 remaining teeth was 13.4% is similar to the one reported by Gurkan et al among Turkish HD patients [7]. This study found that the number of functional remaining teeth is significantly lower in HD patients; also, terminal edentulism (class I and II Kennedy) is significantly more prevalent in dialysis patients. This could be interpreted as a consequence of severe forms of periodontal disease and could have a negative impact on the nutrition status of the HD patient (as mastication is impaired) and on the oral health related quality of life.

Periodontal disease can be prevented by practicing good oral hygiene, eating a healthy diet, and not smoking. If periodontal disease does develop, its progression can be slowed or stopped with proper dental treatment, regular checkups, and continued education on oral hygiene [9]. The present study showed that periodontal health is affected at an early age in the HD population, as significantly more HD patients younger than 51 years old had moderate/severe periodontal disease when compared to same age group controls. As most studies report higher prevalence of severe periodontal disease among HD patients the dental professionals should take the proper steps in providing correct dental care for this population and efficient education towards correct oral hygiene habits.

CONCLUSIONS

Oral health regarding number of missing teeth, oral hygiene and the prevalence of moderate and severe periodontal disease is significantly higher in patients with terminal renal insufficiency treated by haemodialysis than in controls. Also, the risk (odds ratio) of having moderate/severe periodontal disease is 3 times higher for haemodialysis patients than for individuals with no renal disease.

There is a need for effective management of the periodontal disease among the haemodialysis patients regarding efficient treatment, regular recalls and education regarding correct oral hygiene.

Acknowledgements

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Inhibitors of the sodium-glucose cotransporter type 2 in the diabetes mellitus treatment



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Abstract

Sodium-glucose co-transporter inhibitors' drugs (SGLT) work by selectively and reversibility inhibiting at the renal level. Through this mechanism, they reduce glucose reabsorption, which is excreted through urine and thus contribute to normalizing blood glucose. Administration of sodium-glucose co-transporter inhibitors induces favorable changes in glycosylated hemoglobin, body weight and blood pressure, as well as a low risk of hypoglycaemia. Although they are a pharmacological group that can be used as monotherapy, they are mostly used as adjuvants in the treatment of patients with diabetes mellitus who receive drug treatment with other normo or hypoglycemic medications and who have not met control goals. It is important to be alert to possible side effects or adverse reactions to discontinue treatment and take appropriate action. Given this problem, a detailed analysis will be highlighted.

Keywords: glyphozins; sodium-glucose cotransporter inhibitors; Mellitus diabetes; treatment; weightloss

INTRODUCTION

Noncommunicable diseases constitute a public health problem worldwide, and among them, diabetes mellitus (DM) has a high prevalence.(1) In people with this disease, the treatment priority is to achieve good metabolic control and thus avoiding the risk of extreme blood glucose values, thus avoiding future complications of this disease.(2) Avoiding hyperglycemia has a particular value, since it is known that it constitutes a marker of severity in critically ill patients, it is associated with worse outcomes and is an independent factor of mortality.(2)

The reasons for the increase in glycemia in these patients are related to non-compliance with the indicated treatment and, in general, to inadequate self-care behaviors for DM. Financial limitations to access the drugs used for the correct treatment of this disease can also contribute to metabolic uncontrol. Meanwhile, hypoglycemia in patients with DM generally occurs as a result of inadequate or erratic consumption of carbohydrates, after the administration of sulfonylureas or insulin. (2) For the proper treatment of DM, there are several groups of medications.

Among these, there are the so-called oral normoglycemic agents and hypoglycemic agents; the latter can be used orally or parenterally. Both groups of drugs reduce blood glucose, but the latter can -in addition- increase the risk of hypoglycemia if not used properly.(3) Hence, the importance of incorporating new hypoglycemic agents into the market that do not compromise the mechanisms of endogenous counterregulation, which would help reduce the frequency of this negative event.(3) Sodium-glucose cotransporter (iSGLT) inhibitor drugs meet this condition and currently iSGLT type 2 (iSGLT2, for its acronym in English) constitute an option for the treatment of type 2 DM (DM2).(4)

The iSGLT2, are also known by the pseudonym "glifozinas", although they can be used in monotherapy, they are considered as a coadjuvant pharmacological group in the control of patients with DM2, who receive treatment with other normal-type or hypoglycemic drugs, which control goals have not been achieved. Like any medication, it should be noted that despite its benefits, it is not free from some adverse effects and side reactions. (4)

However, due to its importance, SGLT2, as a new option, to obtain good glycemic control in patients with DM, it is necessary that our professionals are familiar with its use. Hence, the objective of this article was to describe the role of SGLT2-i in the treatment of DM.

Aim and objectives

This article aims to describe the role of sodium-glucose cotransporter type 2 inhibitors in the treatment of diabetes mellitus.

MATERIAL AND METHODS

The information necessary to write this article was carried out in the first semester of 2023. This was obtained from the evaluation of different review, research and Web pages, which in general were less than 10 years old, in Romanian or English. Specialized books were used as scientific information search engines and the study used 17 specialized books.

RESULTS

Mechanism of action of sodium glucose cotransporter inhibitors

Renal glucose reabsorption is the primary mechanism by which the kidneys influence glucose homeostasis. This function is fundamental and involves renal gluconeogenesis, which

is capable of producing 15 to 55 g of glucose per day, while the kidney can metabolize 25 to 35 g of it in the same period of time. Glucose uptake from the circulation and glomerular filtration constitute another of the mechanisms involved in blood glucose. (8)

Hypothetically, if it is considered that there is a glucose concentration of 100 mg/dl, with a normal glomerular filtration rate of approximately 180 L per day, healthy people should filter approximately 180 g per day of glucose. (8) This is reabsorbed -almost all- in the proximal convoluted tubule and returns to the circulation, in such a way that no glucose should be excreted in the urine of healthy subjects. The efficiency of this system is excellent and allows glucose to be conserved in the body, which constitutes a valuable source of energy. (9)

Although several types of sodium-glucose cotransporters (SGLT) are known to exist, (English), the present review focuses on the activity of two of its types, SGLT-1, distributed throughout almost the entire body -intestine, trachea, kidney, heart, brain, testis and prostate, and SGLT2, predominantly located at the renal level, theoretically allowing its local action. (9, 10) The latter are expressed in the proximal convoluted tubules of the kidney -in segment S1- and are responsible for most of the reabsorption of glucose filtered from the lumen of the tubules (approximately 90%). (10)

It has been shown that patients with DM2 have increased renal glucose reabsorption, which may contribute to persistently elevated blood glucose concentrations. (10) SGLT2-i act competitively, reversibly, and selectively at the level of SGLT2 receptors located in the proximal convoluted tubules and its action is independent of the secretion or action of insulin and the evolutionary stage of DM2. The inhibition lowers the saturation threshold for glycosuria in such a way that it starts with blood glucose levels between 60-80 mg/dL (<100 mg/dL). Likewise, it increases urinary glucose excretion by 60-80g/day (22) and decreases renal glucose reabsorption by 30-50%. (11, 12)

Some characteristics about sodium-glucose cotransporter inhibitors as part of the treatment of diabetes mellitus

Oral absorption of SGLT2-i is rapid (C_{max} 1-2 h) and is not modified by food intake. At the same time, they present hepatic metabolism by glucuronidation, with little oxidation and participation of cytochromes, and it is confirmed that 60% of these drugs are eliminated through the feces, while 33% are eliminated through the urine.(12) Based on Given the efficacy and safety profile observed, iSGLT2 can be considered a treatment option in patients with a renal glomerular filtration rate > 60 ml/min, with special caution in elderly patients, who do not present acute or chronic diseases that may cause hypoxia. tissue. (13)

There are several drugs that have the ability to act through SGLT2 receptors and produce an osmotic diuresis; Among them, the following are described: dapagliflozin, canagliflozin and empagliflozin - although more recently rtugliflozin was incorporated into this group. These drugs act independently of insulin concentration or peripheral resistance to this hormone and dependent on plasma glucose concentrations, as well as the glomerular filtration rate of the subject. (13)

Initially, iSGLT2 have been authorized for the exclusive treatment of DM2.(14) However, due to the existing difficulties in the adequate therapeutic management of some patients with type 1 DM (DM1) and the need to search for drugs that can be used in combination with insulin to obtain optimal glycemic control, exploring the use of SGLT2-i in these patients is considered.(14) For this reason, the European Medicines Agency (EMA) has agreed to review submission of the registration dossier for sotagliflozin, which is a dual inhibitor of SGLT types 1 and 2. Oral treatment -if authorized- would be used as an adjunct to insulin treatment to improve glycemic control in adults with DM1. (15)

The FDA- and EMA-approved iSGLT2 agents for T2DM patients are in the commercial phase and are still undergoing long-term clinical safety trials. Their pharmacokinetic and pharmacodynamic properties are presented in Table 1. (15)

Table 1. SGLT2 inhibitor drugs commercially approved in the USA and Europe

Drug	Dapagliflozina	Canagliflozina	Empagliflozina	Ertugliflozina
Tradename	Farxiga	Invokana	Jardiance	Steglatro
Selectivity	1200 times more selective to SGLT-2	250 times more selective to SGLT-2. SGLT-1 low potency inhibition	2500 times more selective to SGLT-2	2000 times more selective to SGLT-2
Half life	12,2 h	11-13 h	22,4 h	16,6 h
Bioavailability	78%	65%	>60%	100%
Metabolism	Hepatic due to glucuronidation	Hepatic due to glucuronidation	Hepatic due to glucuronidation	Hepatic due to glucuronidation
Excretion	Urinary with inactive metabolites	Fecal and urinary as drug	Fecal (41%) and urinary (54%)	Renal and fecal excretion (1.5% renal route and 33.8% fecal route as drug)
Drug interaction	No clinical relevance	No clinical relevance	No clinical relevance	No clinical relevance
Renal adjustment	Not recommended in patients GFR <60ml/min/1.73mp	Not recommended in patients GFR<45ml/min/1.73mp. Limit dose to 100mg in patients with GFR between 45-60ml/min/1.73mp	Not recommended in patients GFR <45ml/min/1.73mp	Not recommended in patients GFR<30ml/min/1.73mp. Not recommended in patients with GFR between 30-60ml/min/1.73mp
Hepatic adjustment	No hepatic adjustment required Child Pugh missing data	Child Pugh A and B do not require adjustment. Child Pugh missing data	Missing data. could be used	Child Pugh A and B do not require adjustment. Child Pugh missing data
Pharmacodynamics	Urinary glucose excretion of approximately 70 g per day	Decreases the renal threshold to 70-90mg/dl. It causes a mean urinary glucose excretion of 100 g per day.	Between 64-78 g daily of glucose excreted via the urine	Between 45-68 g of glucose excreted via the kidneys
Presentation	5mg and 10mg tablets	100mg and 300mg	10mg and 2mg	5mg and 15mg

These drugs are available both as single-ingredient products and in formulations combined with other drugs for the treatment of DM, to make their use more feasible (15) (Table 2).

Table 2. Combination formulations of SGLT2 inhibitor drugs with other drugs for the treatment of diabetes mellitus

Name	Administration	Presentation	Suggested maximum dose
Xigduo XR	Oral 1 tab/day	2.5 mg Dapagliflozin / 1000 mg Metformin XR 5mg Dapagliflozin / 500mg Metformin 10 mg Dapagliflozin / 500 mg Metformin 5 mg Dapagliflozin / 1000 mg Metformin XR 10 mg Dapagliflozin / 1000 mg Metformin XR	10 mg Dapagliflozin / 2000 mg Metformin
Invokament	Oral 2 tab/day	50 mg canagliflozin/500 mg metformin XR 50 mg canagliflozin/1000 mg metformin XR 150 mg canagliflozin/1000 mg metformin XR	150 mg canagliflozin/1000 mg metformin XR
Glyxambi	Oral 1 tab/day	10 mg empaglifozina/5 mg linagliptina 25 mg empaglifozina/5 mg linagliptina	25 mg empaglifozina/5 mg linagliptina
Segluromet	Oral 2 times/day	2,5 mg ertugliflozina/1000 mg metformina 2,5 mg de ertugliflozina/850 mg metformina 7,5 mg ertugliflozina/850 mg metformina 7,5 mg ertugliflozina/1000 mg metformina	2,5 mg ertugliflozina/1000 mg metformina
Steglujan	Oral 1 tab/day	5 mg ertugliflozin and 100 mg sitagliptin 15 mg ertugliflozin and 100 mg sitagliptin	15 mg ertugliflozin and 100 mg sitagliptin

iSGLT2 may play an important role as an oral "antidiabetic" (ADO), this is because they have a similar effectiveness to sulfonylureas and achieve a decrease in HbA1c equivalent to metformin (0.8% to 1.0%), if we start from a value of 8% during two years of treatment.(14) Glycosuria caused by the administration of iSGLT2 induces a decrease in basal glycemia,

glycemia and HbA1c, and is shown to be at the same level as other OADs in the reduction of HbA1c, being higher -in some cases- in weight loss or blood pressure, with an incidence of hypoglycemia similar to that observed with placebo.(14)

All of which provides an element of tranquility for the patient and the doctor. iSGLT2 induce a decrease in albuminuria in patients with DM2 and -as already mentioned- weight loss secondary to the excretion of glucose (calories) in the urine, which is accompanied by a reduction in waist circumference and waist circumference, fat mass, especially visceral adipose tissue. (15) However, the pharmacodynamic response to SGLT2-i decreases in chronic kidney disease, which is related to the severity of renal failure. Hence, the recommendation to consult the prescribing information related to dose adjustments or restrictions in moderate to severe renal dysfunction, where its use is not recommended. (15)

Some results of interest related to the use of sodium-glucose cotransporter inhibitors in people with diabetes mellitus

The use of dapagliflozin has been studied in the specialized literature-the first of these drugs to be approved and marketed in 2012- in patients with DM. The results suggest that it is promising to add this medication to insulin treatment in order to improve glycemic control in these patients. (16) In a study with patients that had DM2 and treatment with dapagliflozin found that the use of this drug did not result in a higher or lower rate of cardiovascular death, myocardial infarction, or ischemic stroke than placebo. However, they did find a lower rate of cardiovascular death or hospitalization for heart failure.

Different results were found in literature, by specialists who observed that dapagliflozin was associated with lower risks of cardiovascular events and all-cause mortality, when compared with dipeptidyl peptidase-4 inhibitors, in a real-world clinical setting and a large population of DM2.

In a systematic review that included twelve randomized clinical trials, with a total of 3986 patients, groups of patients treated with dapagliflozin plus metformin were compared against placebo plus metformin, or dapagliflozin plus insulin versus placebo plus insulin, with follow-up ranging from 12 to 104 weeks. It was observed that the addition of dapagliflozin to conventional DM treatment optimized the control of HbA1c levels and glycemic levels compared to the control group; Furthermore, it was found that the group of patients with DM treated with dapagliflozin reduced body weight, a favorable condition for the metabolic control of these patients. (16)

A retrospective observational study, describe the results of treatment with different iSGLT2 in 2113 patients with DM2 (0.74% of all those treated with OAD); of which 53.10% were men. The average age was 64.54 years and the treatment for a period of more than six months, between 2022-2023. Their results suggest that an adequate prescription of iSGLT2 in patients with DM2 offers a short-term benefit in reducing the figures of Body Mass Index (BMI), HbA1c and cholesterol, without repercussions on blood pressure and renal function.

The CANVAS program integrated data from two trials with a total of 10,142 participants with DM2 and high cardiovascular risk. Participants in each trial were randomly assigned to receive canagliflozin or placebo and were followed for a median of 188.2 weeks. It was shown that patients treated with canagliflozin had a lower risk of cardiovascular events than those who received placebo, which supports its cardioprotective effect, but unfortunately a higher risk of amputation was found, mainly at the level of the toes or metatarsals. (15, 16)

It should be noted that the results of the CANVAS program are similar to those obtained in the EMPA-REG OUTCOME trial, (16) where empagliflozin reduced the risk of major cardiovascular events in patients with DM2 with this condition. Likewise, it is known that SGLT2-i also have a nephroprotective effect, (54,55) a criterion supported by other authors.

In another investigation (17) on the action of canagliflozin on the kidney in 1450 patients with DM2 on treatment with metformin who were randomized to receive daily canagliflozin 100 mg (483), canagliflozin 300 mg (485) or glimepiride (482) in increasing doses between 6-8 mg/day, it was observed how canagliflozin, compared to glimepiride, decreased the progression of kidney disease in patients with DM2 treated with metformin in the two years that the study lasted, and independently of the glycemic decrease, which would suggest a reindeer protective effect.

These effects are probably based on the favorable effects of SGLT2-i on glomerular hemodynamics, decreasing hyperfiltration, reducing glucose tubular toxicity, as well as its beneficial effects on blood glucose, blood pressure, weight, and uricemia. As can be seen, one of the merits currently attributed to the use of SGLT2-i is cardiovascular and renal protection. (17) Some researchers (13,14) have proposed hypotheses that try to explain this behavior, based on the metabolic and hemodynamic effects caused by these medications and which are described below:

- Metabolic effects: includes the reduction of glucotoxicity induced by glycosuria, the reduction of fat mass (especially perivisceral), the hypouricemia effect due to uricosuria and the preferential use of fats instead of carbohydrates, which leads to the increased production of ketoacids, which are a more efficient fuel for the ischemic heart and kidney.

- Hemodynamic effects: osmotic diuresis induced by glycosuria and natriuresis reduces preload (volemia) and afterload (blood pressure) and improves tubuloglomerular feedback and intraglomerular pressure, all without increasing sympathetic tone.

In addition, hemoconcentration increases hematocrit (although there also appears to be an increase in erythropoietin), which may facilitate oxygen delivery to ischemic tissues. Some researchers conducted a phase III, double-blind trial at 133 centers worldwide, randomly assigning 1402 patients with DM1 receiving treatment with any insulin therapy (pump or injections) to receive sotagliflozin (400 mg daily) or placebo for 24 weeks. It was observed that 28% of patients receiving the drug achieved the primary endpoint of maintaining HbA1c below 7%, without severe hypoglycemia or ketoacidosis compared to 15% of patients in the placebo group. In the sotagliflozin-treated group, HbA1c decreased by 0.46%, weight loss was 2.98 kg, blood pressure decreased among those with hypertension by 3.5 mm Hg, and the dose of daily insulin was significantly lowered.

The incidence of severe hypoglycemic episodes was significantly lower compared to the placebo group; instead, the cases of diabetic ketoacidosis were higher. Situation reported previously by the FDA and the EMA, with the use of other iSGLT2. (30, 58, 59) This situation agrees with a 17-year-old patient without ketosis for 9 years with DM1, who started treatment with dapagliflozin 10 mg/day and who unexpectedly had an event of this nature. Logically, the authors believe that this result is of particular concern, due to the use of SGLT2-i in people with DM1, and they support the criteria of Crasto, W. (13) who suggest that health professionals should carefully seek and evaluate the presence of ketoacidosis in these patients. Given this situation, iSGLT2 should be discontinued and appropriate measures taken to correct this health problem as soon as possible.

One of the elements of great interest pointed out by several publications is the beneficial effect exerted by iSGLT2 on weight loss, which had been outlined previously. Its use is postulated to be associated with a dose/response weight loss, mean loss of 2.1 kg compared to placebo after 12 weeks of use, and mean loss of 2.9 kg after two years, compared to other drugs. (17)

The ertugliflozin efficacy evaluation and cardiovascular safety outcomes trial (VERTIS-CV trial; NCT01986881) addressing cardiovascular health in people using this product could not be compared with the results of the research discussed above, as such investigation was ongoing at the time of writing this article.

The aforementioned means that SGLT2-i, at least in theory, can be considered candidates of interest, at least as an adjuvant, associated with metformin, for the treatment of insulin resistance syndrome by contributing to the improvement of its components, despite not to act directly on insulin resistance.

The most common side effects and adverse reactions observed during the use of sodium-glucose cotransporter inhibitors

SGLT2-i inhibitors represent a novel approach in the treatment of DM2. They can be used in combination therapy with other oral normal or hypoglycemic drugs or even with insulin and have potential application in patients who are intolerant to metformin due to gastrointestinal side effects. (17)

SGLT1 inhibition is associated with potentially serious adverse reactions. Although SGLT1 plays an insignificant role at the renal level, this transporter, located mainly in the small intestine, facilitates the absorption of glucose from food and its inhibition can lead to an increase in glucose in the intestinal lumen, with risk of appearance of osmotic diarrhea. (16,17)

The recently introduced iSGLT2 drugs in DM therapy are sufficiently selective over SGLT2 so that no effects are produced on intestinal SGLT1. The ability to inhibit renal SGLT2 without modifying intestinal SGLT1 is important for the practical use of these drugs. Likewise, an increase in glucose in the distal intestine could increase the secretion of glucagon-like peptide 1 (GLP1), (68) which is useful in subjects with DM2.

The most common side effects and adverse reactions observed in people using iSGLT are related to their mechanism of action (17) (Fig. 1).

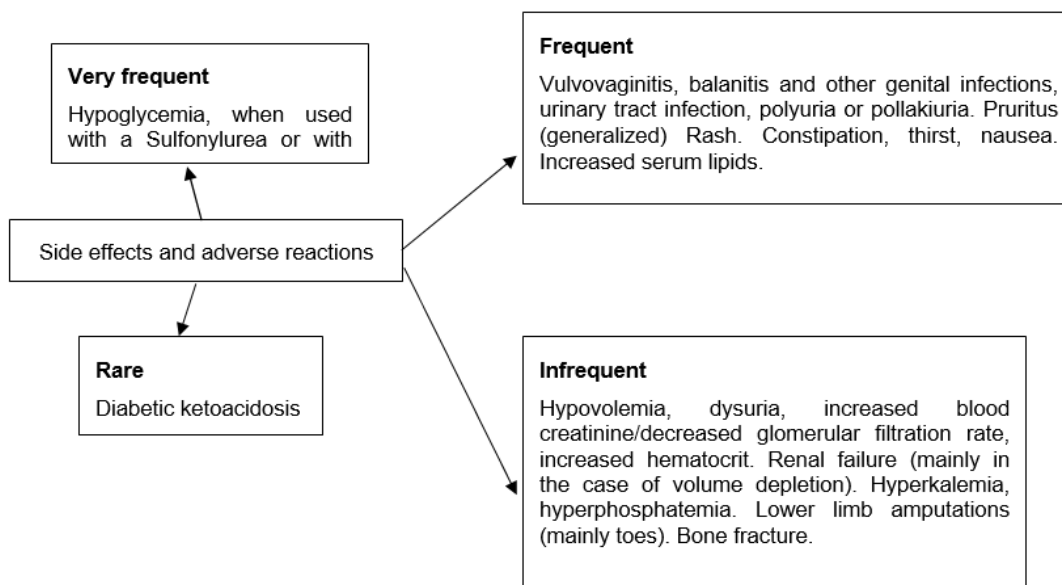


Figure 1. Side effects and adverse reactions that can occur during the use of iSGLT

In fact, Van Bommel (17) indicate that the most common side effects and adverse reactions observed at the population population using SGLT-2-i since their commercialization have been urogenital tract infections, ketoacidosis, and kidney damage. Urinary sepsis was mostly not serious, although ketoacidosis and kidney damage were, generating income and sometimes endangering the lives of patients. Bailey, C.J. (11) in an investigation carried out for this reason, describe adverse effects and secondary reactions to treatment with iSGLT2, although these were low in frequency (urinary tract infections (UTI): 5.31%; vaginal mycosis: 2.65%; hypoglycemia: 0.88%; diabetic ketoacidosis: 0.00%; intolerance to dapagliflozin: 4.42%).

Which, in part, coincides with the majority of adverse effects and side reactions reported by other authors. (12, 14)

Despite the benefits of iSGLT, reality requires careful prescription, consulting the warnings published by the health authorities and notifying any adverse reaction when suspected. In this way, Van Bommel it contributes to their better and more complete knowledge. (17)

DISCUSSIONS

The use of iSGLT-2 not only reduces the reinjection of glucose already filtered from urine into the blood, improving the metabolic control of DM, but also restores tubuloglomerular feedback by increasing glycosuria and distal urinary flow. But the most notable effect is due to the inhibition of glucose entry into the proximal tubular cell, since glycosuria is toxic to the kidney, particularly to cells capable of transporting glucose, that is, proximal cells endowed with SGLT2.

These effects of iSGLT2 in the human clinic have shown their ability to reduce kidney damage and cardiovascular risk in patients with DM2, (12) when they are indicated, taking into account the recommendations for their use. Inhibition of the SGLT2 cotransporter leads to a caloric deficit and a set of metabolic and endocrine adaptations such as: use of lipids as an energy substrate, increased ketogenesis, increased gluconeogenesis, and increased insulin sensitivity. iSGLT2 have been shown to reverse endothelial dysfunction, inflammation, oxidative stress, and interstitial fibrosis, which delays the progression of chronic DM complications and improves the cardiometabolic profile. (10) Likewise, the reduction in effective circulating volume and increase in the activity of circulating renin-angiotensin-aldosterone system blockers, thus creating a nephroprotective effect. (8)

Something especially useful about the use of SGLT-2-i and which has been indicated by several researchers (17) is that the weight loss achieved with its use is predominantly from adipose tissue and particularly from visceral adipose tissue, which represents an important aspect in the improvement of the cardio-metabolic profile of these patients.

Weight loss is mainly due to glycosuria, with an estimated caloric deficit of 50 kcal/day, despite the possible contribution of other mechanisms, such as brown adipose tissue activation and insulin reduction. In patients with DM1 and DM2 treated with insulin and iSGLT2, it is observed that the daily needs of insulin doses decrease and hypoglycemia is less frequent. However, these drugs must be prescribed cautiously, especially in young patients with DM1, due to the greater possibility of ketoacidosis and with a low body mass index, due to the weight loss that it can induce. (17)

CONCLUSIONS

In conclusion, note that the administration of iSGLT2 induces favorable changes in HbA1c, body weight and blood pressure; exhibiting a low risk of hypoglycemia. Although they constitute a pharmacological group that can be used as monotherapy, SGLT2-i are generally used as adjuvants in the treatment of patients with DM2 who receive pharmacological treatment with other normal or hypoglycemic drugs, in case they have not achieved control goals. You must be alert for the appearance of possible side effects and adverse reactions, in this case, discontinue the treatment and take the necessary measures to resolve this situation.

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93%
dintre pacienți

confirmă reducerea
hipersensibilității dentinare⁴

91%
dintre pacienți

apreciază recomandarea
medicului stomatolog⁴



Calmarea imediată* a durerii
din sensibilitatea dentară¹



Calmarea imediată* și de durată începe cu recomandarea dumneavoastră**

*Pentru calmare imediată, aplicați direct cu degetul pe dintele sensibil și masați ușor pentru 1 minut;

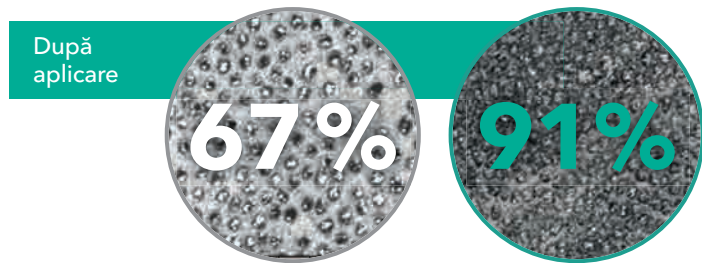
**Doar în legătură cu pasta de dinți

Referințe: 1. Nathoo S, et al. J Clin Dent. 2009;20(Spec Iss):123-130; 2. Docimo R, et al. J Clin Dent. 2009;20(Spec Iss): 17-22.; 3. Report Deon Hines-0003, 2016; 4. Studiu Ipsos cu privire la utilizarea produsului elmex® SENSITIVE PROFESSIONAL Repair & Prevent, efectuat în Polonia, rezultate după 2 săptămâni de utilizare, cu 325 de participanți (2017).

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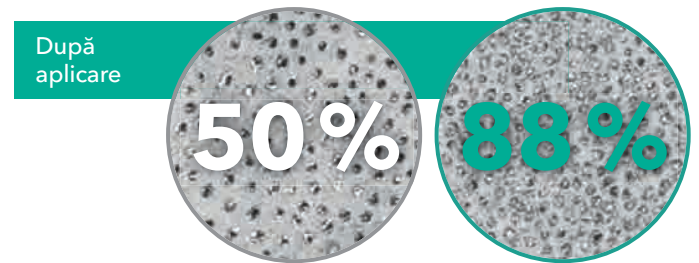
Studiul 1^{1,*}

Tehnologia cu fluorură de staniu/ fluorură de sodiu Tehnologia PRO-ARGIN



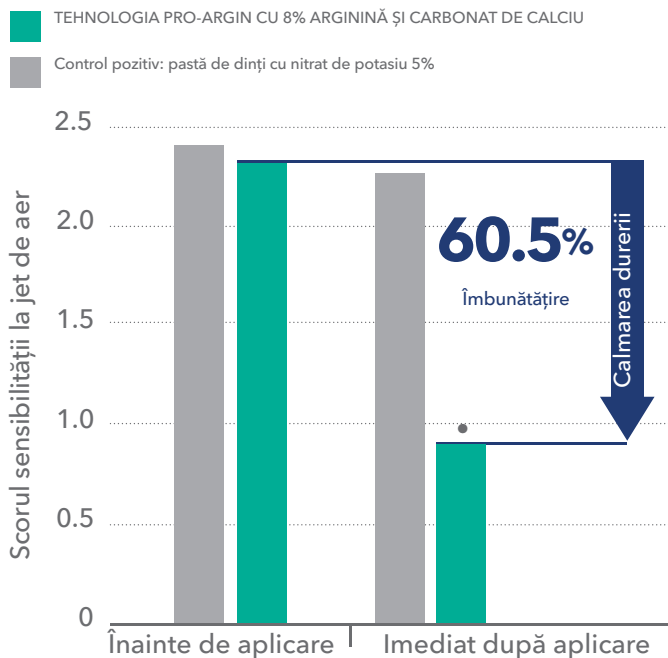
Studiul 2^{2,*}

Tehnologia Novamin/ fluorură de sodiu Tehnologia PRO-ARGIN



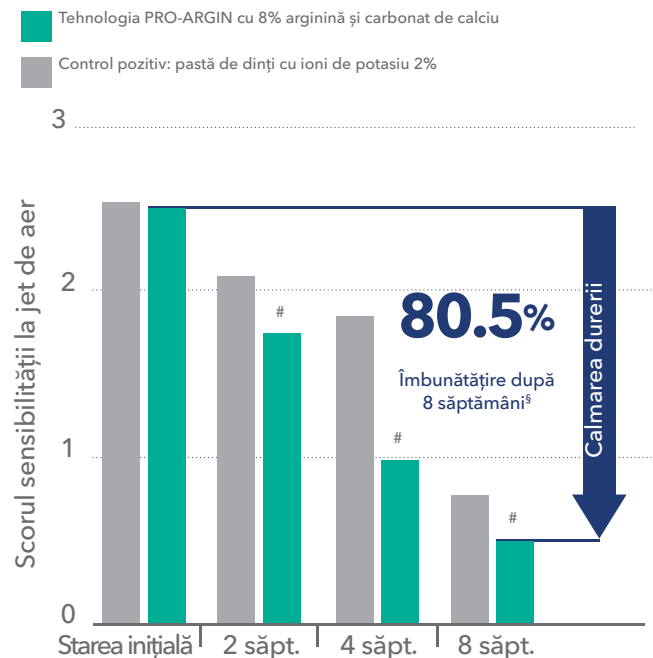
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Calmarea semnificativă a durerii din sensibilitatea dentară instant^{3,†,**}



† În comparație cu starea inițială (sunt prezentate doar datele relevante)
• Semnificativ statistic ($p < 0,001$)

Calmarea semnificativă de lungă durată a durerii din sensibilitatea dentară după 2, 4, și 8 săptămâni de utilizare^{4,§,&}



§ În comparație cu starea inițială
& În comparație cu o pastă de dinți comercială desensibilizantă, ce conține 2% ioni de potasiu și 1450 ppm de fluor (NaF)
Semnificativ statistic ($p < 0,05$)

*Studiu in vitro, imagini reale de microscopie confocală după 5 aplicări ($p < 0,05$);
**Pentru calmarea imediată aplicați direct pe suprafața sensibilă și masați ușor cu vârful degetului timp de 1 minut.
Referințe: 1. Hines D, et al. Poster acceptat, July 2018 IADR. Colgate- Palmolive Company 2018.; 2. Hines D, et al. Poster #0742, March 2018 AADR. Colgate-Palmolive Company 2018.; 3. Nathoo S, et al. J Clin Dent. 2009;20(Spec Iss):123 -130;
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Evaluation of the relation of the apex of the posterior teeth to the mucosa of the maxillary sinus



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Abstract

Aim and objectives: The study aims to determine the risk of maxillary sinus membrane perforations in dental treatments on CBCT investigation. The factors that have been taken in consideration were the relation of the upper molars position with the maxillary sinus as well as the frequency of a certain type of risk.

Material and methods: This observational study analyses the statistical data obtain from 50 patients that presented for the extraction of the upper molars. The criterion for quantifying the position of the upper teeth was created after a standardized position of an implant in relation to the maxillary sinus. Another desired was a special attention to the teeth that have an intimate contact with the membrane of the maxillary sinus observed on the radiographic images.

Conclusions: Following this study, we can affirm that with proper imagistic investigation we can perform a classification of upper teeth position with the maxillary sinus. Additionally, we can assess a specific risk of oro-antral communication and the doctor has the possibility of choosing or adapting a therapeutic protocol according to the risk category in which these teeth are found.

Keywords: maxillary sinus; oro-antral communication; CBCT

INTRODUCTION

The maxillary sinus is one of the four air-filled cavities of the skull, and it is the largest of them. The sinus structure is located in the body of the maxillary bone having a pyramidal shape and has a dominant anatomy in the posterior jaw and midface. The maxillary sinus has a very wide and diverse manifestation in terms of anatomical variation. As a structure it has three main processes: the inferior alveolar process (delimited inferiorly by the alveolar crest), the zygomatic process (delimited laterally by the zygomatic bone) and the infraorbital process (it is delimited superiorly by the orbital floor, and inferiorly by the canine fossa) [1,2].

The proximity of the posterior teeth to the floor of the maxillary sinus is an extremely important element related to the anatomical, physiological and pathological characteristics in dentistry. In the most common situations, the maxillary sinus is separated from the roots of the molars and premolars by a spongy bone street that in certain circumstances has a considerable lack of substance that allows the apex of the teeth to be very close or even project onto the floor of the maxillary sinus [2].

It is very important to establish the position of the roots of the posterior maxillary teeth with the floor of the maxillary sinus before any treatment plan is practiced to avoid possible complications. Most risks are associated with the apex of the roots of the posterior teeth in the vicinity of the floor of the maxillary sinus. [3]

The vertical relationship between the root apex and the maxillary sinus floor is classified into four categories:

- type 0: the maxillary sinus floor is located above the apex of the roots
- type 1: the apex of the roots touches the maxillary sinus floor
- type 2: the maxillary sinus floor is located between the roots
- type 3: the apex protrudes into the maxillary sinus floor

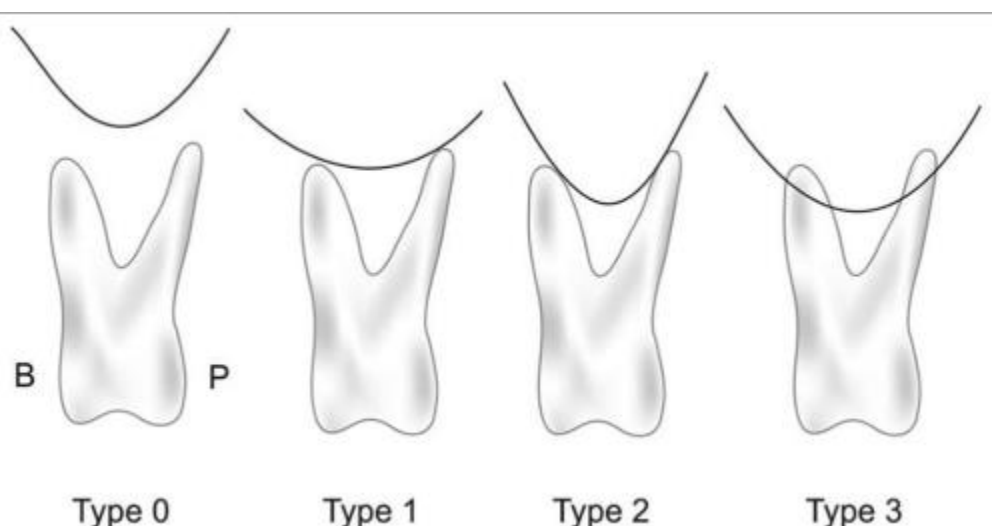


Figure 1. The type of contact of the apex with the floor of the maxillary sinus [3]

During endodontic treatments there is a risk of perforation of the maxillary sinus floor in the relationship between the apex and the floor of the maxillary sinus type 1 and 3. Cases of orbital abscesses resulting from the endodontic treatment of the upper first molar have also been reported in the scientific literature.

The highest frequency in the contact of the apex with the maxillary sinus is given by the mesio-buccal roots of the upper second molar [4]. In another study by Kilic et al. It was

observed that the disto-buccal root most often comes into contact with the floor of the maxillary sinus. [5]

In the study by Ramesh, it was discovered that the mesio-buccal roots of the upper first molars have the greatest proximity to the floor of the maxillary sinus, and in the case of the premolars, the palatal root of the upper second premolar is the closest to the sinus. For molars, the most common type of relationship is type 1, and for premolars type 0 [6].

Aim and objectives

The purpose of this study is to determine the risk of maxillary sinus membrane perforations in dental treatments in relation to the type of position the sinus teeth have with the floor of the maxillary sinus as well as the frequency of a certain type of risk.

MATERIALS AND METHODS

A first stage of the prospective study is the creation of a system for selecting patients who can participate in the study. The patients who needed a surgical intervention and who presented themselves at the Timișoara Oral and Maxillo-Facial Surgery Clinic were included in the study.

The patients who participated in the study expressed their consent in writing, according to Ministry of Health Order 1411 of 12.12.2016, annex no. 1 to the methodological norms - Form for expression of consent of the informed patient.

The selection of patients who were included in the study was based on the examination of radiological investigations.

Patient selection criteria:

1. Adult patients (over 18 years old)
2. Both sexes
3. Indication of extraction of at least one tooth
4. Indication of endodontic treatment
5. Patients hospitalized or treated for a long period to follow the evolution or effects of treatments (endodontic, surgical) in the Oro-Maxillo-Facial Surgery Clinic Timișoara

Patient exclusion criteria:

1. Minor patients (under 18 years)
2. Patients with sinus inflammation, malignant/benign tumors
3. Patients with genetic diseases (Down Syndrome)

The study material is analysed from at least 50 CBCT images from which a sample is created based on the number of selected teeth. The CBCT images accepted in the study are 3D maxillary, extended maxillary, dental, partial, TMJ, frontal sinuses, paranasal sinuses and nasopharynx. The exposure parameter was selected according to the anatomical characteristics of the patient and the therapeutic indications. Patients with radiological evidence of bone pathologies (cysts, tumors), root apex abnormality or chronic periapical lesions associated with maxillary posterior teeth were excluded from the study.

With the aid of Windows photo viewer, on a 27-inch LED screen, 2560x1440 ultra HD resolution, the CBCT images were analysed and scored accordingly. Examiners used local focus image magnifiers and adjusted brightness and contrast as deemed necessary to gain a clearer view of the images. The examiners took the previously analysed images and randomly reanalysed them so that any form of subjectivity through repeated exposure to several different images could be eliminated.

The relationship between the maxillary sinus and the apex of the roots of the maxillary posterior teeth were evaluated and scored according to some previously established criteria. Each tooth was assigned a score according to the following criteria:

- 0, when there is a space of 2 mm or more between the apex of the root and the sinus floor
- 1, when the apex is less than 2 mm from the sinus floor without contact with it
- 2, when the root is projected into the floor of the maxillary sinus but is medial or lateral to it.
- 3, when the root protrudes into the maxillary sinus but does not perforate the membrane of the maxillary sinus
- 4, when the apex protrudes and perforates the floor of the maxillary sinus.

After scoring each image according to the position of the teeth, the type of risk is associated:

- Type 0 position, insignificant risk
- Position type 1, low risk
- Position type 2, increased risk
- Position type 3, serious risk
- Position type 4, absolute risk

At the end of the analysis, division and scoring of the images, statistical calculations will be performed with the help of the Mathstat program in which the frequency of contact, the proximity or lack of contact of the roots of the sinus teeth with the floor of the maxillary sinus will be observed.

This study was carried out on a sample of 150 teeth.

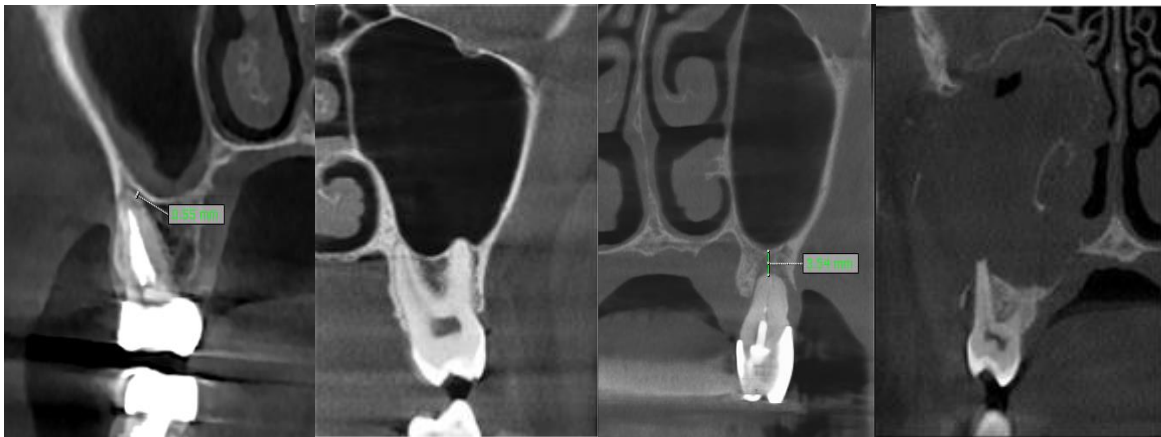


Figure 2. The type of contact of the apex with the floor of the maxillary sinus

RESULTS

In this study, 150 upper and posterior teeth were included from 50 CBCT images taken between January 2021 and December 2023. Of the 150 teeth, 80 were teeth from men and 70 from women. Teeth were divided according to the hemiarch of origin and according to the gender of the patient, and in the last part of the table a general quantification statistic was made without reference to the gender of the participant.

In Table 1 we present the data distribution.

Table 1. Frequency of types of contact with the maxillary sinus depending on the position of the tooth and the gender of the patient

Upper right teeth(%)		Upper left teeth (%)		TOTAL (%)
		Males		
Type 0	17(42.5)	16(40)		33(41.25)
Type 1	14(35)	17(42.5)		31(38.75)
Type 2	5(12.5)	4(10)		9(11.25)
Type 3	3(7.5)	2(5)		5(6.25)
Type 4	1 (2.5)	1(2.5)		2 (2.5)
		Females		
Type 0	16(45.71)	14(40)		30(42.85)
Type 1	12(34.28)	13(37.14)		25(35.71)
Type 2	4(11.42)	5(14.28)		9(12.85)
Type 3	2(5.71)	1(2.85)		3(4.28)
Type 4	1(2.85)	2(5.71)		3(4.28)
		Males+Females		
Type 0	33(44)	30(40)		63(42)
Type 1	26(34.66)	30(40)		56(37.33)
Type 2	9(12)	9(12)		18(12)
Type 3	5(6.66)	3(4)		8(5.33)
Type 4	2(2.66)	3(4)		5(3.33)

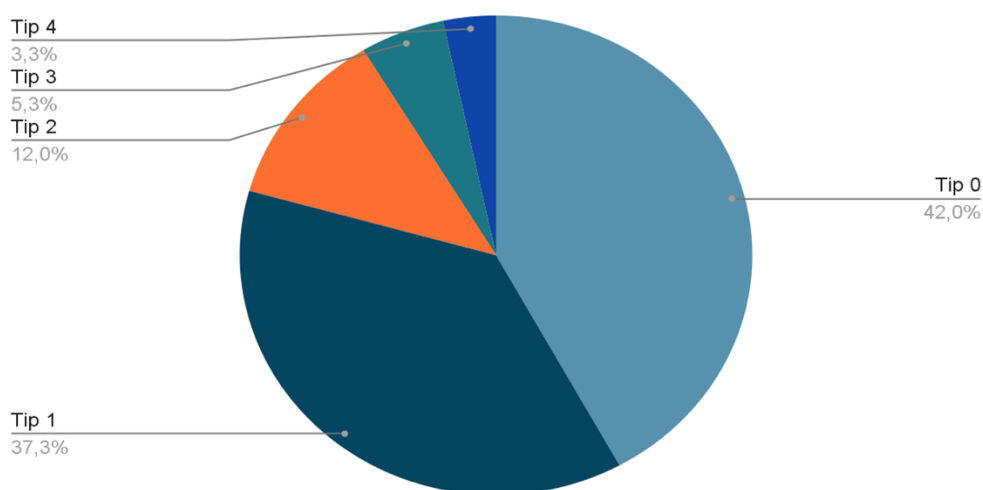


Figure 3. Frequency of types

From Fig. 3 graphic, we can observe that the majority of teeth in proportion of 77.3% have a degree of risk located below or equal to the "low risk" type of risk. This leads us to a favourable prognosis in terms of completing endodontic treatments or tooth extractions without sinus accidents or subsequent complications.

The results of these statistics show very clearly the frequency distribution by type of position of the apex of the sinus teeth with the floor of the maxillary sinus, and this distribution is inversely proportional to the degree of risk. In other words, the higher the degree of risk of the position, the more the frequency with which that risk can be found decreases. This is obviously extremely favourable in dental medical practice within endodontics and dento-alveolar surgery.

DISCUSSIONS

In this study, we wanted to classify sinus teeth according to a very specific risk in accordance with the frequency of that risk and especially the possibility of choosing or adapting a therapeutic protocol according to the risk category in which these teeth are found.

The reason why a very specific classification was wanted is to take into account the general desire in dental medical practice to comply with a protocol very well adapted to the situation and to be easy to understand by future practitioners who have less experience.

This classification has been built in such a way that it is easy to use and easy to understand and for this reason it has been composed in two parts, a numerical one from 0 to 4 to be able to easily express the situation of a case and a descriptive part what can also be used as a case determination, but its main role is to accurately describe the numerical part. The reason why the numbering starts at 0 is to describe an ideal situation, a standard point of reference.

From a comparative perspective with other studies, the same attempt to classify the cases can be observed, namely a numerical and a descriptive part. The form of classification obviously depends from one author to another and can vary from 3 to 4 or even 5 groupings within the classification [7,8].

When we refer to the results of these studies, a repetition with negligible errors of the frequencies of the type of position that the sinus teeth have with the floor of the maxillary sinus can be observed. Studies presenting a more thorough classification classifying position types into four or five groups can easily be reclassified as subgroups that, by correlating data and reducing the classification criteria, can be associated with studies that have more succinct classifications of three groups.

An important aspect that was not taken into account in this study is the age of the patient, something that was well pointed out in other studies in which a strong correlation was demonstrated between advancing age and the reduction of the distance between the sinus floor and the apex of the sinus teeth.

Another aspect that has weight in the frequency variation that was specified in other studies takes into account the biotypes and facial phenotypes of the patients. In the case of the hypodivergent phenotype, a much lower frequency of contact of the sinus teeth with the sinus floor was observed compared to those of the normodivergent and hyperdivergent phenotypic types. In the hyperdivergent one, an increase in the frequency of the approach of the apex to the floor of the sinus was also observed, directly proportional to advancing age. [9]

CONCLUSIONS

This study managed to outline and define an empirical idea, even if it was one that was easy to intuit for experienced clinicians, thus also succeeding in the exact quantification of a frequency that can be reproduced with great accuracy. This is extremely beneficial in developing a deeper perspective on the clinic by future students and especially can be chosen as a criterion in updating work protocols in endodontics and dento-alveolar surgery.

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The healing process of the postextractional alveolar bone of the wisdom tooth



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Abstract

Aim and objectives: Wound healing after tooth extraction is a dynamic process and represents an ongoing challenge in the surgical field. Specialists have focused their attention on healing management, addressing various treatment techniques. In this context, the aim of this study is to research and analyse the factors that influence the healing of the post-extraction alveolar bone of the wisdom tooth.

Material and methods: This observational study analyses the healing process and the complications that appear after the tooth extraction.

Conclusions: The study demonstrated that most patients did not experience post-extractional healing complications. Postoperative management of patients who experienced complications resulted in complete recovery.

Keywords: socket healing; tooth extraction; complications

INTRODUCTION

The healing response has been an essential defence mechanism of the human body since its inception. Injury, in any form, initiates a complex series of closely related and often overlapping biological events, the primary goal of which is to restore tissue integrity [1]

All tissues follow an essentially identical pattern composed of three main phases: inflammatory, proliferative, and remodelling. The type of tissue can influence, however, the specific characteristics of each phase. Delayed healing of certain injuries may be the effect of the surgical procedure itself or the type of wound and may have various clinical manifestations [2].

The surgeon has the ability to identify early signs and symptoms of complications both in the post-extractional alveolar socket and in the peri-alveolar soft tissues. He is also the one who can intervene in a timely manner to ensure the direction of wound healing [2].

In the case of tooth extractions, the post-extractional alveolar bone contains cortical bone, is enveloped by destroyed periodontal ligaments and a band of fixed mucosa. The blood clot that forms after extraction has the role of isolating the bone from the bacterial environment in the oral cavity [3].

The inflammatory phase (postoperative days 3-5) is characterized by vasoconstriction of the injured blood vessels by the aggregation of circulating platelets that adhere to each other to form a fibrin clot. Thus, the bleeding is stopped, and the blood clot is formed. After haemostasis is achieved, vascular permeability is increased, allowing blood plasma and other cellular mediators of healing to pass through the capillary walls. Even now, on the entire surface of the bone cortex, osteoclasts begin to accumulate. Symptoms such as swelling, and pain may occur [4].

The proliferative phase (14 days postoperatively) represents the moment of formation of the granulation tissue containing inflammatory cells, and the body begins the process of collagen secretion. The tissue changes colour and the scar forms. The alveolar bone is covered with osteoid tissue, which prevents the development of the post-extractional bone. At the end of the third and fourth week, the epithelialization process is complete, and the deposition of new trabecular tissue on the bottom of the socket begins. [4]

Complete absorption of the edge of the cortical bone occurs after 4-6 weeks from the moment the tooth was removed. The bone fulfils the socket, and the epithelium covers the upper part, sometimes even reaching the level of the adjacent fixed mucosa. One year after the tooth extraction, only a scarred tissue can be observed at the level of the alveolar ridge.

The factors that can influence the post-extractional healing are represented by local and general factors: haemorrhage, edema, hematoma, dehiscence, alveolitis, smoking, malnutrition, diabetes, radiotherapy etc.

Aim and objectives

The purpose of this study is to research and analyse the factors that influence the healing of the post-extraction alveolar bone of the wisdom tooth.

MATERIAL AND METHODS

The retrospective study includes patients that required extractions of teeth 1.8, 2.8, 3.8 or 4.8. The patients were informed that these cases will be included in a medical research and their written consent was requested, according to Ministry of Health Order 1411 of 12.12.2016, annex no. 1 to the methodological norms - Form for expression of the consent of the informed patient.

All patients who underwent extraction of wisdom teeth under local anesthesia were included in this study. During the clinical examination, the patients' oral hygiene, the position of the wisdom teeth, but also the possible conditions and diseases that contraindicate tooth extraction (malignant tumours, cardiovascular, hepatic, endocrine diseases, etc.) were evaluated.

The inclusion criteria are:

- patients over 18 years;
- female and male patients;
- extraction of 1.8, 2.8, 3.8 or 4.8;

The exclusion criteria are:

- minor patients (under 18);
- patients who, following the clinical examination, were found to have acute infectious processes, malignant tumors;
- patients diagnosed with certain associated conditions that contraindicate dental extraction (malignant tumors, cardiovascular, hepatic, endocrine diseases, etc.)

The patients were examined clinical and radiological. All information obtained was recorded in the patient record.

The degree of difficulty of the procedure is directly correlated with the accessibility of the tooth to be extracted. Accessibility is determined by the radiological examination regarding the position of the teeth in the alveolar, the morphology of the roots and the stage of evolution are observed; as well as neighbouring structures: the bone density of the proximal environment as well as the proximity of adjacent teeth.

RESULTS

In this study, 80 patients were included, during January 2023 – June 2023, 38 males (47.5%) and 42 females (52.5%). The age of the included patients was as follows: 55 patients were between 18 and 30 years (68.75%), 20 patients between 31 and 50 years (25%), respectively 5 patients between 50 and 70 years (6.25%).

In order to pass the selection stage and be included in the study, patients had to require surgery on their wisdom teeth. Thus, for 22 of the cases the extraction of upper right molar 1.8 was necessary (27.5%), for 18 of them the intervention took place at the level of 2.8 (22.5%), for 15 cases the extraction was done on 3.8 (18.75%), and for 25 it was performed at the level of 4.8 (31.25%).

Following the clinical examination, the patients included in the study presented different indications for extraction. Thus, 42 cases were diagnosed with acute apical periodontitis (52.5%), 28 cases presented with carious lesion complicated with fracture of the crown (35%), in 4 cases grade 3 tooth mobility was discovered (5%), and for 6 of the cases the wisdom teeth has orthodontic indication for extraction (7.5%).

Different extraction techniques were required during the surgeries, depending on the patients' needs. The simple forceps extraction technique was used for 31 of the cases (38.75%), elevator extraction through distal dislocation for 28 cases (35%), extraction with root separation for 6 cases (7.5%) and extraction with buccal alveolotomy for 15 cases (18.75%). Suturing the post-extractional socket is not always necessary for all surgeries. In this study, it was used in only 20 of the 80 cases (25%), 60 of the patients (75%) not needing this procedure.

Among the selected patients, for 5 cases postoperative healing was complicated with post-extractional haemorrhage (6.25%), 4 cases presented with post-extractional alveolitis (5%) and 7 cases suffered delayed healing (evaluated at 2 weeks) (8.75%). For the remaining 64 cases, there were no complications in post-extractional healing (80%).

Among the 5 patients who presented with a complicated healing with post-extractional haemorrhage, 3 patients indicated an early haemorrhage occurring a few hours after the molar extraction (60%), while in 2 of them the haemorrhage started late, after a few days from the moment of the intervention (40%).

Post-extractional alveolitis was present in 4 cases, with only one patient indicating its development at the maxillary bone (25%) and 3 patients (75%) at the mandibular bone.

During the control after the surgical intervention, the probing height measured postoperatively on the distal face of the neighbouring anterior tooth with the extracted wisdom molar was also checked. The results showed that it decreased in height by 1-2 mm in 49 cases (61.25%), remained unchanged in 28 cases (35%) and increased in only 3 cases (3.75%) by 1 mm in 2 cases, respectively by 2 mm for 1 case.

DISCUSSIONS

Surgical extractions are defined by the American Dental Association as the surgical removal of an erupted tooth that requires elevation of a mucoperiosteal flap and removal of bone and/or a section of the tooth. [5] Tooth extraction is one of the most common procedures in dentistry in general and in oral surgery in particular.

Surgical extraction may be necessary in cases of impaction of the tooth into bone and/or soft tissue, dental caries below the bone level, insufficient remaining clinical crown, fracture of the tooth or root, the presence of periapical lesions or disease, and other conditions that can only be diagnosed by the dentist. [5]

Throughout time, wisdom teeth have represented a continuous challenge in dentistry due to the diversity of their development and their interaction with the rest of the dentition. Wound healing after tooth extraction is a dynamic process and is an area of major interest in the surgical field. The factors that influence the extraction of wisdom teeth have been the subject of several specialized studies, and researchers have focused their attention on healing management, approaching various treatment techniques. [6]

In the case of wisdom teeth, surgical extraction involves ostectomy and surgical soft tissue implication, resulting in pain and edema as part of the normal inflammatory response. Potential complications include bleeding, infection, nerve damage or jaw fracture. [6]

Risks are influenced by background factors such as the patient's age, gender, and health status, in addition to clinical factors such as bone density, tooth position, degree of impaction, and surgeon experience.

Given that wisdom tooth extractions are prone to more complicated healing, there are numerous studies in the specialized literature that have researched this aspect over time. Although the study carried out in this study did not have such a large number of patients included, the results obtained are similar. For example, a study conducted by Wasiu Lanre Adeyemo et al evaluated the clinical pattern of post-extractional wound healing to identify the types, incidence and pattern of healing complications following tooth extraction. Two hundred eighty-two patients (282) from 318 extractions were evaluated for socket healing. The results of the study showed that most of the post-extractional sockets healed without problems. A few cases of alveolar osteitis were recorded (8.6%), and in a few patients, post-extractional alveolar healing was also complicated by alveolitis (1.2%) [7].

CONCLUSIONS

This study indicates that when the surgical intervention is performed by specialized doctors, patients seriously follow the post-extractional care recommendations and proper oral

hygiene, the healing process proceeds without the appearance of adverse effects and certain severe complications.

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Behçet disease in dentistry



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Abstract

The present study aimed to explore the clinical manifestations of Behçet's disease in six patients using the criteria of the International Study Group for Behçet's Disease. The primary objectives were to determine the prevalence of oral aphthosis and assess potential ocular and vascular complications. For a retrospective analysis of six diagnosed Behçet's disease patients, a comprehensive clinical examination, Pathergy tests, and various biological assessments were performed. Additionally, eye examinations, slit lamp studies, and radiological investigations were carried out to evaluate ocular and vascular involvement. The results of the study reveal that oral aphthosis was present in all patients. Some individuals exhibited ocular and vascular involvement, providing insights into the diverse manifestations of the disease. The conclusions outline the importance of the early diagnosis and comprehensive assessments, that are crucial in identifying potential organ involvement in Behçet's disease, contributing to better management and improved patient outcomes.

Keywords: Behçet's disease, oral aphthosis, ocular involvement, vascular complications, comprehensive assessment

INTRODUCTION

The systemic autoimmune and inflammatory conditions often determine oral manifestations, starting from their early onset, outlining the importance of a proper oral examination as an early diagnosis measure. Behçet's disease is a rare, chronic, multi systemic disorder, progressing in episodes [1]. It has been known as to whether it is an autoimmune disease, an auto-inflammatory disease or both [2,3]. This disease is characterized by the occurrence of mucocutaneous lesions, that involve the dermis, genitalia, ocular and oral mucosa [4,5]. In severe cases, the evolution can determine severe impairment of the patient, affecting the nervous system, and involving the vascular and digestive organs [5,6,7].

The etiology of Behçet's disease is not completely known, being suggested the connection between genetic and environmental triggering factors [5]. Immunological alterations have been reported and approximately 50% of the patients exhibited an increase in the circulating immunocomplex while the disease is exacerbated [9, 10, 11]. Being described as a disease with a chronic evolution, the intensity might decrease with time, but still the complication can be life-threatening, such as myocardial infarct, thrombosis, intestine perforation and blindness [12, 13, 14]. Studies have discussed the incidence of different manifestations, among which the oral symptomatology is encountered in 95-100% patients [7,8].

In the present research, we will discuss about the current knowledge of Behçet's disease [8,9] and the management in the dental office of patients with Behçet's disease. The most important manifestation is a mouth-sex-eye triad which is characterised by oral ulcerations, genital recurrences and eye damage [15, 16, 17]. And all these types of lesions are the main clinical manifestations of Behçet's disease, but they are not always present. We can also note, among other things, skin, joint, neurological and / or vascular damage [18, 19]. Behçet's disease affects women and men [18]. To improve the prognosis, the quick detection of the disease is important when the first symptoms appear, and it is important to use the most appropriate treatment.

Focusing on the management in the dental office of patients with Behçet's disease, it is important to acknowledge the possible symptoms, the complications that a diagnosed patient could have and the potential risks related to certain interventions. This management needs the knowledge of patient treatments in order to avoid the drug interactions and to not conform to contraindicated drugs and acts [20, 21].

With a patient with recurrent mouth ulceration, the dentist must make a positive diagnosis and differential diagnosis of Behçet's disease [9, 10]. The dentist must also be able to prescribe medication for the treatment of mouth ulcerations.

Aim and objectives

The aim of this retrospective study is to identify the manifestations of Behçet's disease and assess its epidemiological aspects. By quantifying the existent symptomatology, the oral manifestations were taken into consideration as a primary sign of this disease. The objectives of this study are to determine the influence of age and gender on the prevalence and severity of Behçet's disease, to discuss the clinical criteria for the positive diagnosis of Behçet's disease and its challenges, to investigate the different clinical manifestations of Behçet's disease, including oral and genital ulcers, eye damage, skin lesions, articular damage, vascular damage, neurological damage, and cardiovascular damage, to assess the prognosis and evolution of Behçet's disease, including potential complications and mortality rates, to evaluate the current treatment options for Behçet's disease, considering symptomatic relief and disease control, to provide insights into the diagnosis of Behçet's disease in dental

practice, including the differentiation of oral lesions and to highlight the importance of complementary examinations in determining the underlying pathology of oral lesions.

MATERIAL AND METHODS

In this study, we retrospectively analysed six patients who exhibited various manifestations of Behçet's disease. The diagnosis of Behçet's disease was confirmed based on the criteria set forth by the International Study Group for Behçet's Disease, which specifically required the presence of oral aphthosis.

Each of the six patients underwent a comprehensive clinical examination, which included conducting the Pathergy test, a non-specific hypersensitivity test used to assess skin reactivity. Additionally, a thorough biological assessment was performed, encompassing several parameters such as the sedimentation rate, complete blood count, C-reactive protein levels, prothrombin level, and activated partial thromboplastin time. These tests were crucial in evaluating the patients' overall health status and identifying potential underlying systemic inflammation.

Furthermore, the patients' ocular health was meticulously evaluated through eye examinations, including fundoscopy, which involves examining the back of the eye to detect any abnormalities. A slit lamp study, which is a specialised microscope, was also utilised to obtain a detailed view of the anterior eye structures, aiding in the identification of any ocular complications associated with Behçet's disease.

As with any retrospective study, there may be limitations, such as incomplete data or potential biases. However, by adhering to the International Study Group's established criteria, we ensured a standardised and reliable approach to diagnosis.

The comprehensive nature of the clinical assessments in this study provides valuable insights into the diverse manifestations and potential organ involvement in Behçet's disease. These findings can contribute to the existing knowledge base on the disease, potentially leading to improved diagnostic approaches and treatment strategies in the future.

RESULTS

Six patients with manifestations of Behçet's disease were included in this retrospective study. The diagnosis was confirmed based on the criteria established by the International Study Group on Behçet's Disease, which required the presence of oral aphthosis. The average age of the diagnosis of Behçet disease was 41 years old. In this study were included people between the ages of 18 and 50, the first sign being given by a 28-year-old person (*Table 1*).

Table 1. Characteristics of the included subjects

Patient	Age (years)	Gender	Duration of Disease (months)
P1	32	Female	18
P2	45	Male	24
P3	28	Female	12
P4	39	Male	36
P5	50	Female	48
P6	34	Male	15

The study participants consisted of three females and three males, with ages ranging from 28 to 50 years. The duration of the disease varied between 12 to 48 months. Besides the demographic details of the patients, the clinical examination provided valuable information regarding the progress of the disease. The oral mucosa, the performance of the Pathergy test and the presence of cutaneous and ocular manifestations was assessed in order to provide a complete clinical description (Table 2).

Table 2. Clinical Characteristics of Behçet's Disease in the included group

Patient	Oral Aphthosis	Pathergy Test	Eye Involvement	Other Manifestations
P1	Yes	Positive	Uveitis	Arthritis, Cutaneous Lesions
P2	Yes	Negative	Conjunctivitis	Arthritis, Genital Ulcers
P3	Yes	Positive	Retinal Vasculitis	Arthritis, Oral Ulcers
P4	Yes	Positive	Uveitis, Optic Neuritis	Arthritis, Gastrointestinal Symptoms
P5	Yes	Negative	Uveitis, Retinal Vasculitis	Arthritis, Neurological Symptoms
P6	Yes	Positive	Conjunctivitis, Uveitis	Arthritis, Vascular Involvement

All six patients met the criteria for oral aphthosis, which was the primary diagnostic requirement. The Pathergy test was positive in four patients and negative in two. Eye involvement was observed in all patients, with uveitis being the most common manifestation. Additionally, other manifestations, such as arthritis, cutaneous lesions, genital ulcers, and gastrointestinal symptoms, were observed in various patients.

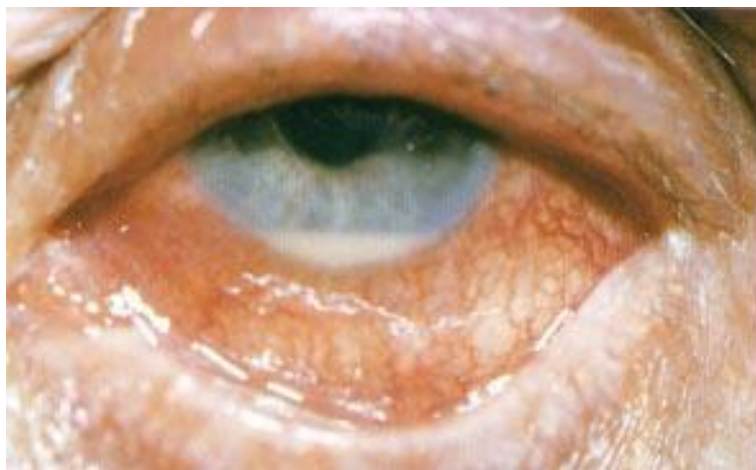


Figure 1. Ophthalmic symptomatology in Behçet's disease



Figure 2. Oral aphthosis in Behcet's disease

The laboratory investigations revealed elevated levels of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in all patients, indicating active inflammation (Figure 1). However, the prothrombin level and activated partial thromboplastin time were within normal ranges.

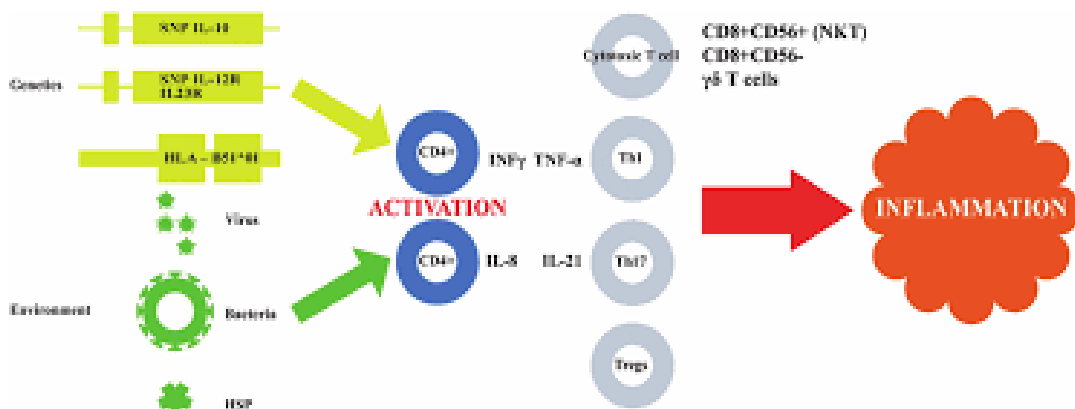


Figure 3. Inflammation process

The radiological examinations, including chest and sacroiliac radiography, revealed no abnormalities in four patients. However, two patients showed signs of vascular involvement, and further examinations with ultrasound Doppler and angio-CT confirmed the presence of vasculitis.

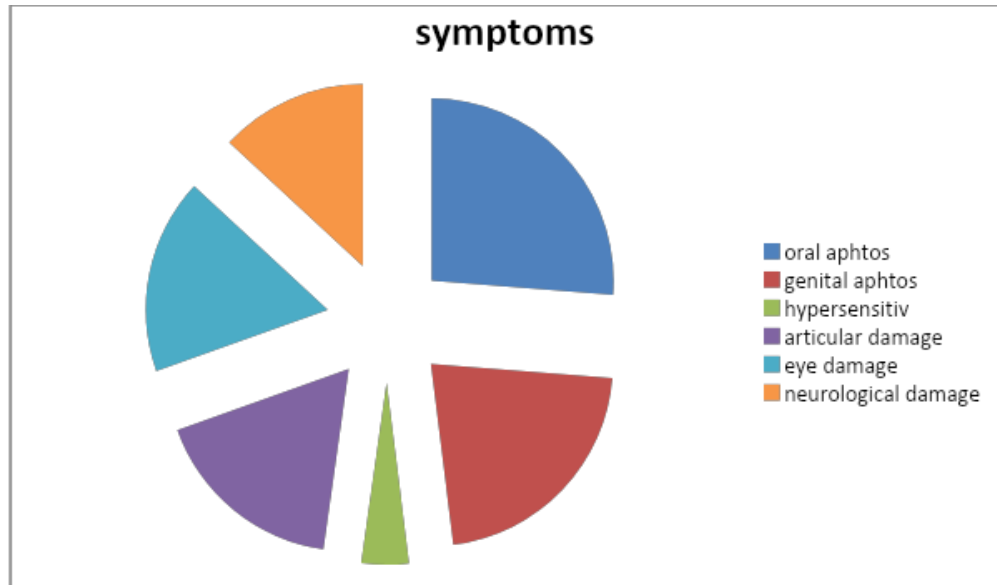


Figure 4. Distribution of the symptoms among the included group

In conclusion, this retrospective study of 6 patients with manifestations of Behçet's disease demonstrated the importance of oral aphthosis as a diagnostic criterion (Figure 4). Eye involvement, particularly uveitis, was a common feature, and some patients presented with systemic manifestations, including arthritis and vasculitis.

DISCUSSIONS

In the discussions section, the study sheds light on important aspects of Behçet's disease without repeating detailed data from previous sections. The historical significance of the disease is emphasised, with records of its existence dating back to ancient times, as described by Hippocrates. This historical background provides valuable insights into the long-standing impact and prevalence of the disease in different regions.

The epidemiological data highlights variations in Behçet's disease prevalence across different geographic areas, suggesting the influence of environmental factors. The study also reveals how age and sex can affect disease manifestation, with men showing more complications than women in certain regions.

Regarding the disease's cause, the study emphasises the complex interplay of genetic and environmental factors, leading to abnormal inflammatory responses and immune dysfunctions. These findings are consistent with previous research, supporting the notion of multiple factors contributing to the disease's onset.

The study emphasises the challenges in definitively diagnosing Behçet's disease due to the lack of specific biological tests. Clinical evaluation remains crucial, with recurrent oral lesions being important indicators. Dentists play a vital role in recognizing oral aphthosis as an initial symptom, highlighting the need for vigilance in identifying potential cases. Various clinical manifestations are discussed, including oral and genital aphthosis, eye damage, skin lesions, and damage to the articular, vascular, and nervous systems. Eye involvement is particularly common and early detection is crucial to prevent severe complications.

Treatment options are outlined, emphasising tailored therapeutic approaches based on disease severity and organ involvement. Corticosteroids are fundamental, while other immunosuppressants are considered for severe cases. The study acknowledges the limitations and potential adverse effects of certain treatments.

The study underscores the complexity of Behçet's disease prognosis, with early care playing a pivotal role in patient outcomes. Prompt treatment is essential to avoid irreversible damage and improve the quality of life for affected individuals.

The special section on Behçet disease in dentistry highlights the challenges faced by dentists in diagnosing the disease. Recognition of recurrent oral lesions and differentiation from other oral pathologies is crucial, emphasising the need for collaboration between dental and medical professionals for early diagnosis and management.

Overall, the study contributes valuable information to the understanding of Behçet's disease, offering insights into its historical context, epidemiology, etiopathogenesis, clinical manifestations, treatment approaches, and prognosis. These findings have implications for future research, aiming to explore more effective diagnostic methods, treatment modalities, and management strategies to improve patient outcomes.

Recommendations of EULAR (European League Against Rheumatism):

1. All patients with involvement of the posterior segment of the eye should be placed under general corticosteroid therapy and azathioprine

2. If the patient has a loss of visual acuity greater than 2/10 and / or retinal damage (vacuitis or maculopathy), cyclosporine A or infliximab should be combined with corticosteroids and azathioprine (the alternative being interferon- α)

3. There is no evidence on the treatment of large vessel involvement. For thrombosis venous immunosuppressants (azathioprine, cyclophosphamide, cyclosporine); for pulmonary or arterial aneurysms corticosteroids are recommended and cyclophosphamide

4. There is also no solid evidence for the use of anticoagulants, antiaggregants and fibrinolytics in thrombosis and venous or arterial lesions

5. There is no evidence for the treatment of intestinal manifestations of MB. Can be used sulfasalazine, corticosteroids, azathioprine, anti-TNF, and thalidomide before surgery, except emergency

6. Colchicines allow arthritis control in the majority of cases.

7. There is no controlled data that can guide the treatment of neuro-Behçet. For corticosteroids, interferon- α , cyclophosphamide, azathioprine, methotrexate and anti-TNFs. For cerebral venous thrombosis, the corticosteroids are recommended.

8. Cyclosporin A is contraindicated (unless expressly indicated for uveitis) in patients having an attack of the central nervous system.

9. The decision to treat mucocutaneous manifestations depends on their perceived severity. patient and doctor. In the front line for aphthosis and acne form lesions, it is recommended to use topical treatments. Colchicines is the treatment of erythema knotty. Azathioprine, interferon and anti-TNF drugs should be discussed in severe cases.

CONCLUSIONS

Behçet's disease remains a challenging and mysterious condition, with its definitive cause still unknown. Experts believe that a combination of genetic and environmental factors contributes to its development. Notably, both men and women can be affected by this disease, but the way it presents may differ between genders, leading to more complications in men [3, 4, 5].

Despite extensive research, there is no specific biological test for a certain positive diagnosis of Behçet's disease. Hence, clinical evaluation plays a vital role, focusing on identifying oral and genital aphthosis and eye damage as the main manifestations [6, 7]. Dentists play a critical role in early detection as oral aphthosis often serves as the initial symptom [9, 10]. Therefore, dentists need to be vigilant and inquire about other potential indicators of Behçet's disease. In some instances, dentists may also find themselves treating

patients with this condition, underscoring the importance of understanding effective management strategies to prevent complications.

The prognosis of Behçet's disease relies heavily on early detection and regular treatment. Patients must be well-informed about their condition and treatment options to effectively manage the disease and improve long-term outcomes.

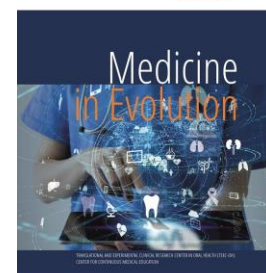
In conclusion, Behçet's disease poses several challenges due to its uncertain cause and the lack of a definitive diagnostic test. Dentists' involvement is crucial in recognizing oral aphthosis and initiating appropriate actions. Educating patients about their condition and treatment is essential for better prognosis and overall well-being. Ongoing research is necessary to unravel the complexities of this disease and develop more targeted therapies.

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The use of artificial intelligence in the diagnosis and prognosis of oral cancer



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Abstract

Artificial intelligence has gained the interest of the medical research field, aiming to introduce it in the diagnosis approaches, the management and monitorization of different diseases. In dentistry, its applicability for multiple pathologies, among which the detection and diagnosis of oral cancer, has proven important advantages. This present review aims to describes the applicability of artificial intelligence in oral pathology, focusing on oral cancer, outlining the advantages, limitations and future perspective for this technology. By introducing this technology in the everyday dentistry practice, for dentists and specialists can undoubtedly improve the quality of care.

Keywords: Oral cancer, artificial intelligence, early diagnosis, oral pathology

INTRODUCTION

Diagnosing oral cancer is of high importance in dentistry. It has however sometimes been neglected due to inconsistency in the competency of diagnosis among clinicians. The motivation for investigating this topic is to bring light on new technological advances that can be used to help both newly qualified and experienced clinicians to provide the best care possible for their patients.

Artificial intelligence started in the 1940-1960s [1] but since then it has become the defining technology of the modern era, improving in leaps and bounds since its beginning. Today, we use AI in our daily lives, most often without notion. AI has encapsulated the world and has made significant changes in the medical field. In dentistry AI can be used in all parts of the patient's care, including diagnosis, prognosis and decision making. AI has been used across the board in dentistry; examples including radiology, orthodontics, periodontics, endodontics and oral pathology. It is mainly used in oral pathology for detecting oral lesions. It can also help to differentiate between lesions that appear radiologically similar but clinically different. The time taken to make this diagnosis is considerably lower than without the use of AI. One study found the difference in time to be 23.1 mins for clinicians diagnosis in comparison to 38 seconds for an AI aided diagnosis [2]. Oral cancer is a malignant disease which leads to many fatalities worldwide. It is unfortunately usually detected in the later stages of the disease and therefore increases the treatment time, cost and morbidity rate. Early detection of oral cancer can significantly improve the survival rate by 75%-90% [3]. Already established screening methods can be used alongside AI to provide a proficient diagnosis.

Large amounts of research has been conducted around the application of AI in oral pathology. AI can be utilized in the diagnosis and prognosis of oral cancer extends beyond screening and initial detection. It also finds application in histological evaluation, where it can assist pathologists in analyzing tissue samples. AI algorithms can process vast amounts of histological data, aiding in the classification and grading of oral lesions. This capability not only enhances the accuracy and speed of diagnosis but also supports treatment planning and decision-making processes [4].

AI plays a dynamic role in dentistry, particularly in the diagnosis and prognosis of oral cancer. Its implementation in various stages, including screening, diagnosis, and histological evaluation, offers substantial benefits. By augmenting the expertise of clinicians and enabling the analysis of diverse imaging modalities, AI improves the accuracy and reliability of diagnoses. As technology continues to advance, the integration of AI in dentistry holds great promise for the early detection and management of oral lesions, ultimately improving patient outcomes [4].

This literature review will set out to review the applications of AI in the diagnosis and prognosis of oral cancer. It will establish criteria to include and exclude articles and proceed to compare results. All articles reviewed will be original experiments.

Aim and objectives

The aim of this review is to assess the existing literature on the use of artificial intelligence in the diagnosis and prognosis of oral cancer, within the last 10 years. Specifically, the objective is to evaluate the effectiveness, limitations, and potential challenges associated with AI-based approaches and provide a comprehensive overview of the progress and implications of AI in oral cancer management.

The scope of this literature review encompasses a broad range of studies that use different modalities of oral cancer screening in conjunction with AI. Different AI methods, such as machine learning, deep learning, as well as their integration with imaging modalities,

molecular data, and clinical parameters are all reviewed. The validity and reliability, advantages and disadvantages are all to be reviewed.

MATERIAL AND METHODS

This literature review seeks to evaluate current research on the use of Artificial intelligence in diagnosis and prognosis of oral cancer within the last 10 years. Search engines were used to find the articles. The search engines used were Google scholar, PubMed, Science Direct, SCOPUS and Cochrane library.

In the advanced search, the words Artificial intelligence (OR Machine learning OR deep learning) AND diagnosis AND prognosis AND oral cancer OR premalignant lesions were all used in the search criteria these key words 101 articles were yielded. Both in vivo and in vitro studies were used in the review. These articles included a vast array of articles that were not specific to the line of study. Many of these articles were also not accessible as they were not free.

An Inclusion and exclusion criteria was then applied in order to precisely find articles that were related to the line of study. The inclusion criteria was limited to articles that have been published in the last 10 years. These articles all had to be written in the English language and had to be full texts. It was also necessary for the articles to have quantifiable results so that it was possible to compare them later in the discussion part of the review. The articles also had to include the key words and could not deviate from this subject. All articles that were reviewed in this literature review were original research.

The exclusion criteria consisted of studies that were not written in the English language, review articles and articles that did not present original data. The exclusion criteria of free full texts markedly reduced the number of articles that were able to be used in this study.

The search was carried out using advanced search on the search engines and the criteria was also checked manually. Preceding this a total of 15 articles were found that fit all the criteria.

RESULTS

The results of different papers were presented in different manners, for example some studies evaluated the results of a network in terms of specificity or sensitivity while others used precision or accuracy or recall. The studies based on the implications of artificial intelligence rely on the focus on deep learning technology and machine learning.

1.1. Deep learning

1.1.1. *Photographic imagery*

Uthoff et al. [5] developed a unique dual-modality, dual-view point of care oral cancer screening device. This device utilizes white light and autofluorescence imaging used on a smartphone platform, allowing for early detection of pre-cancerous and cancerous lesions in the oral cavity. In a study involving 170 image pairs, the device's performance was evaluated against the gold-standard diagnosis of an on-site specialist. The remote specialist, along with a convolutional neural network (CNN), successfully classified the images as either 'suspicious' or 'not suspicious'. Data was taken from 99 patients for the CNN analysis and remote diagnosis. The AUC of the CNN was found to be 0.908. The sensitivity of the convolution neural network was found to be 0.85 and the specificity was found to be 0.8875.

A study conducted by Welikala et al. [6] produced an app named Memosa to record pictures of oral lesions from a smart phone. MeMosa annotate is a separate browser that is made by the company to create a large data set of well annotated lesions which can be then

used by AI algorithm to detect early or potentially malignant lesions. 2155 oral cavity images from 1085 individuals were used in this study. 1744 images were used as the training set and 204 were used as the testing set. The pictures consisted of lesions and pictures without lesions. These images were taken from 3 different source: MeMosa app, images annotated by clinicians, images from the web. Memosa annotate was then also used to add more data to the database and these lesions were separately analysed by 3-7 clinicians. They were of different areas of the oral cavity. Welikala investigated the convolutional neural networks' ability to classify images and identify objects. The precision, recall, and F1 score for classifying images with lesions were 84.77%, 89.51%, and 87.07%, respectively. The object identification of lesions achieved a precision of 46.61%, and a sensitivity of 37.16% [6].

Jubair et al. [7] analysed a dataset of 716 clinical images depicting different tongue lesions. The images were categorized into "suspicious" lesions (236 images) and benign lesions (480 images) and were collected over a four-year period from 543 patients using various cameras and smartphones. To ensure data integrity, the dataset was randomly divided into a training set (79%), a validation set (7%), and a test set (14%), with redundancy checks performed to avoid overlap between the image sets. Jubair et al developed a lightweight convolutional neural network which used a pretrained EfficientNet-B0 as the learning model. Jubair et al found the mean specificity to be 84.5%, with a sensitivity of 86.7% and an AUC for of 0.928 for the EfficientNet-B0 model.

Sunny et al. [8] investigated the usage of a smart tele-cytology point-of-care platform for oral cancer screening. A total of 11981 images were used in the training, development and validation of the model, the model used was an existing ANN named Inception V3. The model produced a sensitivity of 89%, a specificity of 100% and an overall accuracy of 90% (Table1).

1.1.2. Radiologic imaging

Another study conducted by Kirubabai et al. [9] utilizes a deep learning algorithm to classify oral MRI images as either normal or abnormal. The study used a Convolutional neural network classification method and used this to further diagnose cancerous regions in images as either Mild or severe. The study used 160 cancer affected oral images. Kirubabai et al. analyzed the performance of the CNN with and without data augmentation. The study found that using the CNN with data augmentation yielded a selectivity of 98.6%, a sensitivity of 99.1% and an accuracy of 99.7%. The CNN without data augmentation yielded a selectivity of 93.7%, a specificity of 94.1% and an accuracy of 95.6%.

Ariji et al. [10] conducted a study to evaluate the effectiveness of deep learning classification of images for the diagnosis of lymph node cancer. CT images were used of 127 images that were already proven histologically lymph node metastasis and 314 that did not, and 45 with OSCC. The deep learning methods were then compared with experienced radiologist opinions. Deep learning AUC was 78.2% sensitivity 75.4% and specificity 81.0% but these results did not differ considerably from the results of the radiologists (Table 1).

1.1.3. Numerical data

Adeoyo et al. [11] investigated the applications of deep learning to predict the malignant transformation free survival of oral potentially malignant disorders. Data was collected from 716 patients who underwent biopsy for oral leukoplakia, oral lichen planus, or oral lichenoid lesions. 573 patients were used for the training of the algorithm and 143 unseen cases were used as the test set; the patients that were used for the test set were randomly selected in order to remove any possible bias from the results. Adeoye et al investigated 5 different algorithms, cox-ph, cox-time, DeepHit, DeepSurv, and RSF. The C-index and the IBS of the different algorithms were found to be 0.83 and 0.03 for Cox-ph, 0.86 and 0.06 for Cox-time, 0.86 and 0.08 for DeepHit, 0.95 and 0.04 for DeepSurv, and finally 0.85 and 0.03 for RSF.

Adeyo et al found that the DeepSurv algorithm produced the best discriminative performance, while the RSF algorithm produced better calibrated probability estimates.

Kim et al. [12] performed a study on the effectiveness of deep learning techniques to calculate survival predictions of patients who suffered from oral cancer. A total of 255 patients' data was used for the study, with 141 patients being in either stages 1,2 or 3 of cancer, and 114 patients having stage 4 cancer. The data set was split 70/30 into the training set and the testing set. Three different deep learning algorithms were used, DeepSurv, Cox proportional hazard (CPH) and Random survival forest. The Random survival forest yielded a C-index of 0.764, the DeepSurv algorithm yielded a C-index of 0.781, and the CPH produced a C-index of 0.694 for the training sets. The decision tree was much better at predicting survival rates with AUC of 0.840, sensitivity of 0.917, and specificity of 0.576.

Alabi et al. [13] investigated the effects of machine learning applications for the prediction of locoregional recurrences in early oral tongue cancer using a web based prognostic tool. A total of 311 patients' numerical data was used for the study, 165 of those patients being male and 146 being female, with the data set being split into a 70/15/15 ratio of the training set, validation set, and the testing set respectively. An ANN was used as the deep learning algorithm in this study. The ANN yielded an overall accuracy of 92.7%, a selectivity of 71.2%, a specificity of 98.9% and a C-index value of 97.3% (Table 1).

1.2. Machine learning

1.2.1. Photographic imagery

The study conducted by Duran-Sierra et al. [14] aimed to develop a method to classify oral lesions as either precancer/cancer or healthy using a special type of imaging called multiparametric autofluorescence lifetime imaging (maFLIM).

To classify the images, the researchers used four different models: Linear Discriminant Analysis (LDA), Quadratic Discriminant Analysis (QDA), linear Support Vector Machine (SVM), and Logistic Regression. They determined an optimal score threshold through analysis, and if an image's score was above the threshold, it was classified as precancer/cancer; otherwise, it was considered healthy. They also evaluated the effectiveness of combining different types of features (spectral and time-resolved) for classification.

The SVM model performed best with spectral-only features, while the QDA model performed better with time-resolved-only features. So, they created an ensemble classifier by combining the top three spectral features from the SVM model and the top three time-resolved features from the QDA model. They evaluated the performance of each classifier using ROC-AUC analysis. The ensemble classifier achieved the highest ROC-AUC value of 0.81, indicating good performance. The SVM-QDA classification model yielded the highest cross-validation sensitivity (94%), specificity (74%), and F1-score (0.85).

Rahman et al. [15] used Machine learning algorithms with H&E-stained biopsy slides which were collected from diagnostic centres. Microscopic digital imaging was performed, and the images were graded and labelled. Data pre-processing techniques were applied to eliminate staining differences in the captured images. These images were then tested with five classifiers (SVM, KNN, decision tree, logistic regression, and linear discriminant) using five-fold cross-validation. The decision tree classifier performed the best with an accuracy of 99.78%. The decision tree classifier was selected as the most suitable classifier. AUC was 0.99. The accuracy of classifiers generally increased with the size of the training data. sensitivity was found to be 99.7% and specificity 100%

Alhazmi et al. [16] investigated the effects of artificial intelligence and machine learning and its uses in the prediction of oral cancer. The studied developed an artificial neural network (ANN) that aided in predicting the individual's risk at developing oral cancer. The model consisted of one hidden later in addition to the input and the output later. The attributes that were included composed of 29 variables that were given to everyone in

order to develop the final model. 138 cases were selected, with 73 being eligible to the criteria. 22 of the 73 cases were benign or pre-malignant cases, while the remaining 51 cases were malignant. 54 cases were used as the training set and 19 as the testing set. The sex of the patients was split with 36 being male and 37 being female with the mean age of the patients being 55 years. Alhazmi et al found that the average sensitivity of the artificial neural network was 85.71% whereas the specificity of the model was found to be 60%. The accuracy of the network for the prediction of oral cancer was 78.95% (Table 1).

1.2.2. Optical coherence tomography (OCT)

James et al. [17] focused on the automated interpretation of Optical Coherence Tomography (OCT) images for oral cancer detection. Two approaches were implemented: Artificial Neural Network-Support Vector Machine (ANN-SVM) model and a simple score algorithm. Patients undertook incision/punch biopsy from the same lesion site where the OCT images were taken. The majority of patients were male, the median age of the patients being 45 years old and 80% had a history of tobacco usage. A total of 3594 images were used, 70% used for the training set and 30% used for the cross validation set. The algorithm was capable of identifying OSCC from others with a sensitivity of 93% and a specificity of 74%. The algorithm produced by James et al was not able to differentiate between the different grades of dysplasia, however it could be differentiated from normal/benign with a specificity of 76% and a sensitivity of 95% (Table 1).

1.2.3. Numerical data

Omar et al. [18] developed a prediction model using machine learning in order to predict 5-year overall survival among patients with oral squamous cell carcinoma. This model was used in comparison to a prediction model created by the TNM clinical and pathological stage. The study was conducted over a span of 7 years with a total of 33065 patients taken from the national cancer database. The mean age of the patients was 64.6 years, with 59.9% of the population being male and 90.1% being white.

The model was created using the Azure Machine Learning Studio and the data was split 80/20 with the former being used as a training set and the latter as a test set. Several different 2-class models were considered, including decision forest, decision jungle, logistic regression, and neural network. The decision forest classification model was the most robust out of the models investigated with an AUC of 0.8, a precision and accuracy of 71% and recall of 68%. In comparison the same model using only pathological and clinical TNM staging data was less accurate with an AUC of 0.68, an accuracy of 65%, a precision of 69% and a recall of 52%.

Bur et al. [19] conducted a study to predict nodal metastasis in OSCC. Patients who had undergone surgery to primary tumour excision and neck dissection with T1-2N0 between the years 2007- 2013 were identified using national databases and 5 variables were used to predict metastasis. Data was collected on 278 patients and machine learning algorithms were then used to predict nodal metastasis. The decision forest algorithm yielded the highest results with AUC of 0.840 sensitivity of 0.917 and specificity of 0.576 (Table 1).

Table 1. Summarization of studies involving the use of AI

Reference	Type of deep/machine learning	Type of lesion	Sensitivity (%)	Specificity
Uthoff et al. [5]	Deep learning	Pre-malignant/Malignant	85	88.75
Kirubabai et al. [9]	Deep Learning	Normal/abnormal	98.6	99.1
Duran-Sierra et al. [14]	Machine Learning	Pre-malignant/Malignant	94	74
Rahman et al. [15]	Machine Learning	Pre-malignant/Malignant	99.7	100

James et al. [17]	Machine Learning	Normal, benign, potentially malignant	92	79ut
Welikala et al. [6]	Deep Learning	Normal/premalignant	89.51	84.77
Omar et al. [18]	Machine Learning	Oral squamous cell carcinoma	68	71
Adeoyo et al. [11]	Deep Learning	oral leukoplakia, oral,lichen planus, or oral lichenoid lesions		
Alhazmi et al. [16]	Machine Learning	Pre-malignant/Malignant	85.71	60
Jubair et al. [7]	Deep Learning	Suspicious/benign	86.7	84.5
Kim et al. [12]	Deep learning	Malignant lesions	91.7	57.6
Bur et al. [19]	Machine learning	Metastatic lymph node	91	57.6
Ariji et al. [10]	Deep learning	Metastatic/normal lymph nodes/ OSCC	78.2	75.4
Sunny et al. [8]	Deep learning	Potentially malignant/malignant lesions	89	100
Alabi et al. [13]	Machine learning	Previously treated tongue cancer	71.2	98.9

DISCUSSIONS

Oral cancer poses a growing health concern in several low- and middle-income countries, particularly in South and Southeast Asia [5]. To address this issue in high-risk populations residing in remote areas with limited infrastructure, Uthoff et al. [5] developed a unique dual-view point-of-care oral cancer screening device. This device utilizes autofluorescence imaging and white light imaging on a smartphone platform, allowing for early detection of pre-cancerous and cancerous lesions in the oral cavity. This study was able to successfully demonstrate that deep learning methods are able to distinguish between cancer and pre-cancer lesion. Currently the gold standard for cancer detection is done by a clinician to verify the parameters. A limitation of this study could be that the comparison against the deep learning itself is biased and subjective.

Another study conducted by Welikala et al. [6] produced an app named Memosa to record pictures of oral lesions from a smart phone. MeMosa annotate is a separate browser that is made by the company to create a library of well annotated lesions which can be then used by AI algorithm to detect early or potentially malignant lesions. The study investigated CNNs ability to classify images as well as deduce object detections. Image classification describes the ability of a network to classify an image into a certain class according to the images visual content, while the object detection refers to the networks ability to determine where an object is located in the image itself and which class that object belongs to. The results showed that the algorithm was a lot better at classifying images than it was with object identification. This may be because object identification is more obscure as lesion margins or presentation may vary. Once a lesion is established however it is easier to classify into categories of benign and potentially malignant.

From the results that were shown in the previous section in it can be seen that the object classification accuracy is extremely poor, with the precision being nearly half as

accurate as the image classification. This shows that while convoluted neural networks can be very effective at image classification, more training is needed in order to improve the results of object classification, this could be done by using larger data sets, training the network for longer or changing/improving the methodology that the network uses to identify the objects position.

The results for image classification obtained in this study are similar to the research conducted by Uthoff et al. [5] whereby the overall accuracy of the two different CNN models harboured similar results when it came to image classification. The number of images used for the training set in the study (1744 photographs) conducted by Welikala et al. was substantially higher than that of the amount used in the study by Uthoff et al (170 photographs). This could suggest that while the amount of data supplied to the network increases the reliability of the study, the amount of data that is used to build the network does not necessarily impact the results as much as the actual framework and build of the network itself. Thus, suggesting a more rigorously developed CNN could provide better and more accurate results with a smaller data set compared to a less proficiently developed CNN with a larger data reserve as its training set.

The study conducted by Kirubabai et al. [9] used a deep learning algorithm to classify oral MRI images as either normal or abnormal. The oral cancer detection system proposed in this study, employing a CNN classification approach, achieves a detection rate of 99.3%. The CNN network has been used to classify images as normal or abnormal in this study. The efficacy of this study shows very high results in all 3 areas of sensitivity, specificity and accuracy. In comparison to the study conducted by Uthoff et al, the sensitivity and specificity results achieved by Kirubabai et al. were over 10% higher than the prior. The study conducted by Welikala also used CNN networks to classify images for referral. This also showed high precision which connotes that CNN networks are good at classifying images. The study by Welikala however yielded much lower results when it came to object detection. This suggests that although CNN networks may have larger scale use for image classification, when it comes to object detection, it could have limitations. The Welikala et al. [6] study however has limitations of its own, it was not able to find a conclusive way to come to a decision of object detection among clinicians. Hence, this is why the object detection using CNN networks may have been less valid as the manual input itself could have had inconsistencies.

Jubair et al. [7] analysed a dataset of 716 clinical images depicting different tongue lesions. The images were categorized into "suspicious" lesions (236 images) and benign lesions (480 images) from images collected over a four-year period from 543 patients using various cameras and smartphones. 3 different models were used each yielding very similar results. This shows that the model itself may not have a direct impact on the results, instead the input and methodology for harboring the results is more important. Understanding these studies can learn to be more stringent with the methods and imputation of data.

Overall photographs coupled with AI were a very good method of classifying oral lesions. Work still needs to be done on object identification, however having a platform that is easily accessible to prevent those that may potentially be at risk of cancer from late diagnosis can really help the prognosis of the disease. These methods should be continually implemented as the benefits are large.

Arji et al. [10] found that there was no significant difference between the deep learning image classification in comparison to radiologist experienced opinion. The high results and similarity with the radiologists show that deep learning can be used effectively. One limitation is that comparing deep learning with radiologist opinions means that that the deep learning algorithm can never surpass a clinician's opinion as clinicians are currently the only means of validation.

Kim et al. [12] found that deep learning methods can be used to predict the survival of patients that have been diagnosed with OSCC. Predicting the survival rates predicts the prognosis of the disease. When compared another numerical study by Alabi et al. which used deep learning with results also being conclusively good, it shows that numerical data in conjunction with AI can have implications in understanding cancer prognosis and also in patient management.

Machine learning has many applications in classifying oral lesions. This aids both the diagnosis and the prognosis aspect of the disease. The study conducted by Duran-Sierra et al. [14] aimed to develop a method to classify oral lesions as either precancer/cancer or healthy using a special type of imaging called multiparametric autofluorescence lifetime imaging (maFLIM). They used this method on real images taken from patients with oral cancer. To classify the images, the researchers used four different models: Linear Discriminant Analysis (LDA), Quadratic Discriminant Analysis (QDA), linear Support Vector Machine (SVM), and Logistic Regression. They trained these models using 34 maFLIM images of oral lesions and healthy tissue. The models created a map that showed the probability of each pixel being precancer/cancer or healthy. They also calculated an overall score for the image based on the average of the probabilities for all the pixels. They determined an optimal score threshold through analysis, and if an image's score was above the threshold, it was classified as precancer/cancer; otherwise, it was considered healthy. They also evaluated the effectiveness of combining different types of features (spectral and time-resolved) for classification [14].

Overall, the study showed that the optimized SVM-QDA ensemble classifier using maFLIM features was effective in detecting oral precancerous and cancerous lesions [14]. This is a very beneficial use of AI as it not only helps with diagnosis of lesions, however the prognosis of cancer is much better when it is detected early, and this study showed promising results. One aspect of AI in all the studies mentioned is that manual input must be first used to train the datasets. This means that this AI model is able to detect pre-cancer/ cancer only based on its training from its own knowledge, instead it relies on pre-existing data. This questions the validity of the results as if the input data is incorrect then the results from the algorithm will also be incorrect. In comparison to deep learning methods whereby the algorithm is able to build up from existing neural networks studies as such using machine learning may be limited. This study has its advantages of being in VIVO as this becomes time saving. Real time images of the oral cavity allow for high degrees of accuracy and results delivered very quickly rather than have to wait for the lab to confirm results.

Another study conducted by Rahman et al. [15] also used Machine learning algorithms. H&E-stained biopsy slides were collected from diagnostic centers. Microscopic digital imaging was performed, and the images were graded and labelled. Data pre-processing techniques were applied to eliminate staining differences in the captured images. Colour channeling and contrast adjustment were performed. Various nucleus segmentation techniques such as Otsu's segmentation, Watershed segmentation, and MSER were applied, and a combination of Otsu's method with morphological operations (erode and dilate) was used for nucleus segmentation.

James et al. [17] focused on the automated interpretation of Optical Coherence Tomography (OCT) images for oral cancer detection. Two approaches were implemented: a simple algorithm-score and an Artificial Neural Network-Support Vector Machine (ANN-SVM) model. Multiple images of oral mucosal lesions were captured and evaluated by a trained oral physician and a non-reference image quality evaluator. Images with low quality and artifacts were removed from the dataset. The remaining images were analysed alongside their histopathological or clinical diagnosis as the gold standard. The performance of each neural network model was evaluated in terms of sensitivity, specificity, and accuracy for distinguishing malignant and dysplastic images.

Overall, the proposed methods achieved high detection rates compared to previous studies, demonstrating the potential of automated interpretation of OCT images for oral cancer detection. Overall, the study demonstrated the clinical application of OCT imaging in triaging patients for oral cancer detection, with the potential to improve diagnostic accuracy and facilitate timely interventions. The OCT imaging was capable of portraying oral cancer with a specificity of between 78-94% and a sensitivity of between 85-92%, thus showing that the use of OCT imaging can be very effective in the diagnosis of OSCC [17].

Omar et al. [18] conducted a study where they developed a machine learning-based prediction model to estimate the 5-year overall survival of patients diagnosed with oral squamous cell carcinoma. They compared this model with a prediction model based on the TNM clinical and pathological stage. Several 2-class models, including decision forest, decision jungle, logistic regression, and neural network, were considered. The development of the model utilized all available variables. The model's performance was assessed by testing it on a separate dataset, and the results were analysed. Among the investigated models, the decision forest classification model demonstrated the highest robustness, with an AUC of 0.8, precision and accuracy of 71%, and a recall of 68%. In contrast, the same model using only pathological and clinical TNM staging data showed lower accuracy, with an AUC of 0.68, accuracy of 65%, specificity of 69%, and sensitivity of 52%. The study conducted by Omar et al. demonstrates that in settings where abundant data is accessible, the utilization of machine learning and artificial intelligence can significantly enhance healthcare outcomes.

Adeyo et al. [11] investigated the applications of deep learning to predict the malignant transformation free survival of oral potentially malignant disorders. The data, which included demographic, clinical, pathological, and treatment information, was obtained from the hospital's electronic health record system. The main objective of the study was to predict the time it takes for these oral lesions to transform into malignancies. The data underwent cleaning and transformation, and five machine learning algorithms were utilized for modelling purposes. The most effective model was then externally validated using an independent dataset. Finally, the final model was deployed using a web-based interface. Descriptive statistics were conducted using SPSS, while the models were implemented using Python. Adeyo et al found that the DeepSurv algorithm produced the best discriminative performance, while the RSF algorithm produced better calibrated probability estimates. The high results of this study show that the time taken for lesions to become malignant can be assessed. The biggest drawback of this study however is that the patients who presented to biopsy all presented at different times during the manifestation of the disease. This study does however show that when a patient presents with a lesion in a certain stage it is possible to estimate the time it would take for malignancy. This again aids the prognosis of the disease.

One of the notable advantages of utilizing AI in oral cancer diagnosis and prognosis is the potential for enhanced accuracy as research progresses and algorithms are further developed and refined. With continued investigation and the advancement of AI training techniques, the predictive capabilities are expected to improve significantly. The strength and quality of the input data, combined with robust methodologies, will contribute to more reliable and robust results.

CONCLUSIONS

Organize conclusions which emerge from the study. In the end state: a) contributions to be acknowledged but which do not justify paternity right; b) thanks for technical support; c) thanks for financial or material support.

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The implications of phytotherapy in the management of periodontal disease



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Abstract

Periodontal disease has a high prevalence worldwide and is estimated to affect up to 90% of the world population. Currently, the attention was directed towards the effects of the herbal extraction in prevention and management of the periodontal disease. Taking into consideration their antimicrobial activity and immunomodulatory action, plant extracts can influence the treatment outcome. This review aims to provide an overview of the implications of different herbal extracts as adjuvant therapy and the outcome of the cases compared to standard controls. By integrating the herbal plant extracts and essential oils into different oral hygiene products as well, the first signs of inflammation can be controlled. The results highlighted an important antimicrobial action upon the most common pathogens of the periodontal disease, offering important advantages in the management of this disease.

Keywords: phytotherapy; plant extracts; periodontal disease; adjuvant therapies

INTRODUCTION

The periodontal disease is an infectious disease of the oral tissue that induces an inflammatory response. It is described as an inflammation determined by a various microorganism, host mediated, that has as a result the loss of periodontal attachment. Periodontal tissues include the structures surrounding the tooth, such as the gingiva, alveolar process mucosa, alveolar bone, periodontal ligament, and root cementum. The periodontium supports the tooth, protects against oral microflora and makes it possible to attach it to the jawbone. The inflammatory process leads to loss of connective tissue and alveolar bone, resulting in loss of bone support and thus causing pathological tooth mobility.

Defining the periodontal health is very important if we want to have a common point of reference for evaluating periodontal disease and determining meaningful treatment outcomes. Health can be analyzed on a general or clinical level and must be considered both as a preventive starting point and as a therapeutic endpoint [2]. Thus, periodontal health may exist before the onset of disease, but conversely, periodontal health may be restored to an anatomically reduced periodontium [2]. Periodontal diseases can no longer be considered simple bacterial infection. Rather, these are complex diseases of a multifactorial nature involving an interplay between the subgingival microbiome, the host's immune and inflammatory response, and environmental modifiers thus, periodontal health should not be considered solely in the context of plaque/bacteria levels and control but must embrace a systemic analysis and evaluation of all the factors responsible for the onset of disease, as well as the restoration and maintenance of health [3,4].

Phytotherapy is a field of medicine that uses plants either to treat disease or as health promoting agents. It is often mentioned in Western medicine, due to the increased level of vegetation with beneficial properties. The traditional use of phytotherapies usually preserves the original composition and integrity of the original plant, so that it is used for medicinal purposes either the whole plant or only an essential component that undergoes processing with a minimal process of altering the structures and properties. Practitioners and distributors may use either single-herb treatments, as multiple herbs are thought to have complementary properties, or mixtures with non-herbal substances such as minerals and vitamins [5]. Among the most used vegetable agents are the used as active substances in dental medicine and also in periodontal diseases cases are green tea, carotenoids, curcuma (turmeric), vitamin C and vitamin B complex from fruits and vegetables.

Herbal compounds can be a powerful and dominant method for combating the local inflammation caused by periodontitis. The action of the plants is due to the active compounds they contain, so the beneficial effects are potentiated by flavonoids, coumarins, glycosides, phenolic acids, resins, phytoesters, choline, carotenoids, tannins, vitamins, mineral salts (magnesium, iron, lithium) and oils essential. The most popular compounds are flavonoids and essential oils. Depending on these compounds, the plants have different roles: anti-inflammatory, antioxidant, antimicrobial, antihyperglycemic, antifungal and antiedematous [6].

The aim of the present review is to evaluate the reported implications of several herbal compounds in the management of the periodontal disease as an adjuvant therapy, and to quantify the existent results.

Aim and objectives

The purpose of the review is to present the importance of introducing phytotherapy as an adjuvant treatment in the management of the periodontal disease. From the beginning it is relevant to mention that phytotherapy is a complementary treatment. In order to have an

optimal response, it must be associated with a pharmacological or surgical treatment, depending on the patient's periodontal condition. The interest in herbal pharmaceutical preparations is constantly growing, as they are being studied to combat and prevent periodontal diseases. The ideal formula for successful treatment consists of conventional periodontal treatment based on scaling and root planning and adjuvant therapy. The latter containing substances that have antimicrobial, antioxidant, anti-inflammatory properties to reduce plaque and limit the destruction of periodontal tissue caused by the progressive inflammatory process. By presenting these findings, the future treatment of the periodontal disease could be substantially improved by combining all the therapeutical measures.

MATERIAL AND METHODS

Data collection

Electronic databases (MEDLINE-PubMed, EMBASE, Google Scholar, Semantic Scholar, Elsevier and Academia.edu) were searched for relevant articles for the subject of this systematic review. The main inclusion criteria was based on the year of publication, all studies considered are not older than 10 years, thus the publication range is 2014 – 2023. All search results were screened for relevance by titles, abstracts and keywords. Irrelevant records were excluded. Subsequently all full-text articles initially identified were assessed for eligibility based on inclusion and exclusion criteria. Only English language texts that have the full text available were retained for this analysis. Randomized and non-randomized studies on human subjects were included, thus excluding texts based on experimental animal or in-vitro studies, articles of the case series type, letters to editors, comments and abstracts of presentations.

Combinations of terms and keywords were used: periodontitis, periodontal disease, chronic periodontitis, gingival inflammation, phytotherapy and periodontal disease, oral cavity and herbs effect, herbs in dentistry, periodontal regeneration, herbal mouthwash, chlorhexidine and herbal products, natural tooth paste, herbal oral gel, oral care products, oral gels.

The research hypothesis is supported by studies selected based on the PICO (population, intervention, comparison and outcome) technique. The study protocol and inclusion/exclusion criteria were specified in advance. The hypothesis of this study is: Are herbal oral products (mouthwash, toothpaste, gel, chips) effective in patients diagnosed with periodontal disease?

Population: Adult human subjects diagnosed with periodontitis based on clinical examination (probing depth measurement and/or clinical attachment loss, probing bleeding) and/or radiographic evaluation.

Intervention: Use of herbal dental products (mouthwash, toothpaste, gel) before or after subgingival mechanical instrumentation of periodontal pockets by scaling and root planning (SRP)/supragingival scaling (SPD) or without phytotherapeutic treatment.

Control: Use of conventional/non-herbal dental products (mouthwash, toothpaste, gel, chips) or placebo before or after SRP/SPD or no phytotherapeutic periodontal treatment.

Outcome: Periodontal clinical parameters, including measurement of probing depth, gingival inflammation, plaque, bleeding on probing, clinical attachment loss.

Included studies must report on at least one periodontal clinical parameter, including probing depth (PD), clinical attachment loss (CAL), gingival inflammation index (GI) and plaque index (PI).

This systematic review included randomized and non-randomized clinical trials that assessed the effect of any herbal dental products (mouthwash, toothpaste, gel, chips) and compared them with conventional chlorhexidine products or placebo in patients with

periodontitis. These studies also have inclusion criteria for patients: to be examined human subjects diagnosed with untreated chronic periodontitis in the last 6 months, cooperative, who follow the instructions and who want to be monitored over a period of time, the age of the included patients must be at least 18 years old. At the level of periodontal parameters, the following were observed: the existence of at least 15 dental units, the probing depth of at least 4 mm, the attachment loss of at least 3 mm, the presence of inflammation and bleeding on probing.

Data processing

All basic information was centralized in Table 1. Data extraction was performed according to a certain model that followed aspects related to: periodontal parameters examined, adjuvant therapy, treatment information including type of periodontal therapy, adjuvant treatment groups, number of patients, follow-up period and important clinical findings.

RESULTS

After applying the inclusion and exclusion criteria, 28 studies were eligible for this analysis. The main characteristics were presented in Table 1. SRP therapy was associated with the use of herbal products in 23 studies [7,8,9,10,11-22), while in one study adjunctive herbal therapy was initiated one month before SRP was performed of [31]. Other studies included only SPD treatment [6] or no treatment [26,27]. All examined patients were diagnosed with chronic periodontitis. The severity and extent of disease varied between studies. Three studies included patients with generalized chronic periodontitis and severe generalized chronic periodontitis [7,28,25], two studies with mild to moderate chronic periodontitis [29,30], one study with mild to moderate localized chronic periodontitis [8], others with moderate to severe chronic periodontitis [24,22]. A study with chronic periodontitis with residual periodontal pockets after SRP therapy [26] and another in which patients present with grade II furcation lesions [21] are integrated. The follow-up period ranged from one week to three months. Results at one week were presented in 5 studies [28,31,30,10,15] and at three months in 11 studies [7,32,23,9,24,25,12-14,16,19]. The rest of the studies presented results at certain time intervals such as: two weeks, 21 days, one month or 45 days, 6 or even 12 months. Various plaque indices and bleeding indices were used in the included studies. Mainly plaque deposits, bleeding/inflammation and probing depths were reported.

After analyzing the studies [7,31,8,10,22,25,11-22] a considerable improvement of certain initially recorded parameters was found in the subjects who followed an adjuvant herbal therapy compared to the subjects who underwent only SRP therapy with/without placebo effect substances.

All included studies reported significant differences in favor of herbal products compared to placebo or no adjuvants. There are two comparative clinical studies that reported no differences between the parameters of the two groups of subjects: SRP ± placebo and SRP + phytotherapeutic substances. They analyzed the effect of Liquorice (licorice) mouthwash and *Salvia Officinalis* gel [10,15]. However, *Salvia Officinalis* has an influence on the immunological indices, which after the second application of the gel had a significant decrease [15].

Table 1. Table 2: BOP: Bleeding on probing; CAL: Clinical Attachment Loss; CHX: Chlorhexidine; GI: Gingival index; PI: Plate Index; PD: Probing depth; SPD: supragingival scaling; SRP - Scaling and Root planning (subgingival scaling and root planing); MTC: *Matricaria chamomilla* - chamomile; *Punica granatum*, *Piper nigrum* - pomegranate, black pepper; *Salix alba*, *Malva sylvestris* and *Althaea officinalis* - white willow, forest mallow, big mallow; Triclosan - antimicrobial and antifungal organic compound; *Ocimum sanctum* - holy basil, tusli; *Camellia sinensis* - green tea; *Nigella sativa* - black cumin; Licorice - licorice; Lemongrass - lemon grass; Platango

lanceolata – narrow plantain; *Emblica officinalis* - Indian currant; Plant extract - *Mimusops elengi*, *Acacia arabica*, *Punica granatum* (pomegranate); *Quercus bratii* and *Coriandrum sativum* - oak *bratii* and coriander; Alendronate – medicine for treating osteoporosis; Metformin – antidiabetic drug

Reference	Parameters	Adjuvant therapy	Subjects/Groups	Surveillance	Conclusions
[7]	PI, GI, BOP, PD, CAL	Mouthwash	Total subjects= 75 A. 25 = SRP + 1% <i>Matricaria chamomilla</i> (MTC) B. 25 = SRP + 0.12% CHX C. 25 = SRP + placebo	6 weeks, 3 months	MTC has a significant higher effect than the placebo and comparable effect to CHX.
[8]	PI, GI, BOP, PD, CAL	Mouthwash	Total subjects = 45 A. 15 = SRP + 5% green tea B. 15 = SRP + 0.12% CHX C. 15 = SRP	30 days	The green tea has influenced all the parameters compared to CHX and SRP.
[9]	PI, GI, BOP, PD, CAL	Mouthwash	Total subjects = 90 A. 30 = SRP + Triphala B. 30 = SRP + Bakul C. 30 = SRP + CHX	2, 4, 6, 8, 12 weeks	Triphala has the most significant plaque reduction result at 3 months compared to CHX and Bakul
[10]	PI, GI	Mouthwash	Total subjects = 30 A. 15 = SRP + Sweet wood mouthwash B. 15 = SRP	1 week	There is a decrease in plaque and gingival inflammation, but no significant differences between SRP and SRP + licorice mouthwash.
[11]	PI, GI, PD	Subgingival gel	Total subjects = 20 A. 10 = SRP + Aloe vera B. 10 = SRP + distilled water	1, 2 months	PI improved in both groups. Aloe vera gel administration significantly improved GI and PD.
[12]	PI, GI, BOP, PD, CAL	Subgingival gel	Total subjects = 46 A. 23 = SRP + 10% <i>Emblica officinalis</i> B. 23 = SRP + gel placebo	2, 3 months	<i>E. officinalis</i> produces a significantly greater improvement in patients with chronic diseases. There is therapeutic potential to help treat chronic periodontitis.
[13]	PI, GI, PD, CAL	Subgingival gel	Total subjects = 30 A. SRP B. SRP + CHX gel C. SRP + plants gel (<i>Mimusops elengi</i> , <i>Acacia arabica</i> and <i>Punica granatum</i>) D. CHX E. Plants gel	1, 3 months (gel application at every 10 day in a month)	SRP + any gel causes a significant reduction in clinical parameters, compared to SRP. The results of groups B and C are similar, so the herbal gel can be considered as a good adjunctive treatment for chronic periodontitis.
[14]	PI, BOP, PD, CAL	Subgingival gel	Total subjects = 18 A. 9 = SRP + gel: 20% <i>Quercus brantii</i> și 1% <i>Coriandrum sativum</i> B. 9 = SRP + gel placebo	1, 3 months	The results in group A were improved compared to those in B, but no significant benefit was observed. It is possible that by increasing the concentration and repeating the consumption, significant effects can

Reference	Parameters	Adjuvant therapy	Subjects/Groups	Surveillance	Conclusions
[15]	PI, GI, BOP, PD	Subgingival gel	Total subjects = 14 Nr. Periodontal pockets= 28 A. SRP + gel Salvia officinalis B. SRP	1 week, 1 month	be observed. S. officinalis gel has an anti-inflammatory potential observed by monitoring clinical and immunological parameters. The effect accentuated at the 2nd application by the significant decrease of the immunological indices.
[16]	PI, GI, PD	Subgingival gel	Total subjects = 30 A. SPR B. SRP+ Aloe vera gel	1, 3 months	Aloe vera gel improves the periodontal condition. The role is beneficial, without side effects, but requires more studies to certify the antimicrobial, anti-inflammatory properties.
[17]	PI, GI, PD, CAL	Subgingival gel	Total subjects = 30 A. SRP B. SRP + Curcuma gel	1 month	PD is reduced in group B compared to group A, due to the anti-inflammatory effect of turmeric. This plant produces tissue re-epithelialization and neovascularization. The CAL values are not different.
[18]	PI, BOP, PD, CAL	Subgingival gel	Total subjects = 40 A. 20 = SRP B. 20 = SPR + Curcuma gel	1. 2 months	Improvements in PI, BOP, PD in group B. There were no significant differences in CAL.
[19]	PI, GI, PD, CAL	Chips with plant extracts	Total subjects = 30 A. SRP + Cip cu Neem B. SRP+ Cip cu Turmeric C. SRP + Cip placebo	1, 3 months	After 1 month the results were visibly improved in the case of groups A and B compared to C. The results of the parameters after 3 months look the same as those observed after one month. Long-term effects are debatable.
[20]	PI, GI, BOP, PD, CAL	Subgingival gel	Total subjects = 15 A. SRP B. SRP + Curcuma gel C. SRP + 10% Tusli gel	1 month	GI, PD, CAL values improved in group B and C compared to A. Tusli gel shows significant reduction in BOP, PD and CAL and Curcuma gel influences PI and GI. Tusli has antimicrobial properties and

Reference	Parameters	Adjuvant therapy	Subjects/Groups	Surveillance	Conclusions
					turmeric has an effect against plaque itself.
[21]	PI, BOP, PD, CAL	Subgingival gel	Total subiecți = 90 A. 30 = SRP + placebo B. 30 = SRP + Alendronat 1% C. 30 = SRP + Aloe vera gel	6, 12 months	All parameters improved semi-significantly in groups B and C compared to A.
[22]	GI, BOP, PD, CAL	Subgingival gel	Total subiecți = 90 A. 30 = SRP + placebo B. 30 = SRP + Aloe vera gel C. 30 = SRP + Metformina 1%	6, 12 months	In groups B and C, the parameters show a statistically significant change, compared to group A. Metformin has the best effect on PD and CAL, but aloe vera also shows considerable improvements compared to the placebo gel.
[23]	PI, BOP, PD	Subgingival irrigation with solution	Total subjects = 30 A. 15 = Punica granatum Linn., Piper nigrum Linn B. 15 = 0,2% CHX (one month before SRP)	2, 4, 8, 12 weeks	Irrigation with plant extract solutions has a significant effect upon reducing the dental plaque. CHX has a significant effect in reduction of gingival inflammation.
[24]	PI, BOP, PD, CAL	Mouthwash	Total subjects = 40 A. 20 = SPD + plante mouthwash (Propolis, Salvia officinalis, Platango lanceolata and essential oils) B. 20 = SPD + mouthwash placebo	12 weeks	The herbal mouthwash showed a significant decrease in the bleeding score and plaque build-up.
[25]	PD, CAL	Mouthwash	Total subjects = 45 A. 15 = SPD + lemongrass mouthwash B. 15 = SPD + CHX mouthwash C. 15 = SPD	12 weeks	Mouthwash with lemongrass oil can be a good alternative in chronic periodontitis. Significantly increased effect on PD, CAL in the group that used plants and CHX compared to SPD.
[26]	PI, BOP, PD	Mouthwash	Total subjects = 50 A 25 = Herbal (hidro-alcoholic extract of Scrophularia striata) Mouthwash B. 25 = Irsha mouthwash (Listerine), without periodontal treatment	2,4 weeks	Herbal extract mouthwash has a better effect on chronic periodontitis compared to Irsha mouthwash.
[27]	GI, PI, BOP	Mouthwash	Total subiecți = 60 A. 15 = Aloe vera + ceai verde B. 15 = Matrica chamomilla C. 15 = CHX D. 15 = Placebo	2 weeks	Indices in the placebo group did not change. All other substances improved clinical parameters after administration. GI and IP significantly

Reference	Parameters	Adjuvant therapy	Subjects/Groups	Surveillance	Conclusions
					changed in groups A and C compared to B. Between A and C there are no significant differences.
[28]	PI, GI	Mouthwash	Total subjects = 50 A. 25 = SRP + triphala B. 25 = SRP + 0.2% CHX	7, 30, 45 days	Triphala mouthwash has a reduction effect upon the dental plaque, and gingival inflammation. It proved to be more efficient than SRP+CHX.
[29]	PI, GI, BOP, PD, CAL	Toothpaste	Total subjects = 30 A. 15 = SRP + green tea B. 15 = SRP + triclosan solution	4 weeks	Green tea use was associated with a higher influence in the gingival inflammation reduction and CAL.
[30]	PI, GI, BOP, PD, CAL	Mouthwash	Total subjects = 40 A. 20 = SRP + 0.05% green tea B. 20 = SRP + 0.2% CHX	7 and 21 days	Green tea was more efficient than CHX at three weeks.
[31]	PI, GI, BOP, PD, CAL	Subgingival irrigation with solution	Total subjects = 30 Nr. Periodontal pockets A. 180 = SRP + extract of S. lappa B. 180 = SRP + 0.2% CHX C. 180 = SRP	7, 14, 28, 42 days	S. lappa irrigation showed benefits compared to SRP. S. lappa and CHX showed similar results and S. lappa had a higher effect upon the 4-5 mm periodontal pockets.
[32]	PI, GI, PD, CAL	Subgingival irrigation with solution	Total subjects = 30 Nr. Periodontal pockets A. 15 = SRP + 4% Ocimum sanctum B. 15 = SRP + 0.2% CHX	30 days	Ocimum sanctum irrigation showed similar benefits as CHX upon the gingival inflammation reduction and plaque reduction. CHX had a superior effect in PD, CAL reduction.
[34]	PI, GI, BOP, PD, CAL	Mouthwash	Total subjects = 30 A. 15 = SRP + Nigella sativa B. 15 = SRP + saline solution,	2 weeks	Nigella sativa and the saline solution had beneficial effects. There were no significant differences between the 2 administered solutions.

Plant-based substances acted only on some specific parameters, others not registering significant differences. However, after analyzing the groups of subjects subjected to SRP/SPD and SRP/SPD + phytotherapy, the reduction of periodontal parameters is significant in the groups in which adjuvant therapy was also administered.

The effects of the various adjunctive therapies that accompanied the basic periodontal treatment were compared in Table 4. Mainly it was aimed to record the results collected from certain groups of subjects, more precisely the groups of patients who followed SRP/SPD + chlorhexidine therapy and those in which patients underwent SRP/SPD + phytotherapy.

Most of the studies did not record significant differences between the two adjuvant therapies, at the level of the researched parameters [7,32,25,13]. Mouthwashes with extracts of *Matricaria chamomilla*, *Althea officinalis*, *Salix alba*, *Malva sylvestris* and Lemongrass oil had an influence similar to chlorhexidine on periodontal elements. As in the case of the previously exposed plant products, the gel from plants (*Mimosa pudica*, *Acacia arabica* and *Punica granatum*) administered subgingival, had similar results to CHX [13].

Effects of gels with subgingival administration

Subgingival delivered gel has been shown to be effective in 11 studies [11–22]. They highlighted the fact that all the parameters measured and compared between the groups of patients, treated by conventional periodontal therapy with or without the administration of placebo substances and those under conventional periodontal treatment supported by adjuvant phytotherapy, were considerably improved thanks to the plants. Four studies based on Aloe vera gel [11,16,21,22] and three on Curcuma gel [17,18,20] recorded semi-effectively increased beneficial effects, the analysis being done, after the SRP, between the group test and control group (placebo). Aloe vera gel did not cause allergic reactions. The result of the other gels was variable. Gels of *Quercus baronii* and the plants *Mimosa pudica*, *Acacia arabica* and *Punica granatum* positively influenced probing depth and loss of ligamentous attachment [12,13]. Conversely, *Coriandrum sativum* and *Salvia officinalis* did not show significant changes [14,15].

Effects of mouthwash

Herbal extract mouthwash was found in 12 studies, plants such as *Matricaria chamomilla* (28), *Althaea officinalis*, *Salix alba* and *Malva Silvestris* extract [7], *Triphala* [28,9], green tea (30, 8), *Nagilla sativa* [10], *Scrophularia striata* [26], Licorice [10], Propolis, *Salvia officinalis*, *Platango lanceolata* and essential oils [11], Lemongrass [25]. The measured parameters showed significantly improved values in the test patients compared to those in the patients belonging to the control group. These results being as good as those obtained following the administration of CHX mouthwashes (7, 28).

The comparison of adjuvant therapy based on green tea mouthwash and triclosan mouthwash had positive results in favor of green tea administration. Gingival index and probing bleeding were reduced, and a gain in attachment was observed in the test patient group. This may be attributed to the anti-inflammatory and antioxidant properties of green tea [29]. The previously mentioned features positively influenced the measured parameters, the results being significantly improved compared to the administration of chlorhexidine mouthwash [30,8].

The administration of licorice mouthwash was analyzed over a short period of time and in a small sample of people. The reduction in plaque and gingival index were insignificant in the test group compared to the control group. However, it is believed that licorice mouthwash can be a safe and natural substitute for chlorhexidine. Over time, chlorhexidine caused adverse reactions, and patients did not like the taste either [10].

Effects of other pharmaceutical forms of administration

Solutions for subgingival irrigation with extract of *S. lappa* [31], *Ocimum sanctum* [32], recorded positive effects on the periodontium, similar to those obtained following the administration of the CHX solution. In the case of the compound with *Punica granatum* Linn and *Piper nigrum* Linn, the results are slightly different, so that chlorhexidine registers significant changes, with statistical significance [24].

Applying Neem and Turmeric chips subgingival, more precisely inside the periodontal pocket, then stabilizing them by covering the gingiva with periodontal cement to protect postoperative wounds (coe-pak), demonstrated its effectiveness by decreasing all recorded parameters. It should be noted that the long-term effects are debatable, since the results of the parameters after one month are the same as those measured after 3 months [19].

The toothpaste showed a significant reduction in gingival inflammation and periodontal attachment loss compared to toothpaste containing triclosan. This may be attributed to the anti-inflammatory and antioxidant properties of green tea [29].

DISCUSSIONS

Periodontal disease is a major public health problem worldwide and is the most common cause of tooth loss. The success of periodontal therapy depends on the treatment of adverse environmental and behavioral factors and the elimination/reduction of pathogenic bacteria. Patients began to become aware of the adverse effects of synthetic antimicrobial products, thus increasing interest in natural substances. Natural phytochemicals can be considered good alternatives. Phytotherapy uses complexes or substances derived from plants, which are homogeneous compounds with a certain chemical structure. Phytotherapeutic agents have various bioactive components, which possess a very good medicinal value with the fewest side effects [26].

The main objective of periodontal therapy is to reduce the microbial load, thus improving clinical parameters. Scaling and root planing have remained the gold standard of periodontal therapy, with numerous other agents currently being used as adjunctive therapeutic modalities [16].

The use of plant extracts in the form of toothpaste, mouthwash, gels, solutions, chips with local administration at the level of the periodontal pockets, has been considered effective for the prevention/treatment of the disease, but also for the periodontal maintenance after SRP/SPD (26).

In the case of using the gel with subgingival administration containing extract of *Quercus bratii* and *Coriandrum sativum*, a notable improvement was observed only at the probing depth level. Polyphenols and tannins are the main substances in oak and coriander. They have hemostatic, antibacterial, anti-inflammatory and analgesic activities. Although the use of the herbal gel containing oak and coriander resulted in more improvements in clinical parameters than the placebo gel, these were not considered significant benefits. Thus, emphasis is placed on the major role of SRP as the main method of periodontal treatment. It is possible that by increasing the concentration of the gel, its sustainability or repeated consumption, some significant benefits can be observed [14].

A very good combination that can successfully replace mouthwash with CHX is Aloe Vera with Green Tea. Mouthwash with these plant components showed similar effects to CHX on periodontal structures. Anti-inflammatory, anti-plaque properties and lack of side effects contributed to these conclusions [27]. The antimicrobial property of *Embilca officinalis* (Indian gooseberry) fruit is mainly supported by flavoids, phenols and tannins. Phenolic compounds contribute to the relief of acute and chronic inflammation. The test group showed significant results such that probing depth and clinical attachment loss were significantly improved compared to the placebo control group [12].

Another form of productive administration of plants following SRP/SPD is mouthwash. Outcomes among patients who also received adjunctive therapy with green tea or lemongrass extract mouthwash were significantly improved. All monitored parameters were positively influenced by these plant substances [8,25]. The results of the study suggest that lemongrass mouthwash, used as an adjunctive treatment following periodontal treatment, significantly reduced clinical signs. A significant reduction in probing depth and periodontal attachment loss was observed, as were systemic markers of infection. In the study, both antimicrobial and antioxidant activities were achieved with 0.25% lemongrass essential oil. This mouthwash has been shown to be a good herbal alternative to 0.12% chlorhexidine mouthwash, with no significant changes in parameters between the two [25].

Green tea extract mouthwash significantly influenced all periodontal variables compared to chlorhexidine solution [30,8]. Green tea was much more effective, this fact was demonstrated in a control performed three weeks after periodontal therapy [30].

Chamomile mouthwash is used to manage chronic periodontitis due to its antimicrobial, superior healing, anti-inflammatory and immunomodulatory properties. The main mechanism of action is given by the anti-inflammatory property due to apigenin. Chamomilla matrix improves the clinical and microbiological picture of chronic periodontitis [7].

Curcuma, the main yellow bioactive component of turmeric, has been shown to have a broad spectrum of biological actions, such as anti-inflammatory and antibacterial, suggesting its potential for use as a subgingival agent [18]. Studies evaluate the effectiveness of turmeric gel when used with SRP as a topical gel, compared to SRP alone. The results indicated the effect by improving the plaque index, bleeding on probing and probing depth in patients with chronic periodontitis [17,18,20]. There was a slight, but not statistically significant, reduction in the average level of attachment in the test group compared to the control group. This may be due to increased levels of growth factors in the healing tissues; thus re-epithelialization occurs earlier, stimulating neovascularization and collagen production [17,18]. Within the limits of clinical trials, it can be concluded that curcumin gel administered subgingival was effective in reducing plaque, gingival inflammation and subsequently, probing depth. However, longer-term studies are needed to determine the effect of topical application of curcumin gel in improving periodontal attachment [17,18,20].

The presented studies confirmed that plant materials could be successfully used in the management of periodontal diseases. Herbal therapy has a favorable safety profile, with few, if any, side effects compared to conventional agents such as chlorhexidine or the drug substances alendronate and metformin. Adverse reactions are a negative factor on patient adherence to periodontal therapy.

CONCLUSIONS

Herbal extract mouthwashes and gels have been shown to be the most widely used phytotherapeutic pharmaceutical forms in the management of periodontal diseases. They are affordable and easy to integrate into patients' routines. Even after the first month of administration, the gels significantly changed the evaluated periodontal parameters, and the results continued to improve during the follow-up period. Although the effects of the mouthwashes were analyzed after the first week of treatment, statistically significant results were recorded after longer administration.

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HPV implications in oral cancer carcinogenesis: a systematic review



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Abstract

The topic itself is unknown to the majority of the population and HPV is not yet correlated by non medical people as an infection that can lead to oral carcinogenesis. HPV infection has no borders, in every population over the globe people are infected with at least one subtype of the human papilloma virus even unbeknownst to them. There is extensive research of how subtypes of the human papilloma virus infect the genital area, and especially the female genitalia, due to the fact that it is the main cause of cervical carcinogenesis. The following review of research articles related to the effects of HPV infection on carcinogenesis of the oral squamous cell carcinomas. Research articles were obtained from the database of National Library of Medicine of the United States of America, known as PubMed and the online database Google Scholar, relating to the age and sex of the patient, the site on which oral squamous cell carcinoma developed and the detection method. This systematic review aims to provide a comprehensive analysis of the existing literature regarding HPV-related OSCC, synthesising current knowledge and identifying key research gaps. Further research is needed on the field of cellular and molecular mechanism of infection, prevention, other factors that might influence HPV related carcinogenesis, early detection of biomarkers treatment methods and strategies, long term outcomes of patients treated of oral squamous cell carcinoma and patient that are infected of the subtypes sixteen and eighteen and finally its epidemiological character as an independent type of cancer.

Keywords: Oral cancer, Human papillomavirus, Papillomavirus infections, neck cancers

INTRODUCTION

Human papillomavirus (HPV) is a small, non-enveloped deoxyribonucleic acid (DNA) virus that infects skin or mucosal cells. Its size is approximately 5748 to 8607 base pairs. Of the more than 100 known HPV genotypes, at least 13 have been linked to cervix cancer as well as other anogenital and head and neck cancers [1]. About 70% of all cases of cervical cancer are caused by the two most prevalent "high-risk" genotypes (HPV 16 and 18) [2]. It is the most common sexually transmitted infection (STI) in the United States, with an estimated 79 million Americans currently infected [3]. It is estimated that around 80% of people will be infected with at least one type of HPV at some point in their lives, and the majority of these infections will not cause any serious health problems [4]. Most people will be infected due to the various ways of contamination, since HPV fomites or virions may not even be susceptible to medical or dental clinical disinfectants [5]. Depending on the type of HPV there are different diseases that may evolve, if the patient becomes symptomatic [6].

The warts that affect the skin of the body are not the same that affect the mucosal epithelial tissues or even the skin around the anogenital region [7]. Even though there are numerous types of human papilloma virus that manifest as anogenital warts, the main causative types are type 6 and type 11 [8]. When types 6 and 11 infect the larynx and the lining of the respiratory tract they can form warts / papillae on the epithelial lining causing laryngeal papillomatosis [9]. Penile cancers -50% of those caused by HPV- caused from HPV are caused, again, from HPV type 16 [10]. Either cervix or penis, the probability of cancerous lesion multiplies significantly in patients who are also affected by human immunodeficiency virus (HIV) [11]. The virus spreads by desquamation, -also a reason why it is such a common sexually transmitted infection [12]. Detection through biomarkers, such as ctDNA (circulating tumourDNA) or cHPV (circulating HPV DNA), in blood is promising but further advancements on the molecular tools is needed [13]. Diagnosis for men is not commercially ready, but researchers are trying to identify which regions are more prone to be infected by human papilloma viruses [14]. A complete oral oncological screening takes 2' to complete and can be done also without any additional equipment, although devices like Velscope and OralID are proven that can detect abnormalities of the mucosa on an earlier stage [15]. Condoms may reduce just slightly the probability of transmission of the infection [16]. In total there are three injection vaccines, with the second injection performed at least after 4 weeks from the first dose and the third injection performed at least 5 months after the first dose [17].

As can be quite logical, lack of vitamins A, C, D, E affect directly the oral mucosa and it is believed that deficiencies can induce the pathogenesis of oral lesions, but further investigations are needed for the metabolic pathways to be understood [18]. A cluster of differentiated 8 lymphocytes -T suppressor cells- predominates in the infiltrate, suggesting that immunosuppression is associated with disease progression [19]. The lower lip, the anterior floor of the mouth, and the lateral margins of the tongue are all high-risk areas for oral cancer [20]. It is of importance to mention also verrucous carcinoma -a subtype of OSCC- that can be identified from its distinct clinical features [21]. Fixation of nodes to neighbouring tissue as a result of cell invasion is a late event that indicates aggressive disease [22]. The diagnosis of oral squamous cell carcinoma is based only after histological examination in combination with a proper anamnesis of the patient and the clinical evaluation of the lesion [23]. Staging helps determine the extent of oral cancer, guide treatment decisions, and assess the prognosis of the patient based on the size of the primary tumour, lymph node involvement, and the presence of distant metastasis [24]. Radiation therapy can be used pre- and post- operatively in cases where dimensions of the tumour are wide [25]. Research

reveals that the earlier the stage on which the patient initiates the treatment the less risk for a recurrence [26].

Aim and objectives

This study is intended to identify how the infection with the human papilloma virus affects locations where oral squamous cell carcinoma is more prone to develop. It is intended to investigate the differences between the two sexes and the different age groups of the patients that were positively diagnosed with HPV related OSCC. It is important to be able to differentiate lesions that can have a specific area of infection in order to be able to continue the research on the topic regarding the action and the mechanisms of both the infectious cause-HPV- and the host-humans- of how they interact. With a better understanding and more exposure of the subject it is more likely for techniques to be developed that can improve all the aspects of treatment planning. From an earlier detection, to better understanding the risk factors, to refine treatment approaches, to evaluate prognostic factors for recurrence, to more thorough evaluation of HPV vaccines and how those affect the oral and oropharyngeal expression of the Human papilloma virus.

MATERIAL AND METHODS

Search strategy

The databases of PubMed and Google Scholar were searched to extract the needed research articles. The search for the articles for PubMed, on the search bar was the following: (((HPV [Title]) OR (Human papillomavirus [Title]) OR (Human papillomavirus [Title])) AND ((OSCC [Title]) OR (oral squamous cell carcinoma[Title]))) NOT ((systematic review [Title]) OR (meta-analysis [Title])). Additional parameters were added, the article must be published in the year 2013 or later. The database gave a hundred and nine different articles. While on the Google Scholar database the search was: allintitle: ((HPV) OR (human papillomavirus)) AND ((OSCC) OR (oral squamous cell carcinoma)). Again with the additional parameter to have been published in the year 2013 or later. The database gave us 94 results of articles and reports.

Inclusion and exclusion criteria

In order for the research articles to be included in the review they needed to have the following criteria. First and foremost, the articles must be published in the year two thousand thirteen and later. Inside their content, the researchers must specify the age and sex of the patients that the cancerous lesion was documented and its location. It also must contain the detection method of HPV of the tumour and it must contain high risk types of HPV. The tumours included must be primary, non recurrent and not previously treated by chemotherapy or radiation therapy. The studies must be in vivo and contain clear data regarding the age and sex of the patients as well as the location of the OSCC. Studies that are secondary (systematic reviews, literature reviews and meta-analyses) are excluded. Excluded are also letters to editors, case reports and articles that are not written in the English language. In its contents the articles that describe detection through serological analysis are excluded. If any of the criteria are not met, the article is not included in the review.

RESULTS

From the articles that were the outcome of the search from the PubMed database, the selection process began. Each individual's article abstract was read and depending on the content and its relevance to the goal thesis review was determined. Once an article's abstract showed relevance, it was read in its complete form to determine the presence of the inclusion

criteria. If the articles were relative to the subject but did not include all the inclusion criteria they were not added on the review. In total out of the 109 articles that resulted from the search in the PubMed database only 9 had all the inclusion criteria. Then, the selection process began on the Google Scholar database. In a similar manner, articles that were relevant to the subject had their abstract read and if they showed similarities to the goal of the review, they were read in full content to determine their inclusion. From the database of Google Scholar, 94 articles were the result of the search bar. After selection of the articles that align with the inclusion criteria and subtracting the duplicate articles that were found from the PubMed database, the resulting articles were 4. The selection and determining of inclusion criteria was done by the undergraduate student writing this review and their evaluation was done by the coordinating professor. Examples of the articles that were included are Chen et al. Oral human papillomavirus infection, sexual behaviours and risk of oral squamous cell carcinoma in southeast China: A case-control study [40]. Even though it has all but one criteria met - the location of the HPV related oral squamous cell carcinoma- it can not be included with the rest of the articles. Another is Yang et al. [41]. Even though the article contains valuable data, it is not specific to the sites of the OSCC and lacks the detail to collect the independent data of the HPV-related patients.

Starting with the articles that were retrieved from Google scholar there are: Silveira et al. [27], Tsimplaki et al. [28], Tokuzen et al [29] and Phusingha et al. [30]. Silveira et al. [27] research is based on the survivability of previously treated patients with oropharyngeal or oral squamous cell carcinoma. The patients included were HPV tested to divide in subgroups the patients infected or not and if infected with which type of the human papilloma viruses. In its immunohistochemical method there were p16INK4a, cyclin D1, p53 and Ki-67 antibodies used for detection of a tissue microarray. Out of all the data received, in the current review we extracted the patients that were tested positive to the antibodies of the human papillomavirus. That resulted in 12 cases of oral squamous cell carcinoma and in 32 cases of oropharyngeal squamous cell carcinoma. The first group consists of 12 males whose average age is 47 years old. The malignant lesions were previously excised from various locations of the oral cavity. Specifically, out of the 12 OSCC, 5 of those were excised from the tongue, 4 from the floor of the mouth, 2 from the retromolar area and 1 from the gingiva of the patient. The other group, HPV related OPSCC, consists of 25 male and 7 female patients, that average 60 years of age. The malignant lesions were found and excised by the following locations. From the 32 OPSCC, 13 were found on the pharyngeal tonsils, 6 on the base of the tongue, 5 on the soft palate, 1 in uvula and 7 in other non disclosed locations.

Following, Tsimplaki et al. [28], is a study that focuses on the OSCC that occurs in the tongue and how high risk HPV infection and expression of E6/E7 mRNA affects 53 Greek patients. In the data correlating the research includes if the patients were smokers or consumed regular alcohol. The detection was done by, analysis of HPV DNA and E6/E7 mRNA presence, PapilloCheck® for HPV detection and genotyping. Out of the 53 samples just 6 were positive for HPV DNA, 2 of those in male and 4 in female patients. 4 of the patients were younger than 45 years old while 2 were older with an average of 51 years. In the collected data only one patient (M73) from the HPV related was a smoker and consumed alcohol. Thus concluding that 9.4% of the 53 patients with OSCC of the tongue were positive for E6/E7 mRNA expression.

Tokuzen et al. [29], concentrates on the HPV infection and the expression of the enzyme p16. Through immunohistochemical processes, RNA extraction and qualitative reverse transcription-PCR (qRT-PCR) HPV infection by p16 expression was evaluated. 100 cases were reported, 10 of which were positive for p16 expression, 7 cases were from male and 3 cases were from female patients. The median age of the positive patients is 71 years old.

The primary sites of the OSCC are 3 in mandibular gingiva, 2 in maxillary gingiva, 2 in the floor of the mouth, 1 in the buccal mucosa, 1 at the tongue and 1 on the lip.

Phusingha et al. [30], is a case control study in which its main focus is the increasing trend of HPV-related OSCC in Northeast Thailand. Through buccal mucosa cells, DNA was extracted and with PCR was assessed for HPV DNA detection. Samples that were positive, through reverse line blot hybridisation (RLBH) were genotyped. All samples were sectioned as tissue microarray (TMA), stained, a tumour biopsy punched in each. block to perform in situ hybridisation (ISH). Out of the case control patients the only results taken are from the formalin-fixed paraffin-embedded lesions that were HPV positive (82). Out of the 82 cases, 29 were male and 53 were female patients, with a mean age of 62, that form the following age groups. Below and equal to 50 years old there were 14 patients, between 51 and 64 there were 16 patients and equal and above 65 years old there were 52 patients. The sites of the OSCC that were identified are 39 at the buccal mucosa, 17 at the tongue, 13 on the lip, 7 on the palate, 5 on the floor of the mouth and 1 on a non-specified location. With the different ways of examining the HPV DNA and mRNA on exfoliated cells and on lesion cells from FFPE, they create a reproducible study that can be repeated in later years in cohort studies.

Kouketsou et al. [31], is a cohort study that fixates on the detection of the HPV in OSCC on Japanese patients. There were 174 OSCC cases that were examined immunohistochemically for p16 expression levels. The positive p16 OSCCs were by in situ hybridisation (ISH) for HPV DNA and their genotypes by PCR. The results were associated with the clinical-pathological characteristics. From the samples from 174 cases were only 24 that tested positive on p16 suppressor enzyme expression. The age groups were sectioned by decades. Between the ages 30 to 39 there were 2 patients, ages 40 to 49 only 1 patient, ages 50 to 59 there were 2 patients. Starting from 60 to 69 years there were 7 patients that tested positive to expression of the p16 enzyme. Between the ages 70 to 79 there were 4 positive patients, from age 80 to 89 were 7 patients and finally from age 90 to 99 just 1 patient. The split between the two sexes was 8 male and 16 female patients. The site of the OSCC that was HPV related was on the following sites and amount. On the tongue there are 8 cases, 7 cases are on the lower gingiva, 6 on the buccal mucosa, 2 on the lower lip and 1 on the floor of the mouth. The study concludes that p16 immunohistochemistry and HPV genotyping by PCR may be beneficial for assessing HPV infection in OSCC.

Kane et al [32], focuses on the predictability of the positivity of HPV infection on advanced OSCC. Out of 124 patients 16 patients were tested HPV-positive. Another factor that was tested was tobacco use that was statistically correlated with HPV positivity. The HPV detection was done by immunohistochemical analysis of p16 enzyme expression in the tumour cell nucleus. From the 16 HPV positive samples, 14 corresponded to male patients and 2 to female patients, while all of those patients were above the age of 30. The locations of the sites were determined to the tongue, with 3 samples, and other sites with 13 sites. The study concludes on the result that, a 12.9% of the samples of T4 OSCC is associated with p16 enzyme expression, oral tobacco consumers are the one third of HPV-related OSCC and clinical-pathological variables can not on their own be used as predictors for HPV related OSCC.

Chuerduangphuiet al. [33], targets on the relationships between HPV-related OSCC and the amplification of genes in the epidermal growth factor receptor signalling cascade (EGFR). From 142 FFPE tissues with OSCC, DNA was extracted to investigate the number of EGFR, KRAS, c-myc and cyclin-D1 genes by real time-polymerase chain reaction (RT PCR). Immunohistochemical examination of TMA OSCC samples was performed to detect c-myc expression and HPV infection. HPV E6/E7 RNA detection was done by in situ hybridization. HPV infection was investigated also by PCR and RT-PCR. Of 142 samples, 81 were HPV positive. 24 of those were from male patients and 57 from female patients. The majority of the

patients were women (57) and men numbered 24. The age groups were divided to those below 60 years old (22) and those above 60 years old (59). The main site that was tested HPV-positive was the buccal mucosa with 38 samples. Following were the tongue and lip with 16 each. The gums numbered 2 samples while the floor of the Mouth and the hard palate numbered 5 and 4 respectively. The study concludes that in the EGFR signalling cascade, amplified genes - c-myc, cyclin D1, EGFR- are partially caused by HPV infection.

Kim et al. [34], investigates the HPV subtypes in OSCC via microarray technology (TMA). With a DNA chip kit 187 samples were tested for high and low risk HPV after histopathological analysis of the main mass of the tumour. Of those, 13 were tested positive at HPV DNA presence. The high risk HPV infections were 8 out of those 13 samples. Patients' age was mostly below 65 years old (7 patients) while above 65 years just 1 split in two locations. Four of the OSCC were on the tongue and additional four were on the gums of the patients. It finalises the study by noting that TMA can accurately detect simultaneously known subtypes of HPV but it is limited to detect new subtypes.

Purwanto et al. [35], focuses on the prevalence of HPV infection in Indonesian patients with OSCC. 78 OSCC FFPE samples were tested for HPV DNA plus 79 samples from normal healthy individuals. PCR was performed to identify HPV gene regions of L1 HPV16 and HPV16/18. From the 78 samples 14 were tested positive for HPV DNA. 9 samples were obtained from male and 5 samples from female patients. The age groups were about even with 8 patients below the age of 47 and 6 patients above the age of 47. The site of the OSCCs was mainly the tongue with 11 samples, then the lips with 2 samples and finally from the maxilla mucosa just 1 sample. The study ends with the verdicts that the prevalence of HPV16/18 in OSCC cases is significantly high (17.9%). HPV18 occurred more often, in HPV-related OSCC patients, than HPV16 (86%). This is supported by the high HPV18 prevalence among Indonesian cervical cancer patients studied in 1995 and 2006. Contrary, the prevalence of HR-HPV remains low in the normal Indonesian population (3.8%), but is more frequently detected in non cancer patients. De Lima et al. [36], is a study that centres on the expression of p16, MLH1 and MYC, in HPV-related OSCC. One hundred OSCC samples were sent for in situ hybridisation and later immunohistochemical detection of the HPV proteins. Thirty one samples were detected with HPV proteins. Twenty of those came from male patients and 11 from female patients. The age groups show that 8 patients were below 54 years old and 23 above 54 years old. The location of OSCC of the patients is mainly on the tongue with 8 samples, the floor of the mouth with 8 more samples and also their combination is represented by another 7 samples. There are 4 samples removed from the lips, two samples from alveolar ridge and further 2 from the hard plate. The study reinforces the hypothesis of HPV-related OSCC carcinogenesis from the expression of p16 and MYC and suppression of MLH1.

Saleh et al. [37], fixates on the localisation of the HPV16 on Oral squamous cell carcinoma. From archives, 114 OSCC specimens were obtained and tested for HPV16 by p16INK4A immunohistochemistry (IHC) and HPV16 E6/E7 mRNA by in situ hybridisation (ISH). A comparison of the two techniques was done and revealed that 16 samples were positive by p16INK4A while 14 were positive by HPV16 mRNA ISH. It was taken in consideration of the 14 samples by ISH. Four of those were in male patients while 10 in female patients. The main site was the hard palate with 5 samples. Then, the tongue with 4 samples, the gingiva with 3, followed by the floor of the mouth and the buccal mucosa with 1 sample each. The study recommends the use of p16INK4A as a substitute marker for HPV detection in OSCC, complemented by RNA ISH to identify the HPV subtype if needed.

Pérez-Islas et al. [38], is a cohort study that puts on the spotlight the DNA of the Human papilloma virus in patients with OSCC. Formalin fixed paraffin embedded tissues of OSCC were used to extract DNA and evaluate the presence of HPV DNA. Of the total 119

sample tissues 23 were tested positive on HPV DNA, 13 of which came from male patients and the rest of 10 from female patients. The mean age of all patients is 61 years old while on the HPV positive patients, 55 years old. The main sites are the tongue and the hard palate that number 10 and 4 tissue samples respectively. Then, tissues obtained from the gums and the lips are 3 samples each and finally in the retromolar area there are 2 samples and 1 from the floor of the mouth. The study concludes that HPV positivity is strongly associated with better prognosis and recurrence of the OSCC.

Khalesi et al. [39], is a cross-sectional study of 40 samples that emphasises on the cervical metastasis of the HPV-related OSCC. The samples were paraffin embedded and Immunohistochemical processed for the analysis of the p16 enzyme in the sample cells. In those 40 samples 19 were collected from males and 21 from females. The mean age of the patients is 59 years old. The OSCC site of the samples is mainly the tongue with 27 samples, followed by the alveolar mucosa with 10 samples. On a smaller amount are sites of buccal mucosa with 2 cases and the floor of the mouth with 1 sample. The study results in the statement that p16 expression is significantly higher in non-lymph node metastasis in comparison with lymph node metastasis, which can ultimately affect the prognosis of the patient.

Combining the articles, the sum of them is thirteen with a total of 361 patients of both male and female sexes, in a ratio 167/194, that HPV DNA or proteins was found from the affected cells of the tumour.

The sites of the oral cavity that are documented are the tongue, the buccal mucosa, the alveolar mucosa/gums, the lips, the floor of the mouth, the hard palate and added the option of others for further inclusion. Once added from all the above articles there are 120 samples that were collected from the tongue (there are 7 additional that are described as tongue and floor of the mouth but are not included in this category), 87 samples from the buccal mucosa, 38 samples of alveolar mucosa or gingiva or gums or alveolar ridge, 40 samples from the lip, 28 samples from the floor of the mouth (the 7 additional that are described as tongue and floor of the mouth are not included in this category), 22 samples from the hard palate and 25 on other. On the other are the retromolar areas that amount to 4, the described tongue and floor of the mouth samples that amount to 7 and 14 more samples that were not described in their reviews making a true ratio of non described to known 14: 347.

Table 1. Total acquired data of the included articles

Study	Malignancy	No, Sex(M/F)	Age Mean/ Groups	Location
[27]	OSCC	12, 12/0	x=47	Tongue: 5, FoM: 4, Retromolar: 2, Gingiva: 1
[28]	OSCC	6, 2/4	x=51/4<45, 4>45	Tongue: 6
[29]	OSCC	10, 7/3	x=71/ 14<50, 16 51-64, 52>65	Tongue: 1, Buc. Mucosa: 1, Gingiva: 5, Lip: 1, FoM: 2
[30]	OSCC	82, 29/53	x=62	Tongue: 17, Buc. Mucosa: 39, Lip: 13, FoM: 5, Hard Palate: 7, Other: 1
[31]	OSCC	24, 8/16	x=68/30-39: 2 40-49: 1 50-59: 2 60-69: 7 70-79:4 80-89: 7 90-99:1	Tongue: 8, Buc. Mucosa: 6, Gingiva: 7 Lip: 2, FoM: 1
[32]	OSCC	16, 14/2	16 >30	Tongue: 3, Other: 13
[33]	OSCC	81, 24/57	22<60, 59>60	Tongue: 16,, Buc. Mucosa: 38, Gingiva:2 Lip: 16, FoM: 5, Hard Palate:4
[34]	OSCC	8, 6/2	7< 65, 1>65	Tongue: 4, Gums: 4

[35]	OSCC	14, 4/10	8<47, 6>47	Tongue: 11, Buc. Mucosa: 1, Gingiva:2 Lip: 2
[36]	OSCC	31, 20/11	1 15-44, 7 45-54, 9 55-64, 14 >64	Tongue: 8, Gingiva:1 Lip: 4, FoM: 8, Hard Palate: 2, Tongue+FoM: 7
[37]	OSCC	14, 4/10	x=65 / 1<50, 2 51-50, 5 61-70. 4 71-80, 1 81-90, 1 91-99	Tongue: 4, Buc. Mucosa: 1, Gingiva:3,FoM: 1, Hard Palate:5
[38]	OSCC	23, 23/10	x=63	Tongue: 10, Gingiva:3 FoM: 1, Hard Palate:5
[39]	OSCC	40, 19/21	x=59	Tongue: 27, Buc. Mucosa: 2, Gingiva:10 FoM: 1, Hard Palate:4, Retromolar:2

Table 2. Representation of the sites of the HPV-related OSCC corresponding to the study from which they were obtained

Study	Tongue	Buccal Mucosa	Alveolar Mucosa/ Gums	Lip	Floor of the Mouth	Hard Palate	Other
[27]	5	-	1	-	4	-	Retromolar:2
[28]	6	-	-	-	-	-	-
[29]	1	1	5	1	2	-	-
[30]	17	39	-	13	5	7	1
[31]	8	6	7	2	1	-	-
[32]	3	-	-	-	-	-	13
[33]	16	38	2	16	5	4	-
[34]	4	-	4	-	-	-	-
[35]	11	-	1	2	-	-	-
[36]	8	-	2	4	8	2	Tongue+FoM: 7
[37]	4	1	3	-	1	5	-
[38]	10	-	3	3	1	4	Retromolar: 2
[39]	27	2	10	-	1	-	-
Total	120	87	38	40	28	22	25

Individually each article came to the following conclusions. Silveira et al. [27], concluded that when assessing for HPV DNA both low risk and high risk can be detected and high risk HPV associated also with low risk HPV- related carcinomas of Oropharynx and oral cavity showed worse survival than just high risk HPV-related OSCC. Kouketsu et al. [31]finalised the study by stating that p16 immunoreactivity and HPV genotyping by RT-PCR, may be used as markers for HPV detection in OSCC.Kane et al. [32] states that standard clinicopathological variables are not able to predict HPV-positivity. Although history of no smoking showed statistical trends towards predicting HPV-positivity in oral cancer patients. Chuerduangphui et al. [33] completes the study with the amplification of genes in the EGFR signalling cascade, cyclin D1 should be noted, is partially induced by a HPV infection. Kim et al. [34] validates that TMA can efficiently detect known subtypes of human papilloma viruses but is limited to detect new ones. Purwanto et al. [35], resolved to identify a higher prevalence of HPV-18 on OSCC - rather than HPV-16 which is detected more frequently in non-cancerous populations- which is supported by data of cervical cancer occurrence resulting from HPV-18 infection. de Lima et al. [36], ends with a conclusion that exclusively cytoplasmic staining for p16, mlh1 and myc were associated with advanced tumours. Saleh et al. [37], concludes that p16 marker can be used for detection of HPV in

OSCC and for identifying the HPV subtype in situ hybridisation. Pérez-Islas et al. [38] states that HPV-positivity is not associated with a longer overall survival of OSCC patients, but a better prognosis was associated and so was recurring or progressing disease. Khalesi et al. [39], the study states that OSCCs without cervical lymph node metastasis had a significant increase in p16 enzyme expression compared to samples with cervical lymph node metastasis. The presence of HPV DNA was higher in samples with less lymph node metastasis and possibly a better prognosis.

DISCUSSIONS

It is apparent that research regarding HPV-related oral squamous cell carcinoma is being conducted all over the globe. In the research out of the thirteen articles there are ten different countries that add on the research work and innovation for a better understanding of the OSCC. The articles originating from Brazil [27], [36] and Japan [29], [31] should be mentioned that are of different university entities. Such an achievement is quite admirable because it shows that there are active biomedical personnel that advance into scientific research. The more research done by professionals the better expertise that can be provided to the future doctors, dentists, biologists for a better understanding of physiology and pathophysiology, hence a constant evolution and improvement in the field of medicine. The latter have been considered as a high risk factor for OSCC because of their addictive nature and harmful to the oral mucosa effects. It is also important to note that the two studies with the most patients [30], [33] were both from Thailand from the same University. Such behaviours can not only create oral tumours but transform oral lesions to malignancies [43]. It is also apparent that the countries involved have the following aspect in common, the majority of the population is placed in the main cities of the country with minimal rural areas that can provide sufficient education and prevention of the HPV infection. The next factor, age can reveal the character of which an infection like HPV acts on the mucosa. On the review the articles were each one with its own subgroups that make the valuation as a whole much more difficult. Studies like [31], [37] chose to create subgroups per decade and it was seen scarcely on the articles that were examined for inclusion. The articles have made their subgroup into older or you than an age around the 45th to the 65th year of age. Their reasoning is that this age is 'hot' for malignancies to form. The immune system is generally weakened, bodies usually fatigued in the modern era of stress and lack of exercise influence the state of the immune system. Hence, it is quite difficult to determine if HPV-related OSCC is the single and independent cause or it is a cause of the cumulative exposure to the HPV DNA in combination with other risk factors or age related changes in the body's defences against carcinogenesis. Age can impact the treatment considerations due to the overall health status, physiological changes associated with ageing that can impact treatment tolerance, response and outcome. The data from the articles regarding which sex is more prone to develop HPV-related OSCC shows less than 10% difference between the two sexes. Specifically, the 167 male samples represent 46.2% of the samples, while the female samples amount to 53.7%. The main focus of the study is portrayed on the location from which the HPV-related OSCC originated (Table 4.17). As was seen out of 361 samples 120, a third, of those were on the tongue. The tongue being a receptor of all the external factors is mainly affected because of its position. Vicious habits such as nail biting, thumb sucking, biting of objects that are exposed to microbiota such as pencil caps or other types of plastic, may be a factor for a microbiological change in the oral flora. The buccal mucosa is the next site with the higher number of HPV related OSCC, which are 87. The anatomy of the buccal mucosa is related to a thin epithelium which is often subjected to trauma from stress biting. Such habits have a direct correlation to the prevalence of ORSCC [42]. The buccal mucosa is in direct

contact and is immediately affected by any substance entering the oral cavity. Smoking and alcohol being the major high risk factor in non HPV OSCC that after chronic cumulative interaction it the mucosa is influenced especially when combined with drinking (common as seen in a social interaction that combines both alcohol consumption and smoking) alcohol due to the synergetic factor that they have. Hence, if the patient is not punctual or he does not visit the dentist or the otorhinolaryngologist such lesions may be not diagnosed until they are apparent to the patient which otherwise could have been avoided. Following, with almost the same amount of samples, is the lip with 40 samples and the alveolar mucosa/gingival with 38. The lip is more exposed to risk factors such as sunlight, which is a high risk factor for OSCC, smoking and trauma from biting or accidents. Most importantly though, the epithelial lining of the lip is much thinner relative to the gingiva, making it much more susceptible to host viruses as a primary infection site. Further, there are 28 samples that were obtained from HPV-positive OSCC of the floor of the mouth and 22 samples from the hard palate. The hard palate due to its wide keratinised layer of the epithelium, is harder for micro wounds that reach the basal layer to form, thus making it harder for HPV to have as a primary site of infection the hard palate. On the other hand, the floor of the mouth is not subjected to many direct risks such as smoking, alcohol or chewing tobacco. Also, the sublingual and submandibular glands excrete saliva onto the floor of the mouth thus creating clearance of the site. The percentages out of 100 the prevalence of HPV-related OSCC on the locations are as follows: tongue 30%, buccal mucosa 24%, lip 11%, alveolar mucosa/ gums 10.5%, floor of the mouth 7.7%, Hard palate 6%, other site 6.9%.

CONCLUSIONS

The review used strict search methods and inclusion criteria on the databases PubMed and Google Scholar in order to collect the 13 articles. The contents of those articles provide us with the following conclusions. HPV-related Oral Squamous Cell Carcinoma can be found in most populations of the earth. The subtypes of HPV that cause most of the HPV-related OSCC are HPV-16 and HPV-18. Such tumours are more often seen on the tongue (30%) and the buccal mucosa (24%) while sites like the lip (11%), the gingival (10,5 %), the hard palate (6%) and the floor of the mouth (7,7%) can also be seen but more rare. An important clinical finding is that the age range that is mostly affected is between the age of 45 to 65 years old but also with occasions occurring in younger and older ages than that. Between the two sexes the review reveals that women are slightly more likely to develop HPV related OSCC with a percentage of 53.7%, making the difference with men (46.2%), less than 10%. By addressing research in the direction of prevention -vaccination and education- and diagnosis -molecular advancement- we can advance our knowledge of HPV-related OSCC and significantly improve the outcome of future patients.

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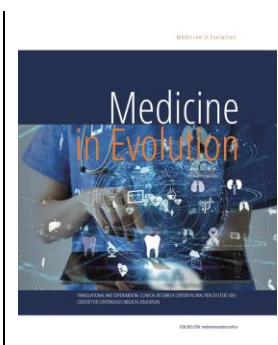
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Systemic correlations of the oral microbiome and salivary parameters in smoking patients



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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 al (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].

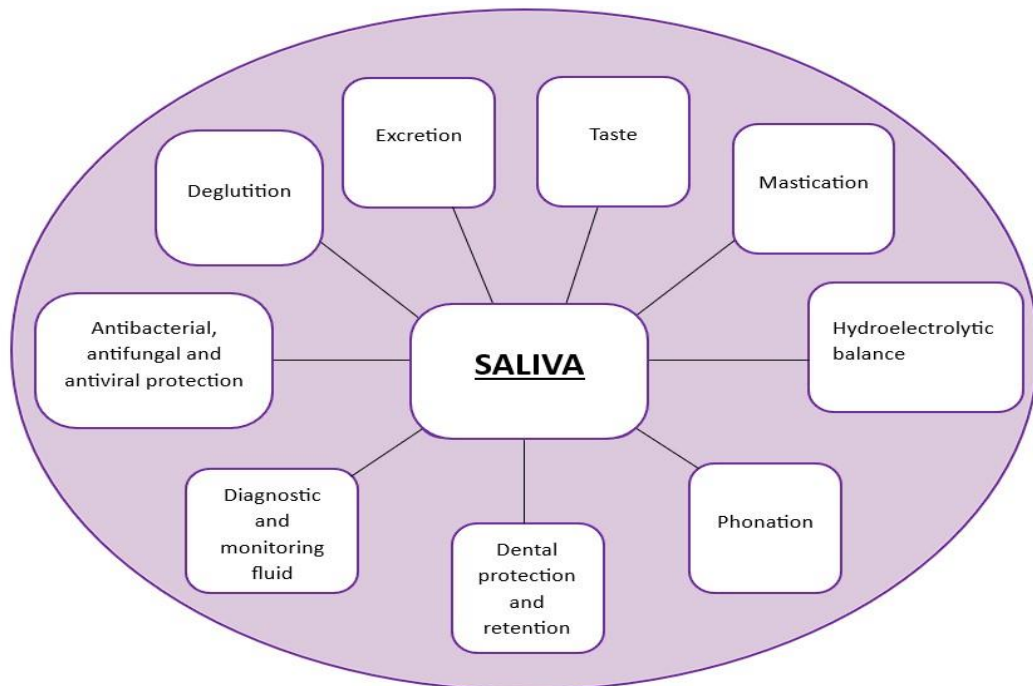


Figure 1. The saliva roles in the body

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, japhd, 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.

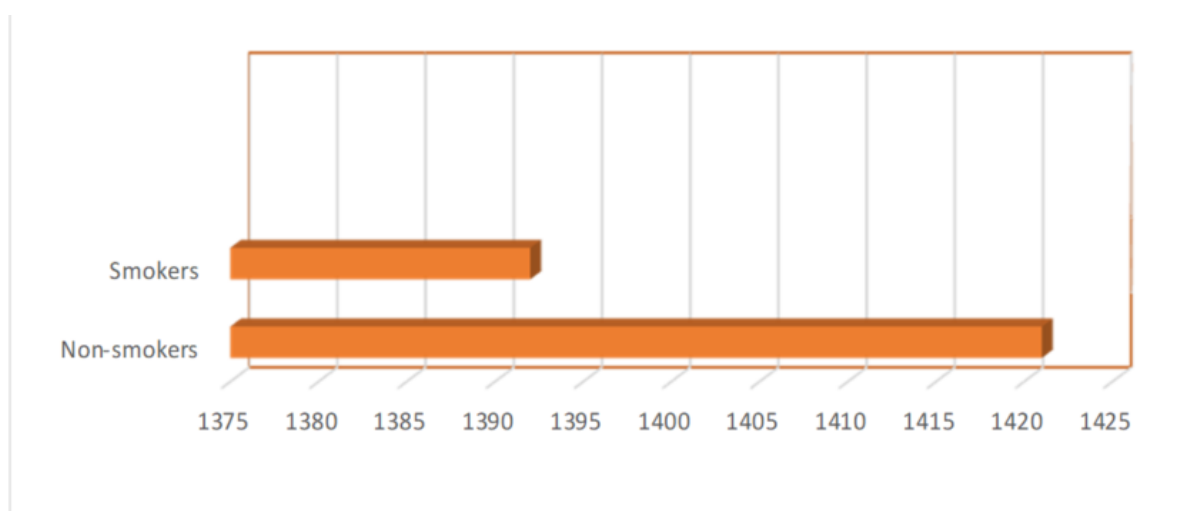


Figure 2. Control groups and case groups

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.

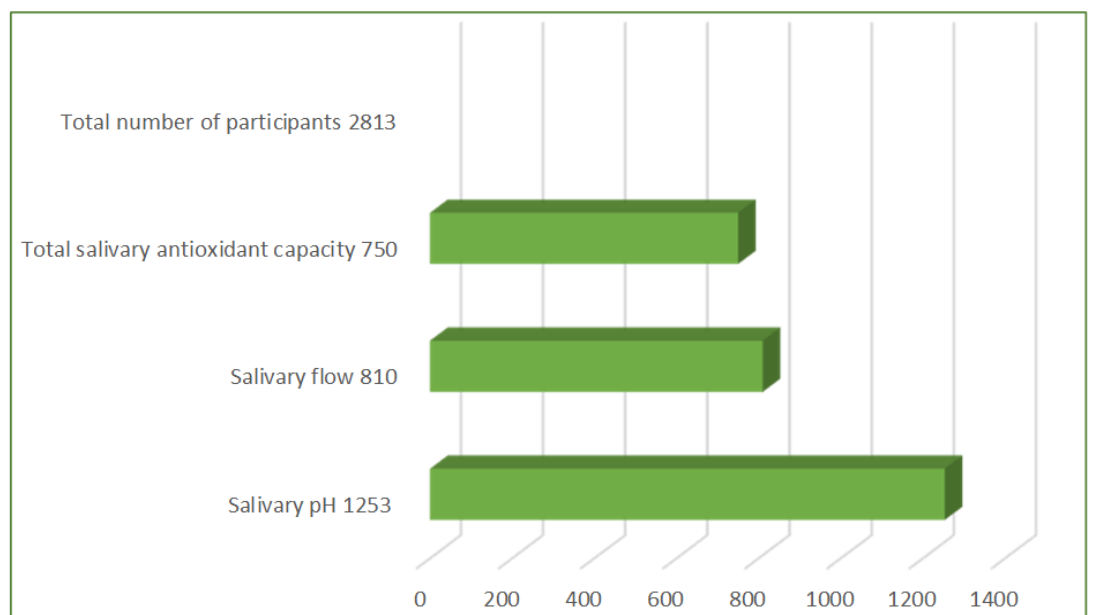


Figure 3. Distribution of study participants according to the monitored parameter

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 individual research.

Table 1. Determination of pH

Name of author, year	Country	pH determination method	Participants		pH value	
			Non-smokers	Smokers	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.

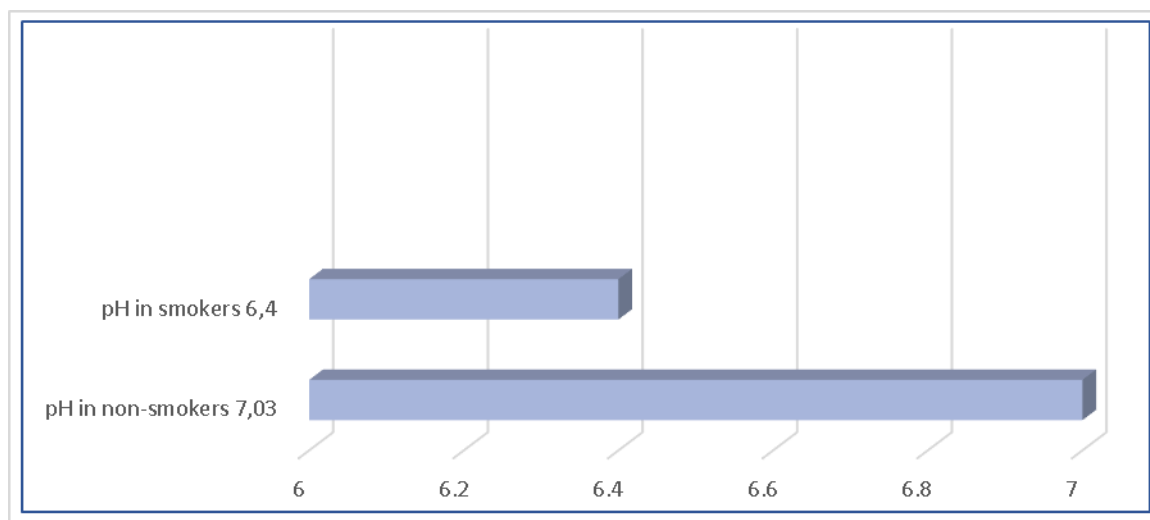


Figure 4. Average pH value in smokers/non-smokers

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 Ömeroğlu 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.

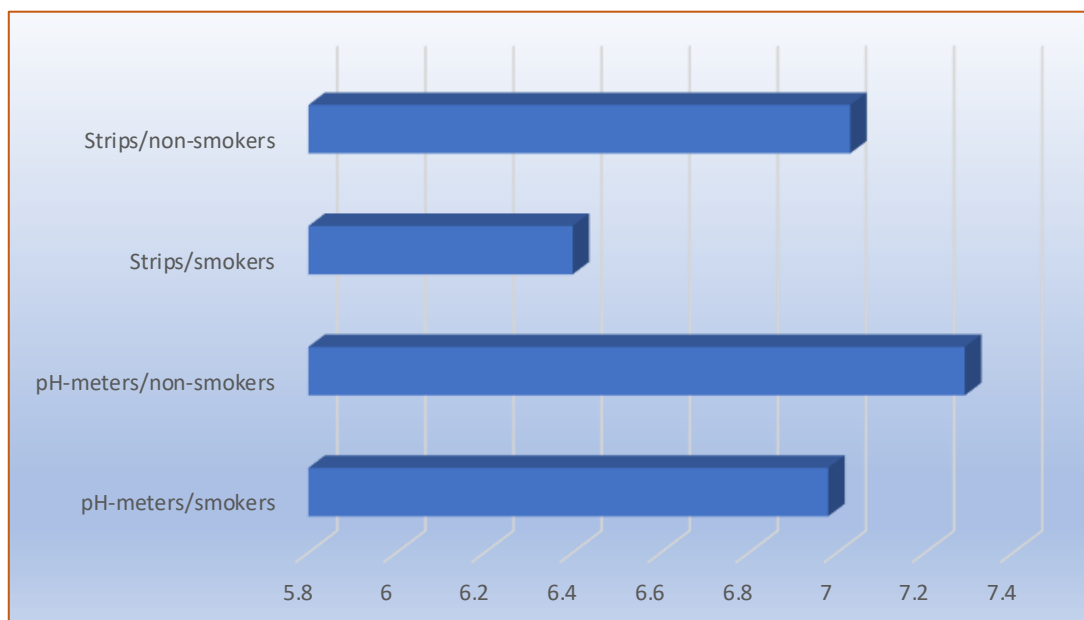


Figure 5. Difference between pH measured with strips and pH meter

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		The value of salivary flow	
				Non-smokers	Smokers	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 Alae, 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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Effect of glycerin on the optical proprieties of PEEK



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Abstract

The aim of this study is to evaluate the optical proprieties of PEEK and the influence of a coupling medium (G-glycerin), by testing the hypothesis that glycerin can influence translucency. Eight standardized specimens were fabricated from CopraPeek (WhitePeaks Dental Solution, Germany) Medium A2 dentine 10 mm x 15 mm polished to 1.0 in thickness and Nexco Paste Dentine veneer material shade A3, with thicknesses of 0.5, 1.0 and 1.5 mm. Color and CIELAB coordinates were evaluated using Vita 0.5, 1.0 and 1.5 mm. Color and CIELAB coordinates were evaluated using Vita Easyshade.

All evaluations were repeated using glycerin and the values were recorded. Translucency is the relative amount of light that passes through a unit thickness of a material. Translucency parameter (TP) and contrast ratio (CR) were calculated. Also the value of color change (ΔE^*) was calculated and statistical analysis was performed [1].

Expected results: The mean TP values of the studied samples were in the range of 1.22-3.81, which is lower than those reported for natural teeth [2][3][4]. The OP values of PEEK were recorded in the range of 0.78-3.11, also lower than those of natural teeth. Although mean translucency values were significantly different for each method (TP and CR), they were highly correlated ($r^2=0.70$), even when G was used ($r^2=0.56$).

Conclusion: The coupling medium significantly influenced the mean values of ΔE and translucency. Clinical assessment of perceptibility and acceptability thresholds of translucency is needed

Keywords: PEEK, glycerin, optical proprieties

INTRODUCTION

PEEK belongs to the PAEK family and is a biocompatible and chemically stable material for most organic and inorganic substances. Due to its excellent biological and physical properties, this material is used both in general medicine and in dentistry. However, there are also some aesthetic disadvantages that limit the use of PEEK as a monolithic element. The optical properties of PEEK include low translucency and a gray color [1][3][4]. Therefore an additional layer of composite resin is required for plating. This presents a challenge in achieving adequate strength between the RDC and PEEK physiologic component [6][7].

Conditioning the polymer surface with sulfuric acid or piranha solution increases the adhesion strength. However, given the risks of using these materials in the oral cavity, their applicability is limited. Other studies have evaluated the degree of strength of the bond between PEEK and material intended for plating being a resin intended for provisional restorations, applying various surface treatments (mechanical etching by sandblasting or a chemical conditioning), respectively various primers used for conditioning [5][8][9][10].

Only one study tested the adhesion between the PEEK surface and RDC physiognomic components. In that study, the surfaces were etched, but no adhesives were used.

Aim and objectives

The aim of this study is to evaluate the color and translucency of polyetheretherketone (PEEK) and the influence of the binding medium (glycerin) on the optical properties, testing the hypothesis that glycerin influences the optical properties regardless of the evaluation method.

MATERIAL AND METHOD

A CopraPEEK disc (WhitePeaks Dental Solution, Essen, Germany) was used for this study. There were 6 lots of 8 plates each, having the following dimensions 10x15x1mm thick. The plates were polished using 450-2000 micron grit silicon carbide paper, the final gloss being achieved with a brush and a universal polishing paste (Ivoclar Vivadent). The final thickness of each pad was measured using a caliper. The first 3 batches were conditioned by sandblasting with 110 micron aluminum oxide sandblasting, then VisioLink primer was applied according to the manufacturer's instructions. Afterwards the plates were coated with Nexco color A2 photopolymerizable composite resin in layers of 0.5; 1 and 1.5 mm. The following batches were also conditioned by sandblasting with Al₂O₃, application of VisioLink primer, a layer of Nexco opaquer in one brush and later plated with Nexco light-curing composite resin in layers of 0.5, 1 and 1.5 mm.

Color and CIELAB coordinates were evaluated using a Vita Easyshade spectrophotometer on a white background and a black background [11][12][13]

All evaluations were repeated using glycerin as the binding medium and the values were recorded [3][14][15]

Translucency is the relative amount of light that passes through a unit thickness of a material.

The translucency parameter (TP) was calculated using the following formula

$TP = [(L^*b - L^*w)^2 + (a^*b - b^*w)^2 + (b^*b - b^*w)^2]^{1/2}$ and the contrast ratio (CR) was calculated using the following formula [4][9][21][22]

If the material is completely opaque, the TP value is zero. As the TP value increases, the translucency of the material increases [15][16][17].

Also, the total color change value ΔE^* was calculated and statistical analyzes were performed (t-test, Pearson correlation test).

To quantify color change levels to a clinical standard, ΔE^* values were converted to NBS units according to the following formula [4][18][19][22].

$$\text{NBS} = \Delta E^* \times 0.92$$

RESULTS

The mean TP values of the study samples were in the range of 1.22-3.8, which is lower than the values reported for natural teeth. Average TP values for 1mm thickness of human enamel and dentin are 14.7; 15.2; 18.7; 16.4.

The OP values of the samples were recorded in the range of 0.78-3.11, being also lower than the values of natural teeth, which recorded a value of 4.8.

Samples that had an intermediate layer of opaquer tended to be more opaque compared to those without an opaquer. A possible reason could be the opaquer layer that intensifies the yellowish color of the samples.

Other statistical analyzes are:

TP-TPg = The value of r^2 , the correlation coefficient, is 0.5281. This is a moderate positive correlation, meaning that there is a tendency for high scores on variable X to go with high scores on variable Y (and vice versa).

OP-OPg = The value of R^2 , the correlation coefficient, is 0.0669. Although technically a positive correlation, the relationship between the variables is weak (nb. the closer the value is to zero, the weaker the relationship).

CR-CRg = The value of R^2 , the correlation coefficient, is 0.2464. Although technically a positive correlation, the relationship between the variables is weak (n.b. the closer the value is to zero, the weaker

TP-TPg = The P-Value is $< .00001$. The result is significant at p values $< .01$ (P Value from Pearson (R) Calculator)

OP-OPg = The P-Value is .086579. The result is significant at values p $< .10$ (P Value from Pearson (R) Calculator)

CR-CRg = The P-Value is .000408. The result is significant at p values $< .01$ (P Value from Pearson (R) Calculator)

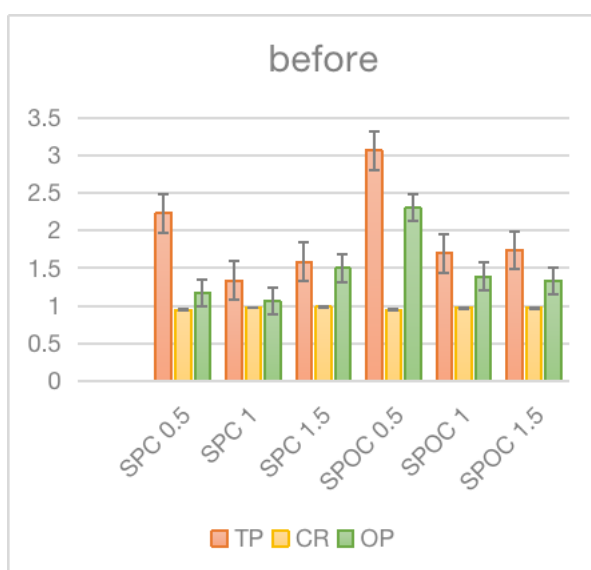


Figure 1. Mean TP, CR, OP values for all groups

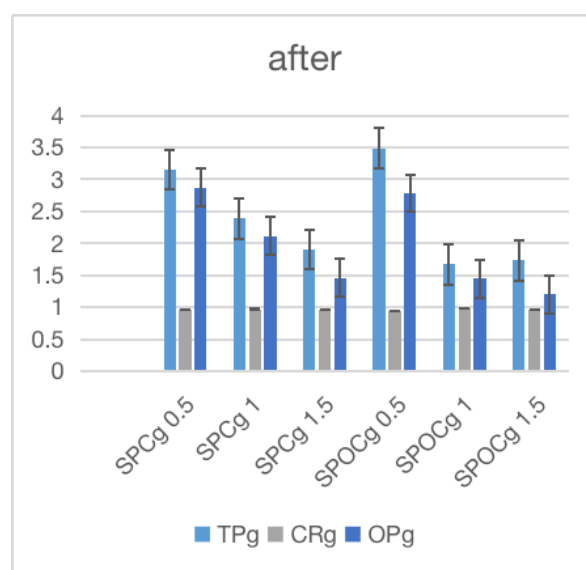


Figure 2. Mean TP, CR, OP values for all groups with binding medium

E recorded increased values in batches with a thickness of 0.5mm SPC and 1mm SPOC.

The lowest values were recorded in the 1.5 SPOC and 1.5 SPOCg thick batches.

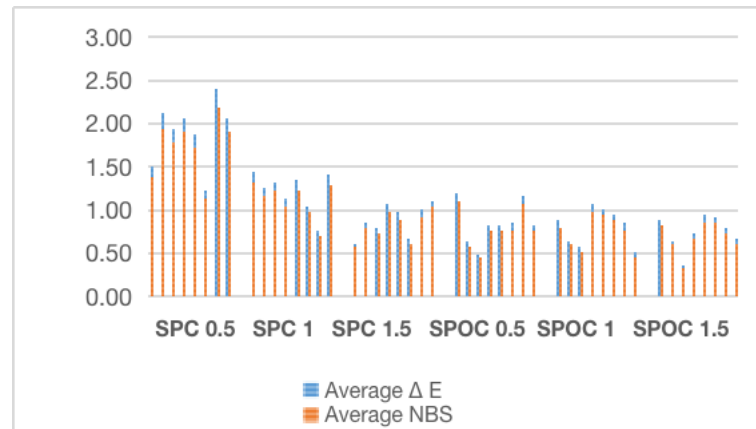


Figure 3. Mean values of ΔE and Translucency

DISCUSSIONS

The use of glycerin as a binding medium significantly influenced the mean values of ΔE and translucency. The groups where glycerin was used had higher values of the parameters compared to the control groups.

The thickness of the physiognomic component in the RDC also influenced the mean translucency and opalescence values. 0.5mm thick plates show higher TP values than 1.5mm thick plates. The highest OP values were recorded for the 0.5 mm thick lot and with an intermediate layer of opaquer.

CONCLUSIONS

It can be stated that the intermediate layer of opaquer influences the optical properties of the analyzed specimens.

The use of a binding medium (glycerin) will lead to perceptible optical changes, according to the recorded NBS values of 1.5-3.0 in batches with a thickness of 0.5 SPC and 0.5 mm SPOC, and in the rest of the batches barely perceptible changes were recorded.

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Dental emergencies during the COVID-19 pandemic in Oradea, Romania: patients' gender and living environment, and the location of affected teeth



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Abstract

Aim and objectives. The aim of this study was to investigate the location of the affected teeth in patients who were treated in the Dental Emergency Department from Oradea, during the lockdown period (2020), and to compare the characteristics identified during the lockdown with those identified in the pre-lockdown year (2019) and post-lockdown year (2021). **Material and methods.** This retrospective study was conducted on patients who were treated for a dental emergency in the Department of Dental Emergency from Oradea, in the following time periods: March-May 2019, March-May 2020 and March-May 2021. The investigated variables were patients' gender, patients' living environment and the location of the affected teeth. **Results.** 634 patients were treated in 2019, 784 in 2020, and 560 in 2021. In all investigated time periods, the most affected teeth were located in the lower posterior area of the dental arches (from 38.8% to 55.7%). In most of the investigated months, patients' gender was not statistically significant associated with the location of the affected teeth, but in April 2020, it was observed that female patients were more frequently associated with treated teeth located in the lower posterior area (54.4%) than in the upper posterior area (37.8%), while male patients were more frequently associated with treated teeth located in the upper posterior area (62.2%) than in the lower posterior area (45.6%). In most of the investigated months, there were no associations between patients' living environment and the location of affected teeth, but in April 2021, it was observed that patients from the rural environment were more frequently associated with treated teeth located in the upper anterior area (54.2%) than treated teeth located in the lower anterior area (0%) while patients from the urban environment were more frequently associated with treated teeth located in the lower anterior area (100%) than treated teeth located in the upper anterior area (45.8%). **Conclusions.** Preventive programs that address male and female patients, as well as rural and urban patients would be helpful.

Keywords: COVID-19, dental emergency, Romania

INTRODUCTION

The 2019 coronavirus disease or COVID-19 was caused by a new type of coronavirus, SARS-CoV-2 [1], a virus that has spread rapidly at a global level [2]. The COVID-19 disease is manifested by various symptoms such as fever, cough, dyspnea, fatigue, but it can also be accompanied by atypical neurological, dermatological [3] or even oral [4] symptoms. The evolution of the disease can be negative and can rapidly cause the death of the affected individuals [5], especially in patients with associated systemic diseases [6]. Arterial hypertension, diabetes and cardiovascular diseases are among the most common systemic diseases associated with a high death rate in patients with COVID-19 [6].

The disease is highly contagious and can be transmitted through direct and indirect contact. It can be transmitted through saliva droplets, direct contact between people, contaminated objects or through air [7]. In order to limit the spread of the virus and to control the disease, the World Health Organization (WHO) declared a global pandemic of COVID-19 and recommended a global lock-down [8]. Apart from the lockdown, a series of preventive measures were instituted, all very useful in limiting the spread of the virus. Social distancing, mandatory wearing of face masks, and closing national borders were some of the measures instituted during this period [9].

In Romania, the lockdown caused by the COVID-19 pandemic was instituted on March 21, 2020 and was suspended on May 15, 2020 [10]. The lockdown had a strong impact on people's mental health, negatively influenced people's social life, but also impacted the economic system and the health system [11]. During the lockdown, the oral health of the patients was also affected, considering the fact that dental offices were closed for routine dental procedures and only dental emergencies could be treated [10]. In Romania, the following dental pathologies could be treated as emergencies during the lockdown period: post-extraction hemorrhages, acute pulpitis, acute apical periodontitis, pericoronitis, post-extraction alveolitis, abscesses, cellulitis, mandibular fractures, dislocations of the temporomandibular joint, and ulceronecrotic gingivostomatitis and only these could be treated [12]. They could only be managed in centers that were authorized by the state [10].

Considering that globally the prevalence of caries is high among both the pediatric and adult population [13], the lack of routine dental treatments during the COVID-19 lockdown could have led to a higher rate of complications. Among the most frequent carious complications are pulpitis, apical periodontitis and abscesses [14]. In addition, the reduction in the number of functional dental offices during the lockdown period could have excessively increased the number of patients who were treated in dental emergency centers, this having a negative impact on the overburdened medical staff [15].

Aim and objectives

The purpose of this study was to investigate the location of the affected teeth in patients who were treated in the Dental Emergency Department from Oradea during the lockdown period (2020), and to compare the characteristics identified during the lockdown period with those identified in the pre-lockdown year (2019) and with those identified in the post-lockdown year (2021), according to gender and living environment.

MATERIAL AND METHODS

The study was designed as a retrospective research and was based on the analysis of the medical records belonging to patients who were treated for dental emergencies at the Oradea County Emergency Clinical Hospital. The emergency dental service is located in the

building of the Faculty of Medicine and Pharmacy of the University of Oradea. Three periods were analyzed: the pre-lockdown period (1 March 2019 – 31 May 2019), the lockdown period (1 March 2020 – 31 May 2020) and the post-lockdown period (1 March 2021 – 31 May 2021).

Initially, all patients who came to the dental emergency service during the previously mentioned periods were included in the study, but later, patients who did not have complete information in the medical records, or who did not sign the informed consent, were excluded.

In order to carry out this research, the following variables were investigated: the gender of the patients (male, female), the living environment of the patients (rural, urban), as well as the area on the dental arches where the dental emergency was treated (upper anterior, upper posterior, lower anterior, lower posterior). To establish these areas, the following aspects were taken into account: the upper anterior area (UA) was represented by upper incisors and upper canines (deciduous or permanent), the upper posterior area (UP) was represented by upper premolars and upper deciduous or permanent molars, the lower anterior area (LA) was represented by lower incisors and lower canines (deciduous or permanent), and the lower posterior area (LP) was represented by lower premolars and lower deciduous or permanent molars.

The study was approved by the Ethics Committee of the Oradea County Emergency Clinical Hospital (IRB No. 22143/06.07.2022) and complied with the principles stated in the Declaration of Helsinki from 2008, as well as the principles stated in the amendments added later to the Declaration of Helsinki. All participants signed an informed consent in which they agreed to the anonymous use of the data obtained as a result of the treatment carried out in the dental emergency service, and for those who were under the age of 18 years, the informed consent was signed by parents or legal guardians.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 25 and Microsoft Office Excel/Word 2013. Qualitative variables were expressed in absolute form or as percentages, and were compared using Fisher's Exact or Pearson Chi-Square tests. Z-tests with Bonferroni correction were performed to detail the results after obtaining the contingency tables.

RESULTS

The final sample included a number of 1978 patients, of which 634 were treated between March and May 2019, 784 were treated between March and May 2020, and 560 were treated between March and May 2021 [16]. In the investigated period of 2019, a number of 301 female patients (March - n=85; April - n=119; May - n=97) and 333 male patients (March - n=112; April - n=124; May - n=97) were treated. In 2020, 421 female patients (March - n=129; April - n=177; May - n=115) and 363 male patients (March - n=108; April - n=205; May - n=124) were treated. In 2021, 292 female patients (March - n=111; April - n=95; May - n=86) and 268 male patients (March - n=104; April - n=80; May - n=84) were treated (Figure 1).

Depending on the living environment, in 2019, between March and May, 302 patients from rural areas (March - n=100, April - n=100; May - n=102) and 332 from urban areas (March - n=97; April - n=142; May - n=92) were treated. In the same investigated period, in 2020, 343 patients from rural areas (March - n=106; April - n=153; May - n=84) and 441 from urban areas (March - n=131; April - n=229; May - n=155) were treated. In 2021, 235 patients from rural areas (March - n=94; April - n=79; May - n=62) and 325 from urban areas (March - n=121; April - n=96; May - n=108) were treated (Figure 2).

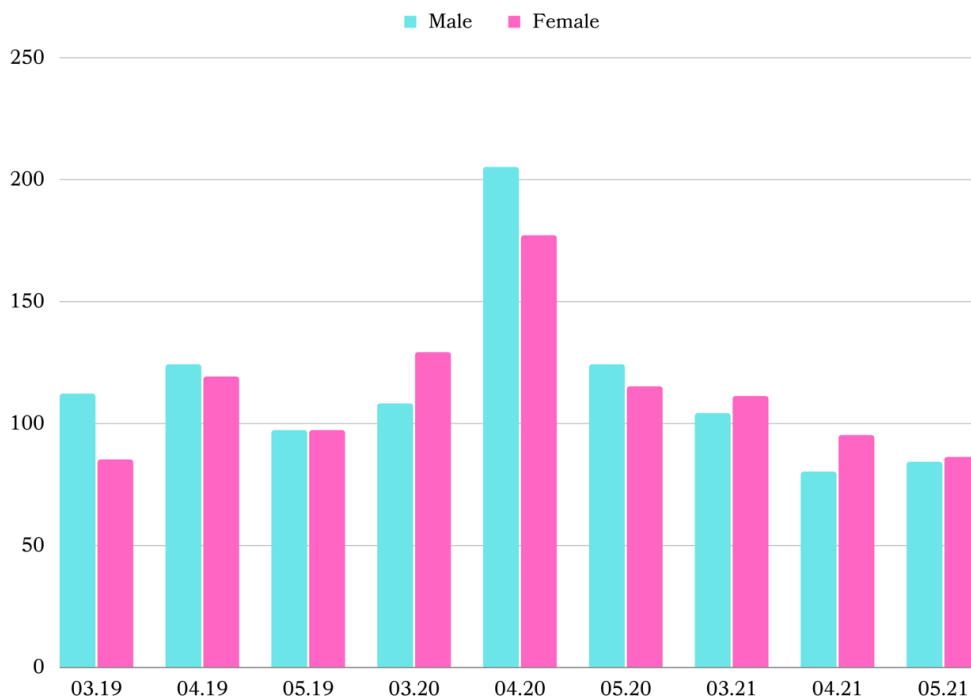


Figure 1. Distribution according to gender

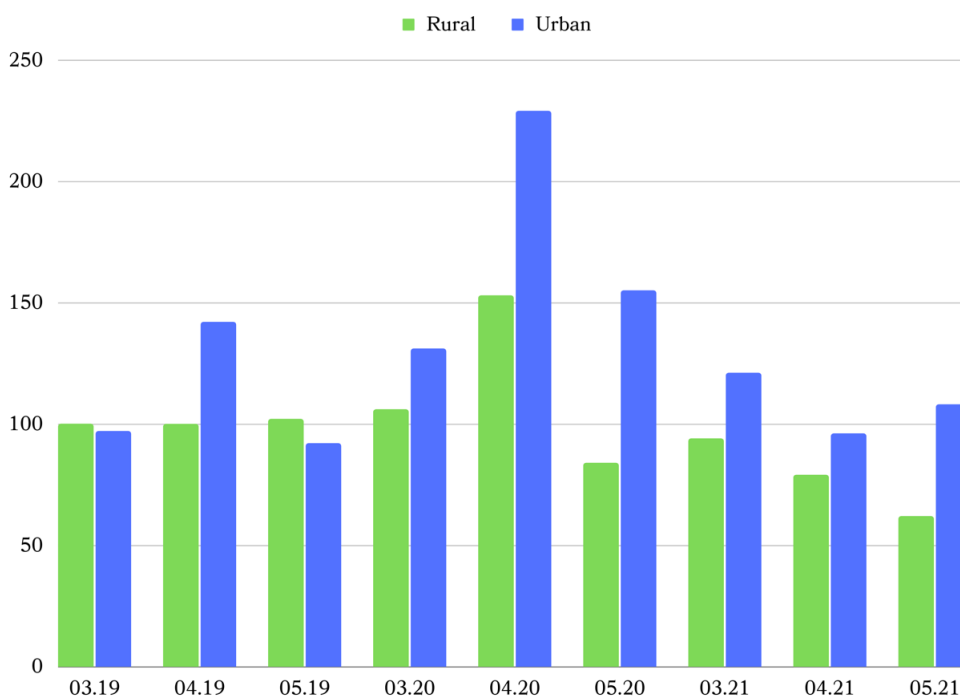


Figure 2. Distribution according to living environment

The data regarding the topography of the teeth treated during the investigated periods are shown in Table I. In all 3 years, the teeth that were treated were most frequently located at the level of the lower posterior arch, and the least treated teeth were those in the lower anterior arch.

Table I. Location of treated teeth

PERIOD	UA	UP	LA	LP
2019				
MARCH	24 (12.2%)	68 (34.5%)	8 (4.1%)	97 (49.2%)
APRIL	33 (13.6%)	85 (35%)	11 (4.5%)	114 (46.9%)
MAY	21 (10.8%)	63 (32.5%)	9 (4.6%)	101 (52.1%)
2020				
MARCH	24 (10.1%)	71 (30%)	10 (4.2%)	132 (55.7%)
APRIL	47 (12.3%)	143 (37.4%)	21 (5.5%)	171 (44.8%)
MAY	22 (9.2%)	86 (36%)	10 (4.2%)	121 (50.6%)
2021				
MARCH	27 (12.6%)	85 (39.5%)	16 (7.4%)	87 (40.5%)
APRIL	24 (13.7%)	56 (32%)	8 (4.6%)	87 (49.7%)
MAY	29 (17.1%)	65 (38.2%)	10 (5.9%)	66 (38.8%)

UA - upper anterior; UP - upper posterior; LA - lower anterior; LP - lower posterior

The data in Table II represent the distribution of patients observed in 2019 according to the location of the teeth and gender. According to the Fisher's Exact test and Pearson Chi-Square tests, the associations between teeth location and gender were not significant in any of the studied months.

Table II. Distribution according to topography and gender for 2019

	UA	UP	LA	LP	p*
March 2019					
Female	12 (50%)	25 (36.8%)	6 (75%)	42 (43.3%)	0.182
Male	12 (50%)	43 (63.2%)	2 (25%)	55 (56.7%)	
April 2019					
Female	16 (48.5%)	40 (47.1%)	6 (54.5%)	57 (50%)	0.959
Male	17 (51.5%)	45 (52.9%)	5 (45.5%)	57 (50%)	
May 2019					
Female	9 (42.9%)	34 (54%)	1 (11.1%)	53 (52.5%)	0.089
Male	12 (57.1%)	29 (46%)	8 (88.9%)	48 (47.5%)	

UA - upper anterior; UP - upper posterior; LA - lower anterior; LP - lower posterior; *Fisher's Exact Test, **Pearson Chi-Square

In 2020, the data obtained showed that there were no significant associations between the location of teeth and gender in the months of March and May, but for the month of April it was observed that female patients were more frequently associated with treated teeth located in the lower posterior area (54.4%) than in the upper posterior area (37.8%), while male patients were more frequently associated with treated teeth located in the upper posterior area (62.2%) than in the lower posterior area (45.6%) according to the Fisher's Exact test (p=0.030) (Table III).

Table III. Distribution according to topography and gender for 2020

	UA	UP	LA	LP	p*
March 2020					
Female	12 (50%)	44 (62%)	8 (80%)	65 (49.2%)	0.120
Male	12 (50%)	27 (38%)	2 (20%)	67 (50.8%)	
April 2020					
Female	21 (44.7%)	54 (37.8%)	9 (42.9%)	93 (54.4%)	0.030
Male	26 (55.3%)	89 (62.2%)	12 (57.1%)	78 (45.6%)	
May 2020					
Female	13 (59.1%)	46 (53.5%)	4 (40%)	52 (43%)	0.305
Male	9 (40.9%)	40 (46.5%)	6 (60%)	69 (57%)	

UA - upper anterior; UP - upper posterior; LA - lower anterior; LP - lower posterior; *Fisher's Exact Test, **Pearson Chi-Square

The data in Table IV represent the distribution of patients observed in 2021 related to the location of the teeth and gender. According to the Fisher's Exact test and Pearson Chi-Square test, the associations between teeth location and gender were not significant in any of the studied months.

Table IV. Distribution according to topography and gender for 2021

	UA	UP	LA	LP	p*
March 2021					
Female	14 (51.9%)	40 (47.1%)	10 (62.5%)	47 (54%)	0.654
Male	13 (48.1%)	45 (52.9%)	6 (37.5%)	40 (46%)	
April 2021					
Female	12 (50%)	30 (53.6%)	3 (37.5%)	50 (57.5%)	0.690
Male	12 (50%)	26 (46.4%)	5 (62.5%)	37 (42.5%)	
May 2021					
Female	13 (44.8%)	34 (52.3%)	6 (60%)	33 (50%)	0.843
Male	16 (55.2%)	31 (47.7%)	4 (40%)	33 (50%)	

UA - upper anterior; UP - upper posterior; LA - lower anterior; LP - lower posterior; *Fisher's Exact Test, **Pearson Chi-Square

The data in Tables V and VI represent the distribution of patients observed in 2019 and 2020, related to the location of the teeth and the living environment of the patients. According to the applied statistical tests, no statistically significant associations were identified.

Table V. Distribution according to topography and living environment for 2019

	UA	UP	LA	LP	p*
March 2019					
Rural	11 (45.8%)	30 (44.1%)	4 (50%)	55 (56.7%)	0.410
Urban	13 (54.2%)	38 (55.9%)	4 (50%)	42 (43.3%)	
April 2019					
Rural	20 (60.6%)	20 (35.3%)	5 (45.5%)	46 (40.4%)	0.092
Urban	13 (39.4%)	55 (64.7%)	6 (54.5%)	68 (59.6%)	
May 2019					
Rural	13 (61.9%)	35 (55.6%)	2 (22.2%)	52 (51.5%)	0.238
Urban	8 (38.1%)	28 (44.4%)	7 (77.8%)	49 (48.5%)	

UA - upper anterior; UP - upper posterior; LA - lower anterior; LP - lower posterior; *Fisher's Exact Test, **Pearson Chi-Square

Table VI. Distribution according to topography and living environment for 2020

	UA	UP	LA	LP	p*
March 2020					
Rural	8 (33.3%)	37 (52.1%)	3 (30%)	58 (43.9%)	0.297
Urban	16 (66.7%)	34 (47.9%)	7 (70%)	74 (56.1%)	
April 2020					
Rural	15 (31.9%)	60 (42%)	7 (33.3%)	71 (41.5%)	0.578
Urban	32 (68.1%)	83 (58%)	14 (66.7%)	100(58.5%)	
May 2020					
Rural	7 (31.8%)	28 (32.6%)	4 (40%)	45 (37.2%)	0.881
Urban	15 (68.2%)	58 (67.4%)	6 (60%)	76 (62.8%)	

UA - upper anterior; UP - upper posterior; LA - lower anterior; LP - lower posterior; *Fisher's Exact Test, **Pearson Chi-Square

Regarding the associations between the location of the teeth and the living environment of the patients in 2021, they were statistically insignificant for the months of March and May, but significant for the month of April, where it was observed that patients from the rural environment were more frequently associated with treated teeth located in the upper anterior area (54.2%) than treated teeth located in the lower anterior area (0%) while

patients from the urban environment were more frequently associated with treated teeth located in the lower anterior area (100%) than treated teeth located in the upper anterior area (45.8%) (Table VII).

Table VII. Distribution according to topography and living environment for 2021

	UA	UP	LA	LP	p*
March 2021					
Rural	10 (37%)	40 (47.1%)	7 (43.8%)	37 (42.5%)	0.824
Urban	17 (63%)	45 (52.9%)	9 (56.2%)	50 (57.5%)	
April 2021					
Rural	13 (54.2%)	25 (44.6%)	0 (0%)	41 (47.1%)	0.042
Urban	11 (45.8%)	31 (55.4%)	8 (100%)	46 (52.9%)	
May 2021					
Rural	13 (44.8%)	27 (41.5%)	3 (30%)	19 (28.8%)	0.325
Urban	16 (55.2%)	38 (58.5%)	7 (70%)	47 (71.2%)	

UA - upper anterior; UP - upper posterior; LA - lower anterior; LP - lower posterior; *Fisher's Exact Test, **Pearson Chi-Square

DISCUSSIONS

The COVID-19 pandemic has placed an enormous burden on the global health system, and the lockdown caused by the pandemic had negative and unwanted consequences on the general health and oral health of the population [17]. Certain population groups were more intensely affected, these being mainly people with chronic diseases [17], or elderly people, on whom the pandemic had a particularly strong physical, mental and emotional impact [18]. In dentistry, during the lockdown period, only the emergency treatment of dental problems was permitted, and the use of hand tools was preferred, in order to reduce the production of aerosols [19]. Moreover, in Romania, during the lockdown period, dental emergencies could only be treated in certain authorized centers [16]. This explains the increased number of patients who were treated between March and May 2020 in the emergency dental service, as it emerged from this study. Overcrowding in dental emergency departments can have a strong negative impact on the medical staff, increasing the level of stress and fatigue [20].

The aim of this study was to investigate the topography of the teeth treated for dental emergency in the three analyzed periods, depending on the gender of the patients and the living environment of the patients. The gender of the patients was chosen as a variable because it was observed that there are differences between men and women in terms of the level of knowledge about oral health [21], in terms of the prevalence of dental caries [22], or in terms of dental visits [22]. In this study, the patients who were treated most frequently for dental emergency, during the lockdown period (2020) were female patients, but in the other investigated years (2019 and 2021) male patients prevailed. This aspect is probably also due to the fact that female patients go to the dentist more frequently and choose to go to the dentist even for minor problems [23], compared to male patients who tend to ignore their oral health issues [24].

Concerning the living environment of the patients, in all the analyzed periods, the patients came mainly from the urban environment, but the number of patients from the rural environment was also high. The higher proportion of patients from the urban environment could also be due to the fact that the dental emergency service in which the study was carried out was located in the urban environment. At the same time, the small number of dental offices in rural areas [16], but also the high costs of dental treatments can make rural residents more reluctant to access dental services [25]. Other obstacles that can negatively impact access to dental services, both in rural and urban areas, are the lack of time, the need to travel to other doctors or to other offices for specialized treatments, and fear [26].

A final variable that was considered in this study was the location of the teeth that required treatment. In all 3 investigated periods, the most affected teeth were located in the lower posterior area of the dental arches. The high prevalence of caries for the mandibular first molars (lower posterior area), was also identified by other authors. Sadeghi Mostafa (2007) identified a slightly higher prevalence of caries at the level of mandibular first molars in a group of 12-year-old children from Iran [27], and Que et al. (2021) identified a higher prevalence of caries at the level of mandibular first molars in a group of children from São Tomé Island [28]. Apart from the known etiological factors of dental caries [29], another explanation for the higher prevalence of caries at the level of mandibular first molars could be the faster eruption and at a young age of the lower first permanent molars [30]. However, there are also authors who have identified a higher prevalence of dental pathology at the level of the maxillary arch. Demirci et al. (2010) identified a higher prevalence of caries in the maxillary and mandibular molars compared to incisors, and a higher susceptibility to caries for the maxillary teeth than for the mandibular teeth [31].

We believe that this study brings valuable information regarding the influence of gender and the living environment on the topography of the teeth treated in the dental emergency service from Oradea. It is necessary to implement dental prevention programs that address rural and urban patients. Increasing the number of state centers that offer emergency dental services, would be helpful. Obtaining similar information from other emergency dental centers in the country would be useful for creating an overall picture at the national level.

CONCLUSIONS

In this study sample, the number of patients treated in 2020 (during lockdown) was higher than in the other investigated periods. Most of the treated teeth were located in the lower posterior area. If in 2019 and 2021 no significant associations were identified between gender and the topography of the affected teeth, in April 2020, it was observed that female patients were more frequently associated with treated teeth located in the lower posterior area, while male patients were more frequently associated with treated teeth located in the upper posterior area. It was observed that in April 2021, patients from the rural environment were more frequently associated with treated teeth located in the upper anterior area than treated teeth located in the lower anterior area, while patients from the urban environment were more frequently associated with treated teeth located in the lower anterior area, than treated teeth located in the upper anterior area.

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PRF in modern dentistry: An innovative approach to oro-dental tissue regeneration



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Abstract

In this study we will follow the use of PRF membranes in modern dentistry and implantology. We will explore the role, importance and specific techniques of using PRF membranes in various implant surgery procedures. This work was based on a study of 30 patients of both sexes who underwent treatment using PRF membranes for the first time. Prior to this, patients were informed about treatment plan and intervention process. The first objective analyzed was the presence of post-operative pain. On the first day after the intervention, more than 60% of patients did not experience pain, and of those who experienced pain, 20% experienced mild pain and only 10% of patients experienced moderate pain. No patient described the pain as intense. The second day after the intervention 93% of patients had no pain at all, and 7% had mild pain. On the third day after the intervention, all patients confirmed the total absence of pain. The second objective analyzed was the presence of post-operative edema. On the first day after the intervention, 83% of patients had edema present. The second day after the intervention the number of patients with edema decreased by more than half, reaching 33%. On the third day, only 10% of patients still had edema. The last objective analyzed was the presence of post-operative infection, in order to prove the capacity of PRF membranes to form a protective barrier against infection. Post-operative infections were present in only 6% of the participants. The use of PRF membranes plays a crucial role in maintaining the structure of the alveolar ridge it also helps the preservation of the integrity and stability of the alveolar bone. By restoring gingival tissues, PRF membranes contribute on improving the overall oral health of the patients. The use of PRF membranes reduces the risk of peri-implantitis and peri-implant mucositis. The PRF membranes stimulate the healing process of periodontal defects, favoring the regeneration of the affected tissues. The use of PRF membranes reduces patients discomfort in the initial phase of the healing process, contributing to the absence of pain and offering a fast recovery.

Keywords: PRF, implantology, surgery, osseointegration

INTRODUCTION

The field of dentistry is in a continuous dynamic, constantly making significant progress in terms of the innovative approach to the techniques used in the regeneration of oro-dental tissues. One of these approaches that has received considerable attention is the use of platelet-rich fibrin (PRF) preparations. This study aims to explore the role of PRF membranes in the healing and tissue regeneration process, as well as their potential as an innovative approach in oral implantology. Many important medical discoveries were made by chance. A suitable example of this is osseointegration, which marked the beginning of a modern era of dental implantology. When Per-Ingvar Brånemark (1929-2014) discovered osseointegration, he was investigating something completely different: the microcirculation of blood in the bone marrow. For this study, conducted on rabbits, he developed a system consisting of a titanium cylinder with a small optical camera that, inserted into the rabbit's femur, helped him observe blood circulation in the bone. After a few months, once the study was completed, Brånemark wanted to recover the micro-camera to reuse it in another project, but when he tried to remove it, he had a surprise: he couldn't detach it because it was attached to the bone. Brånemark called this process of integration between titanium and bone "osseointegration". Similarly, platelet-rich plasma (PRP/platelet-rich fibrin - PRF) was discovered by chance by observing a more accelerated and complete healing process in patients who developed hematomas. From that simple observation in the 1980s, the key components of the blood clot responsible for advanced healing were later determined: active growth factors in platelet alpha granules, various molecules responsible for cell adhesion, and specific clot guidance signals of fibrin. [1]

Aim and objectives

In this study we will follow the use of PRF membranes in modern dentistry and implantology. we will explore the role, importance and specific techniques of using PRF membranes in various implant surgery procedures.

MATERIAL AND METHODS

This work was based on a study of 30 patients of both sexes that underwent treatment using PRF membranes for the first time. Prior to this, patients were informed about the treatment and intervention process.

Patient preparation:

Before collecting the blood for the production of PRF membranes, the patient must be properly prepared. This involves a prior medical assessment, the patient's medical history and checking for contraindications to the procedure. It is important to obtain the patient's informed consent and to consider any medications or conditions that may influence the clotting process.

Blood collection:

Blood is collected by phlebotomy at the level of the antecubital fossa of the arm. The amount of blood collected may vary depending on the specific protocol, usually 2-6 tubes, respectively 20-60 ml of blood, are collected. Depending on the type of membrane we want to produce, harvesting is done in glass tubes with a red cap, for solid PRF or in PET plastic tubes with a white or blue cap (orange for some manufacturers).

Blood centrifugation:

The collected tubes of blood are placed in a centrifuge machine and undergo a controlled centrifugation process. This process causes blood components to separate into

distinct layers based on their density. Centrifugation results in obtaining the platelet-rich fibrin (PRF) layer at the top and the red blood cell layer at the bottom of the tube, separated by the so-called buffer zone, which contains the highest concentration of platelets, leukocytes but also erythrocytes.

Separation of blood components:

After the centrifugation process, the blood components are separated. Typically, the layer of PRP is carefully collected using a pipette or similar device and transferred to a separate container. The PRP layer contains the blood platelets, growth factors and other beneficial components that will be used in the tissue regeneration process.

Formation of PRF membranes

The remaining fibrin layer in the centrifuge tube is retrieved and shaped to form the PRF membranes. This is a manual process where the fibrin layer is pushed and compressed to create a solid or semi-solid form. Sometimes, specially designed tools are used to shape the membranes according to the specific needs of the medical or dental procedure.

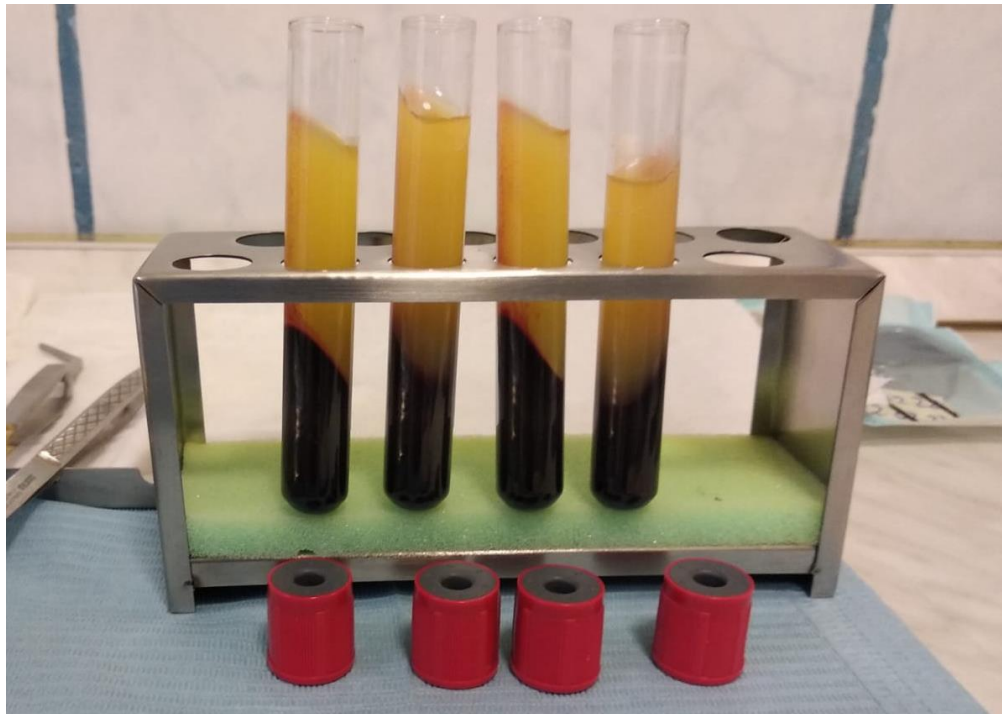


Figure 1. Formation of the solid PRF membranes

Processing and final preparation:

The PRF membranes obtained after modeling the fibrin layer are subjected to a final processing and preparation stage. This step may involve further compression of the membranes to remove excess fluid and to strengthen them. The goal is to obtain well-defined and structured PRF membranes that can be easily manipulated and applied during medical procedures.

Cutting and sizing membranes:

PRF membranes can be cut and sized according to the specific needs of the procedure. Using sterile surgical instruments, the membranes can be tailored to fit the area where they will be applied. This step allows a precise and efficient use of PRF membranes in tissue regeneration.

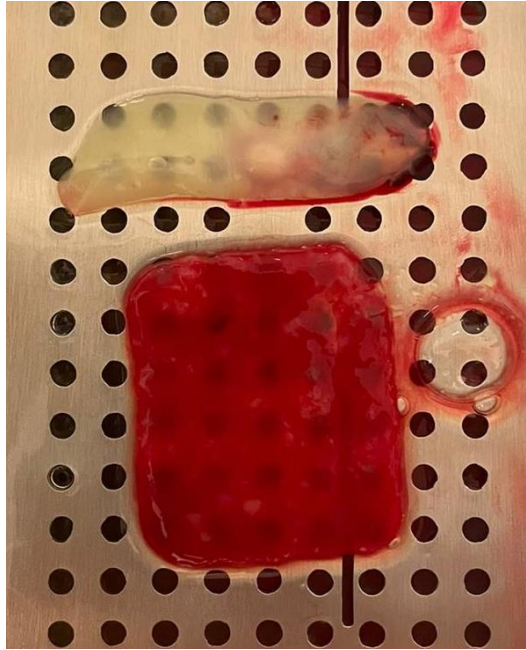


Figure 2. The separation of the solid PRF membrane and the e-PRF membrane

Storage and use:

PRF membranes are kept under appropriate storage conditions, usually in a sterile, sealed container, to maintain their integrity and biological properties. Before use, membranes can be rehydrated in specific solutions or combined with other tissue regeneration materials, depending on the needs of the medical procedure. PRF membranes are applied in areas where it is necessary to stimulate and accelerate the healing and tissue regeneration process.

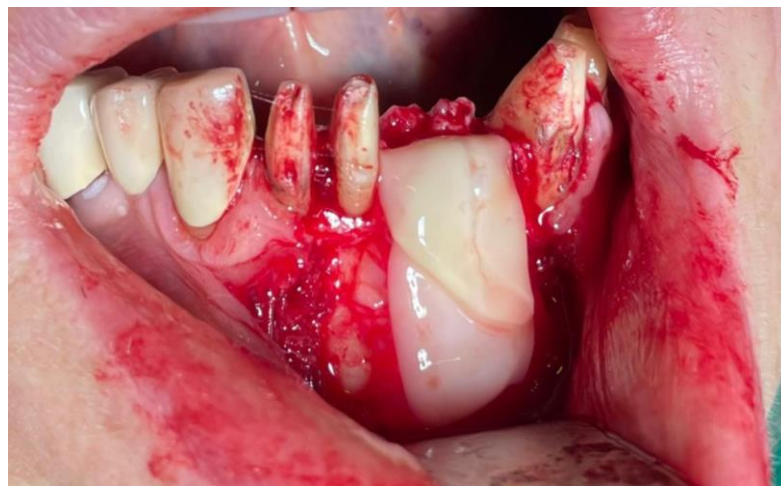


Figure 3. Covering of a post-extractional alveola with a solid PRF membrane in order to obtain a better healing and tissue regeneration

RESULTS

The statistical survey was carried out on the basis of 30 patients of both sexes, with age between 18 and 71 years. They underwent surgical interventions where tissue augmentation was performed with PRF membranes. All currently selected patients were individually and clearly informed about the possible risks and the treatment plan. The objectives pursued in this study were the presence of pain, the presence of edema and the presence of post-operative infections after the use of PRF membranes in the first 3 days after surgery.

Table 1. The gender distribution of the patients

Sex	Number of patients	Percentage
Male	12	40%
Female	18	60%

Table 2. The age distribution of the patients

Age	Number of patients	Percentage
18-25	5	16.6%
25-40	8	26.6%
40-60	7	23.4%
60+	10	33.4%

Table 3. The presence of post-operative pain in the first 3 days after surgery

The presence of pain	Day 1	Day 2	Day 3
Absent	21	28	30
Light	9	2	-
Medium	1	-	-
Intense	-	-	-

Table 4. The presence of post-operative edema in the first 3 days after surgery

The presence of edema	Day 1	Day 2	Day 3
Prezent	25	10	3
Absent	5	20	27

Table 5. Refers to the presence of post-operative infection in the first 3 days after surgery

The presence of infection	Number of patients	Percentage
PRESENT	2	6.6%
ABSENT	28	93.4%

DISCUSSIONS

PRF membranes are as natural as the host tissue and present zero risk of infection or immunological reaction, as they are extracted from the patient's blood. At the same time, the membrane cannot be rejected by the patient's tissue. [2]

The use of PRF increases the healing rate of the grafted bone during surgical treatment.

PRF represents a much more cost-effective option compared to synthetic materials, bringing significant benefits to the economy of dental treatments.

The use of PRF membranes reduces patients discomfort in the initial phase of the healing process, contributing to the absence of pain and ensuring a fast recovery.

The PRF membranes stimulate the healing process of periodontal defects, favoring the regeneration and reparation of the affected tissues. [3]

PRF membranes play a crucial role in maintaining the structure of the alveolar ridge, helping in preserving the integrity and stability of the alveolar bone;

By restoring gingival tissues, PRF membranes contribute on improving the oral health of the patients;

The use of these membranes reduces the risk of peri-implantitis and peri-implant mucositis;

Treatment with PRF membranes is relatively simple and can be performed by most dentists;

Using horizontal centrifugation more cells and more growth factors can be obtained than using fixed angle centrifugation. [4]

It was observed that the formation of PRF depends not only on the centrifugal preparation method, but also on the composition of the cells in the patient's peripheral blood. There is a strong correlation between the patient's hematocrit levels and the sizes of the PRF membranes. Patients with lower hematocrit levels (females and elderly patients) were shown to have significantly larger membranes (30%-40%) compared to patients with higher hematocrit levels (younger males). [5]

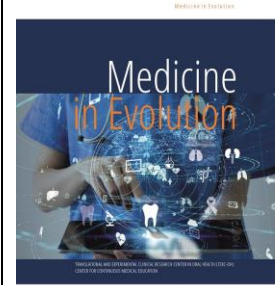
CONCLUSIONS

The first objective analyzed was the presence of postoperative pain. On the first day after the intervention, more than 60% of patients did not experience pain, and of those who experienced pain, 20% experienced mild pain and only 10% of patients experienced moderate pain. No patient described the pain as intense. The day after the intervention, 93% of patients had no pain at all, and 7% had mild pain. On the third day after the intervention, all patients confirmed the total absence of pain. The second objective analyzed was the presence of postoperative edema. On the first day after the intervention, 83% of participants had edema present. The day after the intervention, the number of patients with edema decreased by more than half, reaching 33%. On the third day, only 10% of patients still had edema. The last objective analyzed was the presence of post-operative infection, thus proving the capacity of PRF membranes as a protective barrier against infections, which were present in only 6% of the participants.

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The role of mast cells in oral squamous cell carcinoma



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Abstract

The mast cell is a connective tissue cell that has an active role in inflammation, immune modulation, angiogenesis, and more. Apart from its degranulation in allergic reaction, it is associated with "piecemeal degranulation", i.e., a selective cellular secretion pathway that aggravates the progression of oral potentially malignant disorders (OPMD) to oral squamous cell carcinoma (OSCC) through angiogenic switch. Angiogenic factor released by mast cells ensures survival and progressive capacity. The tumor microenvironment (TME) is composed of many different cell populations, such as tumor-associated mast cells and various infiltrating immune cells and non-cellular components of the extracellular matrix. These crucial parts of the surrounding stroma can function as both positive and negative regulators of all the hallmarks of cancer development, including induction of angiogenesis and activation of invasion and metastasis. This review will focus on describing the role of mast cells in the tumor microenvironment and the dissection of cancer, especially oral squamous cell carcinoma.

Keywords: mast cells, tumor microenvironment, angiogenesis, oral squamous cell carcinoma

INTRODUCTION

Mast cells, described by Paul Ehrlich in 1876, are connective cells originating in a medullary progenitor cell. They are located in vascularized tissues, especially in connective tissues and mucosal surfaces [1]. The phenotype of mast cells differs according to the microenvironment in which they are located, adjusted to the functions they serve [2]. They have an active role in a wide biological spectrum of inflammation, immune modulation, angiogenesis and more.

There is ample evidence regarding the role of mast cells as a promoter of metastasis in some tumor types, but also as an inhibitor in others [3]. The tendency of mast cells to concentrate in the inflammatory and neoplastic focus in the immediate vicinity of blood vessels was observed, which was later shown to accumulate around tumors before the onset of tumor-associated angiogenesis [4].

Inflammation is responsible for both defense and protection against the carcinogen, but at the same time leads to tissue destruction. Also, the pathogenesis of oral potentially malignant diseases (OPMD) and oral squamous cell carcinoma (OSCC) begins with the inflammatory response, mediated by immune cells such as mast cells, neutrophils, lymphocytes, macrophages and others.

Head and neck cancer is considered one of the malignancies with the most severe impact on patients' quality of life, mainly caused by relatively low response to treatment and severe drug resistance [5,6]. It represents a heterogeneous group of tumors that appear from the mucosal surfaces of the oral cavity, nasal cavity, oropharynx, hypopharynx and larynx. Up to 90% of these tumors are head and neck squamous cell carcinomas (HNSCC), which is the sixth most common type of cancer worldwide. Data from the Global Cancer Observatory (GCO) show that the annual incidence of OSCC in 2020 was 377,713 cases worldwide, with the highest number recorded in Asia, followed by Europe and North America [7].

The most important prognostic determinant of HNSCC tumors is considered the presence of lymph node metastases, as lymphatic metastatic spread correlates with a significant decrease in the survival rate of patients [8]. While the main risk factors are tobacco and alcohol consumption, numerous studies suggest the role of HPV infection as a risk factor for the development of HNSCC [9].

In recent years, the perspective on cancer has changed dramatically and the tumor is no longer viewed as a mass of malignant cells, but rather as a complex tumor microenvironment (TME) in which other cell subpopulations are recruited to form a self-sustaining biological structure. The stromal component of the tumor microenvironment is composed of many different cell types, such as tumor-associated fibroblasts, neutrophils, macrophages, regulatory T cells, natural killer cells, platelets, and mast cells. These cell subpopulations interact both with each other and with cancer cells through various secreted cytokines, chemokines, growth factors and extracellular matrix (ECM) proteins.

This review will focus on describing the role of mast cells in the tumor microenvironment and cancer staging, especially oral squamous cell carcinoma.

The role of mast cells in the tumor microenvironment

In the late 1870s, Paul Ehrlich described the existence of MCs in tumor microenvironments (TME). Since then, convincing evidence has confirmed the presence of mast cells in cancerous tissues, now called tumor-associated mast cells (TAMCs) [10]. Remarkably, TAMCs can adopt the "Dr. Jekyll and Mr. Hyde", because they can be both pro- and anti-tumorigenic, or just neutral spectators [11, 12]

Mast cells can promote tumor development by mediating tumor vasculature and by inducing the release of various growth factors such as SCF (stem cell factor) [13]. The increased number of MCs in the tumor mass correlates with a poor prognosis, metastasis and reduced survival rate in several tumor types, including oral squamous cell carcinoma [14]. Mast cell accumulation can occur due to the chemotactic activity triggered by RANTES (regulated upon activation, normally T-expressed) or MCP-1 (chemokine monocyte chemoattractant protein 1).

Mast cell activation is mediated by cross-linking of the IgE receptor (FcεRI) expressed on their surface, which leads to the release of mediators from intracytoplasmic granules. Among the mediators released in the extracellular space are: heparin, histamine, tryptase, chymase, cathepsin G, prostaglandin D2, carboxypeptidase A, leukotriene C4, TNF-α, GM-CSF, chondroitin sulfate and various interleukins [15]. Mast cells release their granule content selectively through piecemeal degranulation [16]. This type of mast cell degranulation has been observed in areas of chronic inflammation or tumors and has been reported to be a preferred secretory pathway of tumor-associated mast cells (TAMC) [17].

Histamine can induce cancer proliferation through H1 receptors and simultaneously suppress host immune defense through another H2 receptor [18]. It should be noted that both histamine receptor binding sites are present on the surface of tumor cells.

Mast cells release fibroblast growth factor 2 (FGF-2) and vascular endothelial growth factor (VEGF) to induce angiogenesis [19, 20]. Therefore, mast cells are often present near CD31+ cells and blood vessels [21]. Mast cells also release tryptase, which contributes to extracellular matrix degradation and vascularization, thereby mediating angiogenesis and tumor growth as well as metastasis [22].

Mast cells can also facilitate tumor progression by modulating the tumor microenvironment (TME) and developing resistance to anticancer drugs. Mast cells can also promote cancer development by releasing specific cytokines. For example, inflammatory IL-6 release can occur independently of histamine [23].

In contrast, MCs can also mediate anticancer responses. For example, MC accumulation in a mammary gland tumor and surrounding lymph nodes can mediate tumor regression. In certain tumor types, mast cells can inhibit the growth of cancer cells by releasing proteolytic enzymes and some cytokines. Mast cell tryptase can also promote protease-activated receptors, such as PAR-1 or PAR-2, stimulated by thrombin and trypsin [24]. Protamine, which neutralizes the anticoagulant properties of heparin, can trigger thrombosis of blood vessels in the tumor mass.

The profile of mediators secreted by tumor-associated mast cells, mentioned above, suggests that tumor-associated mast cells may have both pro-tumorigenic and anti-tumorigenic roles in cancer development. The tumor-promoting functions of TAMC include: angiogenesis through the production of vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF-2) [25]; degradation of the extracellular matrix through the production of metalloproteinases (MMPs) and various proteases, which results in the invasion and migration of tumor cells [26]; inducing the proliferation of tumor cells through the production of histamine [27]. In addition, mast cells produce a variety of chemotactic factors through which they recruit other immune cells to the tumor [28]. Conversely, in some tumor types, tumor suppressive effects of TAMC have been reported, mainly by mediating tumor cell apoptosis through the production of IL-4 and TNF-α [29].

The role of mast cells in the pathogenesis of oral squamous cell carcinoma

Primary tumor growth is associated with the presence of immune cells, which cause inflammation commonly seen in head and neck squamous cell carcinomas (HNSCC). Mast cells influence the primary tumor mainly by producing many pro-angiogenic factors, such as

VEGF, bFGF, TGF, TNF- α , tryptase, heparin and various MMPs, which are associated with ECM degradation, angiogenesis, progression and growth of oral squamous cell carcinoma [30, 31].

The role of mast cells in potentially malignant oral diseases (OPMD), including oral leukoplakia, oral lichen planus [32] and oral submucosal fibrosis [33], has been widely documented. In the presence of a carcinogenic environment, a continuous oncogenic signal drives the conversion of normal cells to OPMD and then OPMD to OSCC [34].

A recent study showed an increase in mast cell density in both OSCC and OPMD compared to normal oral mucosa. Also, the authors observed that the number of mast cells was more reduced in OSCC than in OPMD [35]. A similar observation was presented in the study by Oliviera et al., where it was concluded that once the tumor microenvironment was established, there was a high probability for failure of mast cell migration to the tumor site [36]. Following these results, several hypotheses were formulated by the authors regarding the relevance of mast cell migration. Thus, the authors state that the failure of mast cell infiltration at the tumor level could be due either to reduced chemotactic factors to attract mast cells, or to down-regulation of the c-kit activation pathway, which are prerequisites for mast cell migration. A similar finding was also observed by Singh et al. while evaluating mast cells in OPMD and OSCC using toluidine blue staining [37].

In contrast to the mentioned studies, Iamaroon et al. [38] and Michailidou et al. [39] found a significant increase in the number of mast cells in cases of OSCC compared to OPMD. These findings were correlated with the "angiogenic switch" that could occur in the early stage of malignant transformation. Also, the authors emphasized the role of mast cells in the progression of normal tissue to dysplasia which then leads to the appearance of OSCC. Rojas et al. also observed an increase in mast cell density in squamous cell carcinoma of the lip [40].

The association of mast cells with the pathogenesis of OSCC has been controversial due to the dual role played by these cells. A group of experts believed that mast cells favor tumor progression through angiogenesis and neovascularization. On the contrary, another group of experts supports the cytotoxic function of mast cells that suppresses the tumor growth potential [41,42]. The cytotoxic effect of mast cells is present in the initial stage of tumor infiltration. However, once the tumor is established, the altered tumor microenvironment suppresses mast cell infiltration through various mechanisms, whereby its cytotoxic effect is suppressed favoring its angiogenic potential for tumor growth. This likely mechanism is potentiated with the finding that the cytotoxic effect of mast cells is actively associated with a mast cell-to-tumor ratio greater than 20:1 that reverses when the ratio changes from 10:1 to 1:100 [43].

In oral squamous cell carcinoma, a significant correlation was observed between mast cell density (MCD) and microvascular density (MVD). Following these observations, it was suggested that mast cells can regulate angiogenesis in OSCC, possibly by releasing tryptase from intracytoplasmic granules. Tumor angiogenesis is a complex event mediated by angiogenic factors released by cancer cells and/or host immune cells. Among host immune cells several observations have indicated the role of mast cells in tumor progression by promoting angiogenesis. Tryptase, FGF, IL 4, IL 8, TNF α and β are among the mediators released by mast cell granules and are strong inducers of angiogenesis [44]. The increased densities of mast cells and microvessels in oral squamous cell carcinoma indicate that mast cells may play a role in the upregulation of angiogenesis in this tumor type.

However, three studies in the literature showed no positive correlation between mast cell density and microvessel density in oral squamous cell carcinomas [45, 43, 46]. On the other hand, a study revealed a positive correlation between mast cell and microvessel densities in well-differentiated types, but not in moderately or poorly differentiated types of oral squamous cell carcinoma [47]. On the contrary, one study observed a statistically

significant correlation between mast cell and microvessel densities in poorly differentiated OSCC [44]. However, Sharma et al., in 2010, investigated the correlation of microvascular density with mast cell proliferation and revealed a positive correlation in moderately differentiated types, but not in well or poorly differentiated types [43]. On the other hand, another study stated that there was a significant correlation between mast cell and microvessel density in normal oral mucosa, but not in oral squamous cell carcinoma, regardless of histological grade [48].

Studies in the literature have shown that mast cells in the perilesional and intratumoral area of oral squamous cell carcinoma express CD105, VEGF, VEGFR1 and VEGFR2 and have shown a positive correlation with the angiogenic activity of the tumor [31]. Thus the authors suggest that mast cells influence tumor progression and growth.

Another study investigated the population of cancer stem cells expressing CD44, CD133, and CD117 at the invasion front and intra-tumor areas. Their results suggest that CD 133 and CD117 positive cells were of mast cell origin and could influence angiogenic activity [49].

Mast cells cause much debate today regarding their role in a variety of physiological and pathological processes, including cancer. They act as guardians of the immune system and, in turn, respond to many signaling pathways, thus contributing to the process of carcinogenesis and metastasis. Many studies have shown that the number of mast cells definitely increased with tumor progression. New therapies targeting mast cell mediators and receptors play an important role in controlling the process of tumor progression and metastasis, thus favoring a good patient prognosis.

CONCLUSIONS

Evidence for the crucial contribution of various stromal components, including mast cells, in regulating OSCC development implicates a fundamental role of the tumor microenvironment in providing a supportive niche, thereby substantially promoting OSCC development and metastasis.

While research has previously focused mainly on altered gene expression and aberrant genetic mutations in tumor cells, it is becoming increasingly clear that investigating differences in the stromal composition of the tumor microenvironment in OSCC and their impact on cancer development and progression may help to further better understanding of the mechanisms behind different responses to therapy.

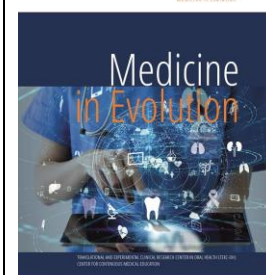
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The attitude regarding dental pain among a group of Romanian adults



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Abstract

Dental pain is a common symptom for oral conditions and most frequently caused by the dental disease. The aim of the present study was the assessment of Romanian adult patients' attitude toward dental pain. Material and method: the cross-sectional study was conducted in 2022-2023 on a sample of 200 adults using an on-line self-administered questionnaire. Results showed that 32.2% of the participants experienced dental pain at least 1 time/year, and the most frequent approaches were scheduling a dental visit (67.5%) and self-medication (42.5%). Only 29% visited the dental office as soon as the pain was felt and the main reason for postponing was the costs of the dental treatments. Analgesics were the preferred medicine for pain management (74%), most frequently ibuprofen (59.5%). Conclusion: Participants in the present showed a suboptimal attitude toward dental pain, tending to postpone the dental visits to address the condition and self-medication with analgesics was largely observed.

Keywords: oral health, dental pain, self-medication, dental attendance

INTRODUCTION

Dental pain is one of the most commonly met symptoms of oral diseases [1]. Although odontogenic pain could be caused by dental caries as well as traumatic lesions such as tooth fracture or dentine hypersensitivity after gingival recession and root dentine exposure [2], it is most frequently present because of the presence of cavities [3,4] because of the high prevalence of this specific dental disease [5]. Dental pain is present in different stages of dental caries evolution: cavity extended to dentinal layer with a reversible inflammation of the endodontic system, cavity complicated with pulpitis on a vital tooth, or in case of involvement of the periapical tissue when the inflammation extends beyond the endodontic space [6-8]. Unfortunately, dental caries has a high prevalence both for temporary and permanent dentition [9], therefore dental pain is an often symptom.

The presence of dental pain has a significant negative impact on the quality of life, affecting not only the functional role of the oral cavity but also the psychological status and social activity [10,11].

However, dental pain is the most frequent reason for dental office attendance [12,13]. This, in spite of the fact that when this symptom appears it is a sign that the dental caries evolved beyond the enamel and reached at least the dentine [8]. Thus, it is a sign of late presentation at the dental office, and a neglected attitude toward the dental attendance [14].

Dental pain could last from a few seconds to hours, lasting longer with the increase in the severity and extension of dental caries [15]. Cold, hot, sweet and pressure in the longitudinal axis of the tooth are the most frequent stimuli for dental pain [8]. On the other hand, in the least extended lesion the pain stops when the stimulus is removed while in more advanced phases the pain is controlled only with analgesic medication [8]. Antibiotics are recommended only in the case of the most serious phases of dental caries, complicated with infection [8].

Thus, in order prevent the dental pain it is recommended for patients to respect the recommended intervals for dental visits so that the diagnosis of dental caries to be established as early as possible, is incipient, non-carious stage or in phase when the dental caries is superficial, affecting only the enamel layer. The presence of dental pain as a symptom of dental caries is a sign of neglect towards oral health and dental attendance because of late detection of the disease.

Aim and objectives

The aim of the present study was to assess the frequency of dental pain and the attitude adopted among a group of Romanian adults.

MATERIAL AND METHODS

The present survey was conducted between December 2022 and January 2023 on a sample of 200 Romanian adults. The survey used for the assessment a questionnaire edited and distributed via internet, with 18 questions, both open and close-ended. The inclusion criteria was the age of at least 18 years and there were excluded participants who worked or studied in the dental field. The filling-in time was 5-10 minutes. All the invited participants were described the aim of the study and were presented the terms of participation according to the Declaration of Helsinki. From the 204 adults who completed the form, 4 were excluded based on the inclusion and exclusion criteria and the final sample was composed of 200 subjects.

RESULTS

The subjects in the present study had an age varying between 19 and 68 years and a mean age of 30 ± 10.4 years. The group was represented by 72.5% females, 26.5% males and 1% did not offer an answer regarding the gender. Ninety two percent of participants were living in urban areas.

Among the participants, one third declared the presence of dental pain at least 1 time per year (Figure 1).

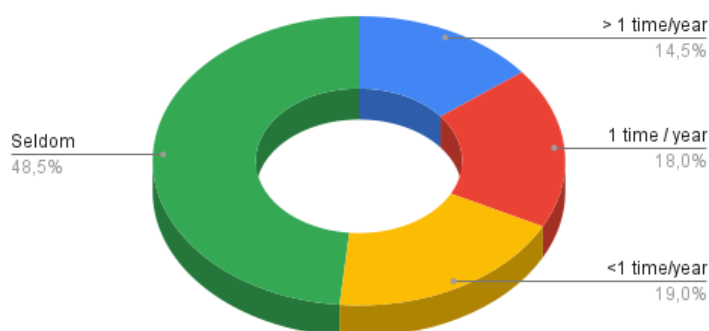


Figure 1. The frequency of dental pain

When it comes to the most frequent approach when participants to the study feel dental pain, only 67.5% (N=135) declare a visit to the dental office while 42.5% (N=85) chose to take medicines. Interestingly, about one quarter of subjects declared they just stay on hold without a specific action to control the pain (Figure 2).

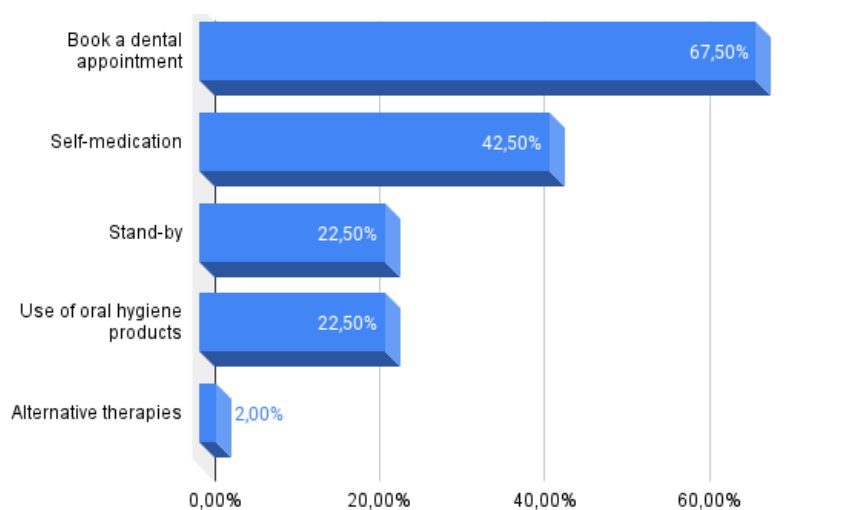


Figure 2. The approach in case of dental pain

Asked about the dental attendance when the dental pain was present, only 29% (N=58) declared they booked a dental appointment as soon as the pain is felt, while most of the subjects (57.5%) declared that they decided to go to the dentist after a few days of persistent pain (Table I). Regarding the main reason for postponing dental visits after the dental pain started, the most frequently mentioned answers were the lack of patients' availability (38.5%) as well as the high costs of dental treatments (31%) (Table I).

Table I. Attitude toward dental office attendance for dental pain

Attitude toward dental attendance for dental pain	% (N)
Timing of dental office attendance for dental pain	
<i>After a few days of dental pain</i>	57.5% (115)
<i>As soon as the dental pain is felt</i>	29% (58)
<i>After a few recurrent episodes of dental pain</i>	10% (20)
<i>When the dental pain evolves to infection</i>	3% (6)
<i>Never</i>	0.5% (1)
Main reason for postponed dental attendance for dental pain	
<i>Lack of necessary time for dental visits</i>	38.5% (77)
<i>High costs of dental treatments</i>	31% (62)
<i>Dental anxiety</i>	23.5% (47)
<i>Lack of felt need of dental treatment</i>	16% (32)
<i>Self-medication was an easier solution</i>	6% (12)
<i>Self-medication was efficient in previous episodes of dental pain</i>	1.5% (3)
<i>Lack of access to dental offices</i>	1.5% (3)

In regards to the medicine taken for pain-relief in cases of dental pain, 22% (N=45) declared they never opted for self-medication while analgesics were used by 74% (N=148). Interestingly, about one quarter of the subjects declared they took antibiotics for dental pain (Table 1). When it comes to the preferred analgesics, ibuprofen and paracetamol were most frequently chosen by the participants to the study (Table II).

Table II. Attitude toward self-medication for dental pain

Attitude toward self-medication for dental pain before going to the dental office	% (N)
Type of medicine taken	
<i>Analgesics</i>	74% (148)
<i>Antibiotics</i>	24% (48)
<i>None</i>	22% (45)
Type of analgesic preferred	
<i>Ibuprofen</i>	59.5% (119)
<i>Paracetamol</i>	24.5% (49)
<i>Ketoprofen</i>	16.5% (33)
<i>Aspirin</i>	6% (12)
<i>Metamizole</i>	1% (2)

DISCUSSIONS

In the present study, one third of the participants declared they had experienced dental pain at least 1 time per year. This is in accordance with the data in the literature [16,17], although an accurate prevalence of the dental pain is improbable because of the inconsistency of the definition of dental pain used in the previous research and the difficulties in discrimination between different causes of dental pain [16,17].

The most accurate predictor for the dental pain is the dental attendance pattern [18]. Thus, the less frequent dental visits, the greater are the changes for dental pain. In our study, it was found that two thirds of the subjects choose to schedule an appointment to the dental office after they feel dental pain. When it comes to the postponed dental visits due to the costs of dental treatments, in our study it was observed that the reason was mentioned by 1 in 3 participants, while a previous study conducted on the UK population revealed a lower frequency, namely 1 in 5 adults [19].

Ibuprofen is the first choice for dental pain relief from the efficacy perspective while paracetamol is recommended when the ibuprofen is contraindicated [15]. Those were the most frequently used medicine by the participants in our survey. However, the analgesic medicine should not impede the dental attendance, symptomatic treatment with medication should be used only along with the dental operative procedure in order to offer the etiological treatment and thus to prevent recurrence of the pain [20].

Self-medication with antibiotics for dental pain is generally ineffective when the pain is caused by pulpitis since it has an inflammatory nature [21]. Then there is a dental infection that causes the dental pain, a surgical approach or endodontic treatment are recommended and not antibiotherapy as a monotherapy [22]. Yet, in our study one quarter of the participants declared they took antibiotics for the episodes of dental pain in the past, irrespective of the cause. Previous research found in the literature showed that up to 85% of antibiotics taken for dental conditions are not indicated [23].

CONCLUSIONS

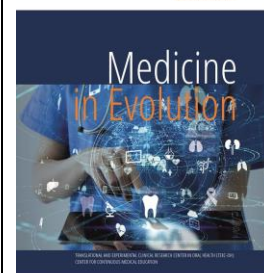
In the present study, participants' attitude toward the dental pain was observed to be of neglect. Although a large proportion of assessed adults schedule a dental appointment after they feel dental pain, in most of the cases, they postpone this decision and the most frequent reason was the costs of the dental treatment. Participants choose self-medication frequently and ibuprofen is the most commonly used medicine. However, an important proportion of the studied population take antibiotics for dental pain, irrespective of the cause of it.

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The esthetics of maxillary frontal group in dental prosthetics



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Abstract

The scientific objectives to be solved within this research are: standardization of an updated work protocol regarding aesthetic restorations, based on differentiated methods of treatment in relation with the etiology of the dysfunction, the sex and age of the patient, the original environment of the patient, his personal desires and his financial possibilities. The material taking into account consisted of a series of 70 patients of different sexes, from different backgrounds, aged between 19-57 years, and presenting carious lesions, chromatic disharmony or frontal edentation. From this study were excluded patients with lesions located only in the posterior teeth. The study was made up of 40 patients from western Romania, who came to our clinic. The actual experiment will include: the elevation of masticatory, phonetic and aesthetic dysfunctions and it was carried out for each patient with the help of clinical and paraclinical examination. These included a series of surveys and investigations. The aim of this study is to highlight the rehabilitation of the maxillary frontal group. What must be remembered is that rehabilitation of the frontal group can be done through several techniques and with various materials. The techniques used for the grinding of the front group are: threshold grinding, tangential grinding, and vestiprep grinding. The materials used for aesthetic rehabilitation of the frontal group are: zirconium and pressed ceramic, and of course we can mention metal-ceramic but the aesthetics are no longer the same because the transparency given by zirconium can no longer be achieved.

Keywords: aesthetics restoration, oral rehabilitation, zirconium, ceramics, smile

INTRODUCTION

For a complete and correct oral rehabilitation, all the functions of the dento-maxillary apparatus must be perfectly restored. In addition to the masticatory function, which is the most important for the patient, the phonation function plays an important role as well as the aesthetic function. In recent years, physiognomic function has gained increased attention, both from doctors and patients who are very concerned with how their teeth look or how they smile. The demands of the patients for dental aesthetics are increasing, and the possibility of achieving the requirements is in full development, thanks to the acceleration of technology and the development of the dental materials industry. [1]

Dental prosthetics is a branch of dentistry that aims to reconstruct the patient's dentition, to give him a balanced aesthetic and a functional smile. Prosthetics can involve the following functions in the oro-dento-facial reconstruction operation: phonation or sounds during speech, mastication, as well as maxillo-facial protection from the effects of bruxism. Dental prosthetics therefore fulfills a complementary and interdisciplinary role in dentistry. [2]

Aim and objectives

The scientific objective to be solved within the research are: standardization of an updated work protocol regarding aesthetic restorations, obtaining differentiated methods of treatment in relation with the etiology of the dysfunction, the gender and age of the patient, the original environment of the patient his desires and his financial possibilities.

MATERIAL AND METHODS

The material taking into account consisted of a series of 70 patients of different sexes, from different backgrounds, aged between 19-57 years and presenting carious lesions, chromatic disharmony or frontal edentation.

From this study, we excluded patients with lesions located only in the posterior teeth.

All the patients included in this study received explanations about their present problems, the future treatment options, the risks and the consequences of the treatment as well as the consequences of the non-intervention. Patients agreed to the prosthetic treatment and the follow-up period necessary to determine whether the prosthetic treatment was successful or not, and whether or not other procedures were necessary to resolve the case.

From the total of 70 patients, we excluded 30 who had lesions only in the posterior area.

The study was made up of 40 patients from western Romania, who came to our clinic.

The actual experiment will include: the evaluation of masticatory, phonetic and aesthetic dysfunctions.

The surveys that were used in this study included: heredocollateral antecedents, personal general antecedents and personal dental antecedents.

We proceed to the inspection of the face from the frontal norm, to analyze facial symmetry, the shape of the face, the layers of the face, the appearance of the lips, the amplitude of the mouth opening and the color of the teguments. Inspection of the face from the lateral norm follows the patient's profile, the ratio of the lips and the lip-chin ratio. The following step was the palpation of the points of emergence of the trigeminal nerve, sinus points, maxillary and mandibular bone outline, palpation of the soft points of the face, palpation of the TMJ, of the masticatory muscles. The endooral examination was made, observing specifically, the color and appearance of the tongue, the sublingual area, the

mucosa of the hard palate, the lute and the palatine veil. Each tooth was analyzed observing the presence of carious processes, fillings, tartar deposits and staining as well as edentations if they were present. Afterwards the radiological examination was realized in order to diagnose the patient and to define a treatment plan related to the reasons for the presentation. The next step was the implementation of the treatment plan.

For the first time, the prosthetic field was imprinted with an additive silicone for the provisional dental restorations. Anesthesia related to the interested areas was carried out. In the case of patients who presented old, unaesthetic prosthetic restorations or which affected the masticatory functions, the former prosthetic restorations were ablated. To prepare the zirconium crowns, the minimum preparations thickness for the zirconium cap on the front teeth is 0.3 mm and for the lateral teeth, 0.5 mm. To prepare the tooth, a 1.5-2 mm preparations of the incisal edge and 1-2 mm in circumference is recommended in the frontal area. In the lateral area, the occlusal surface will be polished by 2-2.5 mm and the circumference by 1-3 mm. Different sized tapered end burs were used as well as threshold end burs. After finishing the preparation, the gingival retraction thread will be inserted to prevent light bleeding and highlight the threshold. A two-time impression will be taken.

The occlusion will be taken with an addition silicone for registration of the occlusion. The impression that was taken at the beginning of the session, will be filled with a biacrylic self-polymerizing resin to create the temporary restoration. After cementing the provisional restoration, the occlusion will be adjusted, with the help of an articulation paper and a fine bur. After completing the clinical session, the laboratory sheet will be filled with the details of the case, which will be sent to the technician together with the impressions and the patient's occlusion. After the doctor-technician consultation, there will be a technician-patient consultation session to determine the optimal color for the prosthetic work using the color key.

RESULTS

Gender distribution of the patients:

Of the total of 40 remaining patients, 25 were female and 15 male.

Table 1. The gender distribution of the patients

Sex	Number	Percentage
Male	15	38%
Female	25	62%

Distribution by age of the patients:

From the total of 40 patients, 8 patients are aged between 19-26 years, representing 20%, 17 patients are aged between 27-35 years, representing 42%, and 15 patients aged between 36-57 years representing 38% of the total of 40 patients.

Table 2. The age distribution of the patients

Age	Number	Percentage
19-26 years	8	20%
27-35 years	17	42%
36-57 years	15	38%

Distribution by the original environment of the patients:

From the total of 40 patients present in this study and who met the study criteria, 28 patients came from the urban environment and only 12 from the rural environment.

Table 3. The distribution of the patients by their original environment

Original environment	Number	Percentage
Urban area	28	70%
Rural area	12	30%

Distribution by the cause of the dental visit:

Of the total of 40 patients, 25 had deep carious lesions, 13 had chromatic disharmony and 2 had frontal edentation.

Table 4. The distribution of the patients by the cause of the dental visit

Causes	Number	Percentage
Dental lesions	25	62%
Chromatic disharmony	13	33%
Frontal edentation	2	5%

Distribution by the type of treatment was implemented to the patient:

Out of the total of 40 patients, 33 required dental crowns and only 7 of the patients required dental veneers.

Table 5. The type of treatment that was implemented to the patients

Dental tratment	Number	Percentage
Dental crowns	33	82%
Veneers	7	18%

Distribution of the patients by the type of material that was use for the restaurations:

Of the total of 35 patients with dental crowns, 29 required zirconia crowns with ceramic application, and 6 patients required all-ceramic crowns.

Table 6. The type of material that was used for the retaurations

Type of merial	Number	Percentage
Zirconium	29	83%
Full ceramic	6	17%

DISCUSSIONS

The techniques used for grinding the front group are: threshold grinding, tangential grinding and vertiprep grinding.

The materials used for the aesthetic rehabilitation of the frontal group are: zirconium and pressed ceramic, of course we can mention metal-ceramic but the aesthetics are no longer the same because the transparency given by zirconium can no longer be achieved. One of the major advantages of zirconium is bio-compatibility. [3] Unfortunately, metal-ceramics can cause a metal allergy called metallosis. For better communication between the doctor and the patient, the wax-up diagnosis is used, which is the wax modeling of the dental arches on the study model to pre-visualize the final result. [4] The mock-up is the transposition of the wax-up in the oral cavity with the help of materials for temporary works. Special attention must be paid to occlusion, functional and parafunctional movements. Ceramic veneering is a highly appreciated and widely used treatment both in the USA and in Europe due to the extraordinary aesthetic effect and the fact that complicated aesthetic problems are solved through a minimally invasive treatment. Ceramic veneers are very resistant, but like natural teeth, they can break or chip in case of accidents. [5] In general, for a significant change in look, it is recommended that all visible teeth be covered by veneers (this means 10 teeth in the upper part and 8 teeth in the lower part). In this way the teeth will be perfectly aligned. The ceramic veneer is the simplest method to change the color and shape of the teeth to achieve a

perfect smile. Dental veneers also help close unsightly spaces between teeth and correct aesthetic defects caused by crowded teeth. Unlike ceramic or metal-ceramic dental crowns, only a small part of the visible portion of the teeth (approximately 0.6 mm) is polished for ceramic veneers. [6] This is basically a minimally invasive cosmetic treatment. Aesthetic analysis remains an absolutely indispensable factor in achieving ideal prosthetic restorations. In this way, specialists, in addition to the technology they use, they must be friendly to interpretations, outline an original form to the patient's style, become innovative, use smile designs that outline the patient's appearance, use their abstraction and the artistic sense in deducing the parameters of the ideal smile, offering that final emotion, in perfect harmony with the concrete present. [7]

CONCLUSIONS

During this work we tried to highlight the rehabilitation of the maxillary frontal group. What we must remember is that the rehabilitation of the frontal group can be done through several techniques and materials.

Following the treatment, the patient has a correct occlusion, the mastication function is performed correctly, the patient's physiognomy is improved, he gains much more confidence in himself, according to them, his mood is positive, wanting to smile much more often. After frontal rehabilitation, people smile more, without realizing it, because it is a natural reaction, this natural reaction has many health benefits, it can make you feel and look better.

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The degree of satisfaction of wearers of fixed prosthetic dentures



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Abstract

Assessment of patient satisfaction with fixed prosthetic treatment should be a consideration of overall prosthetic success. The aim of this study was to evaluate patient satisfaction with prosthetic treatment with ceramic fixed prosthetic dentures (FPD) on metal framework.

Material and method: We selected and surveyed a group of 40 patients aged between 20 and 57 years who required fixed prosthetic treatment. Patients had a choice between 3 types of materials: metal-ceramic (MC), zirconia (Zr) and polymethyl methacrylate (PMMA). Patients were informed about the advantages and disadvantages of each FPD and were asked to choose and justify the choice made. Results showed that the patients who chose PMMA argued this choice for purely financial reasons. Patients who chose Zr for aesthetic reasons argued that it was worth the financial effort. Most patients chose metal-ceramic restorations, reasoning that they present an optimal aesthetic/functionality/cost ratio. Conclusion: Metal-ceramic FPD is still the most commonly used restoration to replace missing teeth, especially in the lateral areas.

Keywords: Satisfaction, fixed prosthetic dentures, metal-ceramic restoration

INTRODUCTION

Dental tissues do not have the ability to regenerate like most other tissues in the human body. Once lost, they need to be restored directly or indirectly [1]. The edentulous state affects aesthetics, mastication and phonation and has influence on the quality of life, on the patients' self-perception [2]. Fixed partial dentures (FPD) are the treatment of choice for replacing missing teeth because they are economical compared to implants [3]. They will improve the functions of the dento-maxillary apparatus, maintain the health and integrity of the dental arches and increase the patients' self-image [4,5].

There is a growing awareness among dentists that assessing patient satisfaction with the value of prosthetic treatment must be a consideration of overall prosthetic success. Fixed prosthetic treatment is often perceived by patients as expensive. That is why it is important to know if patients consider that the treatment provides quality of life, aesthetics and functions of the dento-maxillary apparatus and that they have gained economic value following the prosthetic treatment [2]. There are numerous studies on patient satisfaction with total dentures [6-8], implant-supported partial or total dentures [9-12] or removable partial dentures [13-16]. Thus, the researchers felt the need to evaluate the satisfaction of patients rehabilitated with FPD, looking at both the aesthetic and functional result, as well as the quality of life after the prosthesis [17-20].

The objectives of these studies were to assess patients' awareness of oral health, the need for prosthetic treatments and oral hygiene practices. Prosthodontists must identify and understand patient expectations from consultation to treatment completion [21].

Aim and objectives

The aim of this study was to evaluate patient satisfaction with prosthetic treatment with ceramic FPD on metal framework.

MATERIAL AND METHODS

We selected and surveyed a group of 40 patients aged between 20 and 57 years. The criterion for which this group was selected was their need for fixed restoration. All surveyed and treated patients signed and informed about all treatments performed and participation in this study.

Out of the total of 40 patients, 60% of them are female and 40% male, 20% are aged between 20 and 30, 35% are aged between 31 and 44 and 45% are aged between 45 and 57 years old (Figure 1, Table 1).

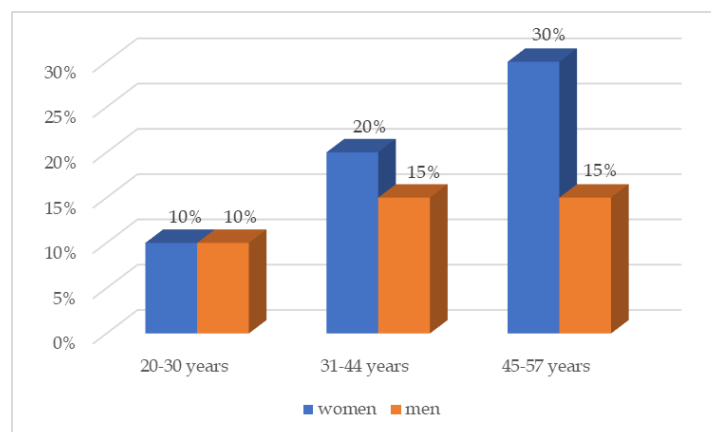


Figure 1. Distribution of patients by age group and gender

Table 1. Distribution of patients by age group and gender

age	Women	men	percentage
20-30 years	4	4	20%
31-44 years	8	6	35%
45-57 years	12	6	45%

From the point of view of income, we divided the patients into three groups: group A - with monthly incomes lower than the minimum wage in the economy (e.g. students, day laborers, unemployed, etc.); group B - with monthly income equal to or higher than the minimum wage in the economy and group C - patients with good living conditions, above average (higher education, stable job) (Table 2).

Table 2. Distribution of patients according to income

Group A	Group B	Group C
15%	60%	25%

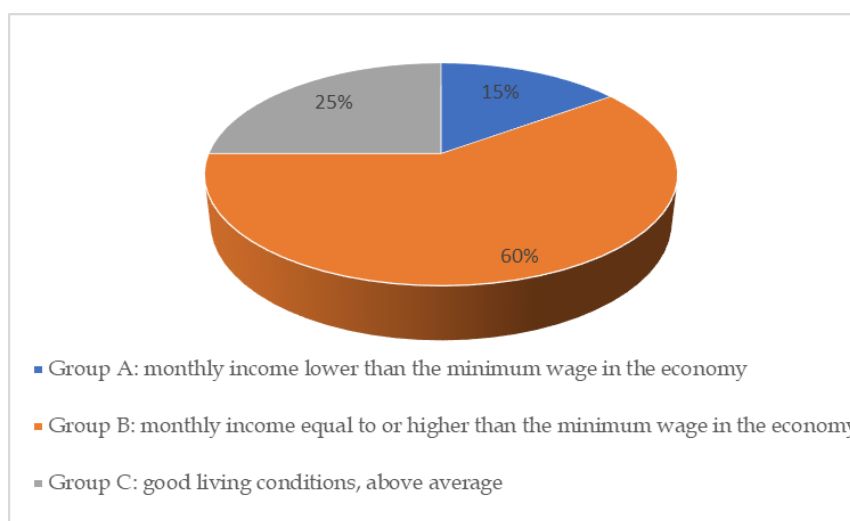


Figure 2. Distribution of patients according to income

Patients had a choice between 3 types of materials: metal-ceramic (MC), zirconia (Zr) and polymethyl methacrylate (PMMA). Patients were informed about the advantages and disadvantages of each FPD and were asked to choose and justify the choice made.

RESULTS

Patients who chose PMMA argued this choice for purely financial reasons. Patients who chose Zr for aesthetic reasons are part of group A and group B in equal proportions, those in group B arguing that it is worth the financial effort (Table 3, Figure 3).

Table 3. Choice of material for FPD

	Group A	Group B	Group C	% of the total
MC	2	16	6	60%
Zr	0	4	4	20%
PMMA	4	4	0	20%

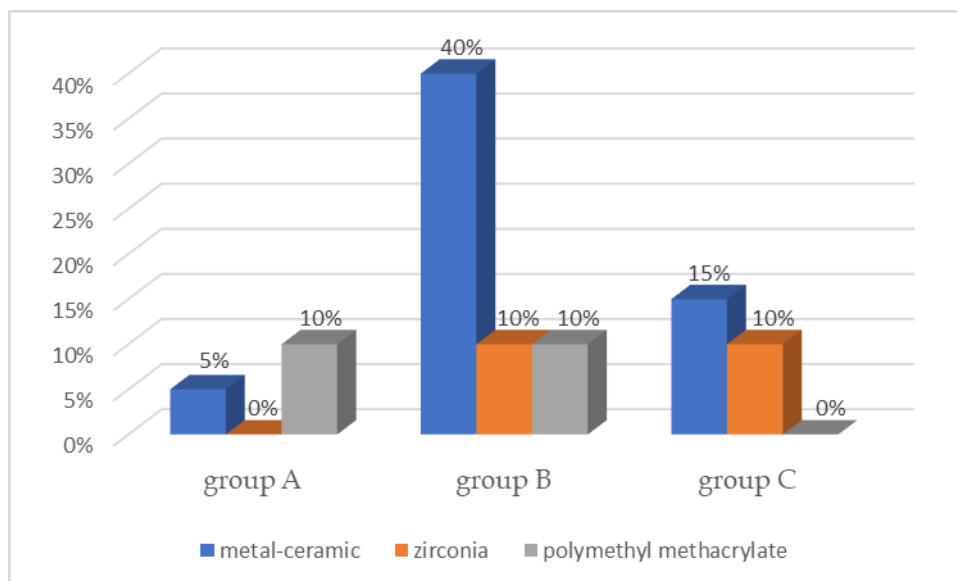


Figure 3. Choice of material for FPD

Patients who chose metal-ceramic restorations argued that the quality-price-aesthetic ratio is very convenient. Out of a total of 40 patients, 34 had or have at least one metal-ceramic crown, of which 27% had at least one such prosthesis less than 3 years, 20% between 3 and 5 years and 53% more than 5 years. The degree of satisfaction with metal-ceramic FPDs was evaluated in these patients. 64.71% declared themselves satisfied, while 35.29% were dissatisfied with fit and aesthetics (Table 4, Figure 4). Patients' complaints over time are related to the aesthetics of the gingival margin, the discolorations that occur as a result of gingival retraction and the accumulation of bacterial plaque.

Table 4. Satisfaction over time of metal-ceramic FPD wearers

Patients	Satisfied	Dissatisfied
Total	22	12
with FPD under 3 years	10	2
with FPD between 3-5 years	8	4
with FPD over 5 years	4	6

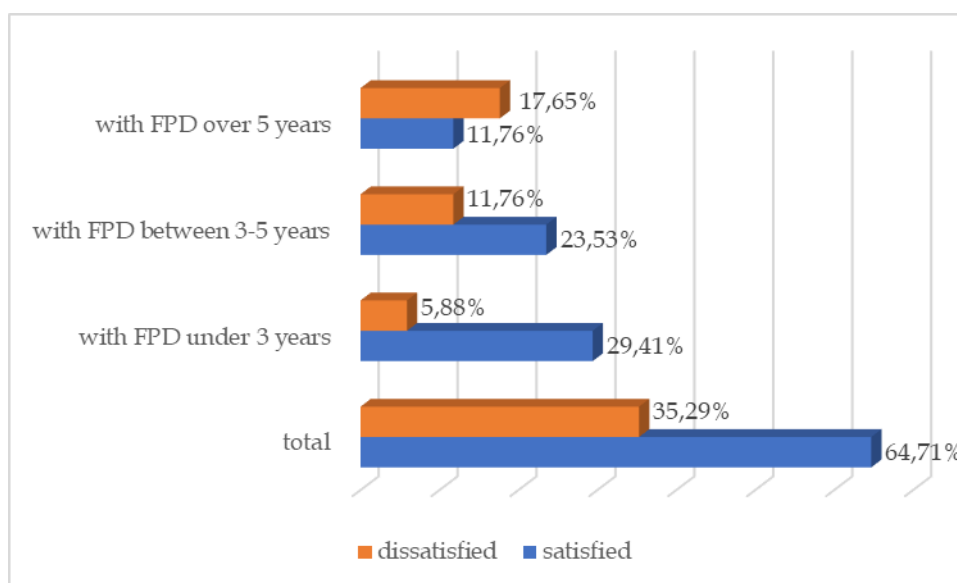


Figure 4. Satisfaction over time of metal-ceramic FPD wearers

DISCUSSIONS

The performance of an FPD is evaluated differently by patients and clinicians. Patients evaluate it based on subjective criteria; aesthetics, mastication, longevity and comfort, while clinicians focus primarily on technical features [22]. Studies consider both clinician and patient satisfaction [23].

In the study by Tan et al., over 90% of patients were satisfied with FPD treatment both functionally and aesthetically, and the costs were considered reasonable [19]. Kashbur et al. reported a very high level of satisfaction (80.9%) in patients undergoing fixed prosthetic treatment [17]. In the study by Zavanelli et al., 72.58% of patients were satisfied with FDP [24]. Kola et al. noted high levels of satisfaction with the functional and aesthetic aspects of the fixed prosthesis [25]. In the study by Shrestha et al., 76.4% of patients were satisfied with the aesthetics of the fixed prosthesis [1]. Geiballa et al. also reported a very high level of satisfaction (80%) with regard to the appearance of the fixed prosthesis [26]. Napankangas and Raustia, in an 18-year retrospective clinical study, evaluated the success rates of metal-ceramic fixed partial dentures made by dental students, concluding that there was good patient satisfaction and few complications in terms of biological or technical [27]. Nayan and Kumari concluded in their study that 90% of patients were satisfied with the functionality of the FPD and 80% were aesthetically satisfied [28].

Banerjee et al. concluded that 94% of patients were satisfied with FDP aesthetics immediately after insertion in the oral cavity, with satisfaction increasing to 98% one week after cementation, respectively 91% of patients were satisfied with masticatory functional capacity. Regarding the assessment of patient satisfaction for hygiene and awareness of oral hygiene techniques, only 58% of patients were satisfied with the cleaning ability of the FDP, with 3% complaining of unpleasant taste and odor [3].

Geiballa et al. found that 94% of patients did not use any additional hygiene techniques to maintain their fixed prosthesis [26]. Maintaining good oral hygiene in FDP wearers is very important to prevent periodontal disease and carious lesions. It is the responsibility of the dentist to inform and train his patients on the techniques and additional means of oral hygiene [23,26,29].

CONCLUSIONS

Metal-ceramic FPDs offer a reliable treatment option, showing good longevity. Metal-ceramic FPD is still the most commonly used restoration to replace missing teeth, especially in the lateral areas.

Most patients were satisfied with the performance of metal-ceramic FPDs in terms of mastication and appearance. Factors such as cleaning and comfort of the fixed prosthesis play an important role in patient satisfaction and the success of the prosthesis.

Counseling patients on FPD expectations and hygiene techniques should be done from the diagnosis and treatment planning stage to ensure a high level of satisfaction and success of fixed prosthetic treatment.

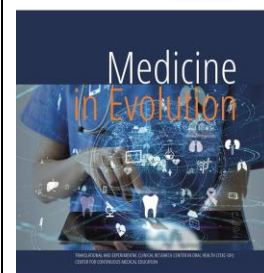
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Prevalence of Dental Caries in Relation to Determinants of Oral Health Status among 11-14-Year-Old Schoolchildren in Western Region of Romania



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Abstract

Aim and objectives: The present study, part of the first national oral health survey for children in Romania, aimed to determine the correlation between the prevalence of dental caries and the impact of dietary factors on oral health status in 11-14-year-old schoolchildren from the western region of Romania. **Material and methods:** Data was collected from a representative sample of 133 schoolchildren (72 boys and 61 girls), average age of 12.26 ± 0.6 , and then examined by calibrated dentists. The University ethics committee approved the study. ICDAS criteria were used to classify visual caries lesion severity. Statistical analyses of the data were performed by SPSS 23, Spearman's rank correlation and the Pearson correlation was used. **Results:** The DMFT index determined for the sample was 2.93 ± 2.70 . The results showed a significant positive correlation among boys who consume fresh fruits (0.17**), cookies, biscuits, cream, sweet pies, sweet rolls (0.27**), jam/honey (0.14**), sugar-sweetened chewing gum (0.38**). Statistically significant positive relationship was highlighted among girls who consume cookies, cakes, cream, sweet pies, sweet rolls (0.33**), sugar-sweetened chewing gum (0.35**), There is a statistically significant positive relationship between the consumption of sweetened beverages (0.24**), candies/sweets (0.18*), sweetened tea (0.24**), and DMFT. **Conclusions:** The prevalence and severity of dental caries in 11-14-year-old Romanian children from the western region of the country are strongly influenced by their socio-economic environment, as well as their specific consumption behaviors, such as the consumption of carbonated beverages, milk, sweets, tea, and cocoa.

Keywords: Dental caries prevalence, oral health status, risk factors, dietary habits, schoolchildren

INTRODUCTION

In the opening statement of its constitution, the World Health Organization (WHO) declares that health encompasses not just the absence of illness and weakness, but a holistic state of well-being that includes physical, mental, and social aspects. However, in the field of dentistry, the dental profession has generally focused exclusively on treating oral diseases and not on promoting oral health in the full sense of the term. Thus, it is necessary to address oral health as an essential dimension of quality of life, which is not limited to the absence of oral disease, but also to a person's ability to communicate and socialise appropriately [1,2].

Optimal oral health has a significant impact on a person's quality of life. Poor oral health can lead to digestive and respiratory diseases, as well as subsequent complications to general health [3]. Oral problems are a major global public health problem and have a profound effect on quality of life. Dental caries, for example, is one of the most common chronic diseases worldwide and has a significant impact on general health and quality of life [4]. The prevalence of dental caries varies by country and socio-economic level, and income inequality is an important factor determining the prevalence of childhood dental caries [1,5].

WHO and other international organisations have recognised the importance of oral health and have launched initiatives and programmes to promote oral health globally. However, there are still many challenges in ensuring optimal oral health for all populations. An integrated and holistic approach is needed, including preventive measures, health promotion and health education, and universal access to oral health services [6,7].

Diagnosing dental caries is crucial for early detection and treatment, and various methods are employed in dental practice for this purpose. One such method is self-assessment, where patients answer questions about their oral health, though the accuracy can be influenced by societal and cultural factors [8]. Another common tool is the DMFT (Decayed, Missing, Filled Teeth) index, which measures active carious lesions, missing teeth, and filled teeth, offering insights into caries severity and prevalence [9,10]. The International Caries Detection and Assessment System (ICDAS) is another diagnostic approach, designed to evaluate caries severity, including early lesions missed in routine exams, providing a standardized, evidence-based diagnostic framework [11,12].

In recent years, the concept of health and the measurement of health status have evolved, with little impact on dentistry. However, the dental profession has remained largely focused on the clinical approach to oral health, equating health with the presence of disease [13]. Thus, dentistry has remained immune to the broad concept of health as defined by the WHO [14]. It is important to understand that quality of life (QOL) measures is not a substitute for, but complementary to, the measurement of outcomes associated with disease. In recent years, there has been an evolution in the concept of QOL related to oral health, as more evidence has become available on the impact of oral disease on social roles [15-18]. Thus, oral health is an essential dimension of people's quality of life and optimal oral health enables a person to fulfil their roles in society. Poor oral health can also have a negative impact on general health and quality of life, potentially triggering digestive and respiratory diseases with subsequent complications for the body. Oral health problems, such as tooth decay, affect millions of people worldwide, with a profound impact on general health and quality of life. Although progress has been seen in reducing the prevalence of caries in some industrialised countries, it remains one of the most common chronic diseases worldwide. In developing countries, the prevalence of dental caries and the number of untreated cases remain high [4,6].

Dental caries has been a global problem and has attracted the attention of many specialists. According to the World Health Organisation, between 60% and 90% of children of all ages have at least one tooth affected by caries, and children have a higher prevalence of

caries compared to adults. In less developed countries, oral hygiene may be poor and regular dental visits may be neglected, affecting prophylaxis [4].

Preventive behaviour plays a crucial role in maintaining good oral health for both adult and young patients. This behaviour includes aspects such as oral hygiene, proper care of gums and teeth, dental services, correct use of hygiene objects, toothpaste and mouthwash [4]. Regular visits to the dentist and oral care measures are essential from an early age to prevent early onset of systemic diseases. Poor oral hygiene can lead to the development of tooth decay, which in severe cases can lead to tooth loss. In Romania, studies show that the level of oral health is in many cases limited to simple tooth brushing, which can lead to frequent complications [19].

Aim and objectives

The aim of the scientific is to analyse the prevalence of dental caries and the impact of dietary factors on the oral health of 12-year-old schoolchildren through a cross-sectional study.

The main objective of the research was to assess the oral health status of 12-year-old children in the western part of Romania by determining the prevalence of caries.

The secondary objective of the research was to identify the relationship between the prevalence of dental caries and the determinants of oral health status of 12-year-old schoolchildren in the western part of the country.

MATERIAL AND METHODS

The cross-sectional epidemiological study was designed and conducted during 2019-2020 [20], with the approval of the World Health Organization (WHO), the support of the Ministry of Health and compliance with the General Data Protection Regulation 2018. The study methodology was approved by the local (each school authority), regional (school inspectorate), national (Romanian Ministry of Health - Opinion No. 3411/05.04.2018, Ministry of Education - Opinion No. 1573/12.03.2019) and University of Medicine and Pharmacy "Victor Babeş" Timișoara, Romania (No. 29/28.09.2018) competent authority.

The study aimed to describe the prevalence of caries and severity of carious lesions in schoolchildren aged 11-14 years residing in rural and urban areas of western Romania in relation to oral health determinants. The participants, 133 children, boys and girls, are students from 9 schools distributed in rural and urban areas of Timiș, Arad, Bihor, Hunedoara, Sibiu, Caraș-Severin counties.

The schools selected in the study were: Avram Iancu Unirea Secondary School, Alba County; Arad Secondary School, Arad County; Nicolae Popoviciu Beiuș Secondary School, Bihor County; Pietroasa Secondary School, Bihor County; Sport High School Banatul Timișoara, Timiș County; Theoretical High School Periam, Timiș County; Theoretical High School I. C. Brătianu Hațeg, Hunedoara County; Măureni Secondary School, Caras-Severin County; Brateiu Secondary School, Sibiu County.

In order to ensure randomisation and stratification of the sample, the total number of pupils was determined as a percentage of the total number of children enrolled in the 8th grade National Examination for each school in Romania. Thus, the percentage share for each county was estimated and the final target number of assessments was obtained according to the type of locality (urban versus rural). Using the randomisation function of MS Excel, one urban and one rural school were selected for each county, resulting in a total of 9 schools distributed in rural and urban areas.

School-level predictors included the Development Index (LHDI 2011 given by Dumitru Sandu), which is a sociological index combining county-level variables such as

education stock, life expectancy at birth, average age of adult population, available living space, number of private cars per 1000 inhabitants and average household gas consumption. Factor analysis was used to aggregate these variables, and the scores obtained were relevant to the assessment of the county's workforce potential and economic potential.

Data were collected using the Children's Oral Health Questionnaire developed by the World Health Organization and described in the WHO Oral Health Surveys - Basic Methods, 5th edition, 2013. The questionnaire was translated into Romanian by two independent translators and differences were resolved in a face-to-face meeting. Two experts in educational and developmental psychology were also consulted to ensure the readability of the Romanian version of the questionnaire.

The questionnaires were distributed to schools through the postal services, sent to the children by the teachers one week before the clinical examination, and completed by the children in collaboration with their parents/carers at home. Completion of informed consent by parents/carers was required for participation in the study (Figure 1). Questionnaires were collected by the examiners on the day of the examination.

National Oral Health Project

Hello,

This study aims, for the first time in Romania, to assess the oral health of children on a national sample. The study is carried out by the Ministry of Health and is funded by the World Health Organization.

Thank you in advance for your agreement. participation.

Q1. You're... a. boy b. girl Q2. How old are you today?

Q3. What class are you in?

Q4. What level of education has your father completed (or your stepfather, guardian or other male adult living with you)?

1. No school	4. Vocational school (10 classes)	7. University studies
2. Primary school (4 classes)	5. High school (12 classes)	8. Do not live with men in the house
3. Secondary school (8 classes)	6. Post-secondary school	9. I don't know / I don't answer

Q5. What level of education has your mother completed (or your stepmother, guardian or other female adult living with you)?

a) No school	d) Vocational school (10 classes)	g) University studies
b) Primary school (4 classes)	e) High school (12 classes)	h) Do not live with men in the house
c) Secondary school (8 classes)	f) Post-secondary school	i) I don't know / I don't answer

Q6. In the last 12 months, how often have you had toothache or feel discomfort from your teeth?

a. never	b. once or twice	c. almost every month	d. almost every week	e. almost every day	f. I don't know / I don't remember
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Q7. How often have you been going to the dentist in the last year?

a) Once	d) Four times	g) I never received dental care / I visited a dentist
b) Twice	e) More than four times	h) I don't know / I don't remember
c) Three times	f) I haven't been to the dentist in 12 months.	

Q8. What was the reason for your last visit to the dentist?

a) Pain or problems with teeth, gums or mouth	b) Treatment or follow-up of treatment	c) Routine checking of teeth	d) I don't know / I don't remember
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Q9. How often do you clean your teeth?

a) never	b) 2-3 times a month	c) once a week	d) 2-6 times a week	e) once a day	f) 2 or more times a day
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Q10. Which of the following do you use when you clean your teeth?

a) toothbrush	b) wooden toothpicks	c) plastic toothpicks	d) dental floss	e) Something else. What's that?
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Q11. Do you use toothpaste to clean your teeth? a) Yes b) No

Q12. How often does your son/daughter consume or drink any of the following foods, even in small quantities?	A few times a day	Once every day	A few times a week	A few times a month	Never
1. Fresh fruit	4	3	2	1	0
2. Biscuits, cakes, cream, sweet pies, buns	4	3	2	1	0
3. Sweetened soft drinks (Coca-Cola, lemonade, fruit juice, etc.)	4	3	2	1	0
4. Sweetness / Honey	4	3	2	1	0
5. Chewing gum containing sugar	4	3	2	1	0
6. Sweets / candy	4	3	2	1	0
7. Milk with sugar / honey	4	3	2	1	0
8. Tea with sugar / honey (sweetened)	4	3	2	1	0
9. Cocoa with sugar / honey	4	3	2	1	0

Figure 1. Oral Health Questionnaire

Clinical examination was performed by calibrated examiners and calibration was performed by examining 21 subjects. The inter-examiner kappa coefficient ranged from 0.74-0.86 and the intra-examiner kappa coefficient ranged from 0.81-0.92. The ICDAS criterion was used to classify the severity of carious lesions, and the ICCMS Guidelines for Practitioners and Educators were used to classify the presence of filling material. Dental plaque or food debris was removed using cotton rollers. The data collected were recorded in a special table attached to the questionnaire and informed consent was obtained from each participant.

Statistical processing of the data was performed in Statistical Package for the Social Sciences (SPSS) version 23 for Windows. Spearman's rank correlation was used to analyse the relationships between variables. The rank correlation coefficient was denoted by rs or rho, and statistically significant values were considered $p < 0.05$. intervals.

RESULTS

The study included a total of 133 school children aged 11 to 14, with an average age of 12.26 years. Of these, 54.1% were boys and 45.9% were girls. In terms of age, the majority of the sample was made up of 12-year-olds (70.7%), followed by 13-year-olds (24.1%) and 11-year-olds (3%). There were only a few 14-year-olds (2.3%).

Table 1. Description of the sample according to gender and age

Variable		N (%)
Gender	Boys	72 (54.1)
	Girls	61 (45.9)
Age	11 years	4(3%)
	12 years	94(70.7%)
	13 years	32(24.1%)
	14 years	3(2.3%)

Parental education

The level of education of mothers and fathers was assessed as a determinant of children's oral health. Of the sample, 19 people reported that their mothers had completed secondary school, 38 had completed high school and 29 had completed university. In the case of fathers, 15 people had graduated from middle school, 25 had graduated from high school, and 24 had graduated from college.

Table 2. Description of the sample according to mother's and father's education

Level of education	Mother's education (%)	Father's education (%)
No school	1 (0.73)	1(0.49)
Primary school (grades 0-4)	3 (2.44)	4 (2.93)
Secondary school (classes 0-8)	19 (14.90)	15 (11.11)
Vocational school (grades 0-10)	18(13.80)	20 (15.14)
High school (grades 0-12)	38 (28.57)	25 (32.72)
Post-secondary school	7(5.37)	5 (3.54)
University	29 (22.10)	24 (18.19)
Don't know/ Don't answer	14 (10.62)	17(12.94)
Not living with parents	1 (0.37)	2(1.95)

Visits to the dentist

The frequency of visits to the dentist was analysed according to gender and the children's place of residence. It was found that 11.5% of boys and 8.5% of girls had never been to the dentist. In the last 12 months, 20.3% of boys and 17.4% of girls had never visited the dentist. For those who had visited the dentist, the percentage of visits ranged from 1 to 4 in a year.

The frequency of oral hygiene habits by gender was analysed. It was observed that the percentage of boys who had never been to the dentist was higher than that of girls. In terms of performing oral hygiene habits during a day and week, significant differences were observed between girls and boys.

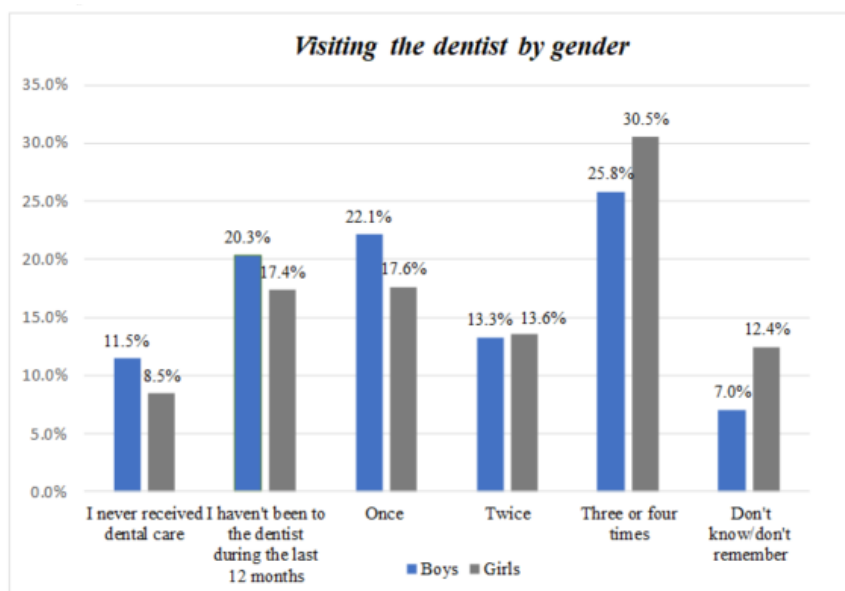


Figure 2. Dental visits by gender

The behaviour and clinical status of children from urban and rural areas were compared. It was found that the percentage of rural children who had never received dental care was higher than that of urban children. The percentage of students included in the study who have never received dental care is 13% for the rural environment, while for students coming from urban areas, it is lower, specifically 7.4%. Within the studied sample, 25.1% of rural students visited the dentist three to four times in the last year, while the percentage was notably higher in urban areas, at 30.8%. Around 13.2% of all rural students and a similar percentage of 13.5% among urban students reported visiting the dentist approximately twice a year. In conclusion, the frequency of annual dental visits within the studied sample is comparable between rural and urban students.

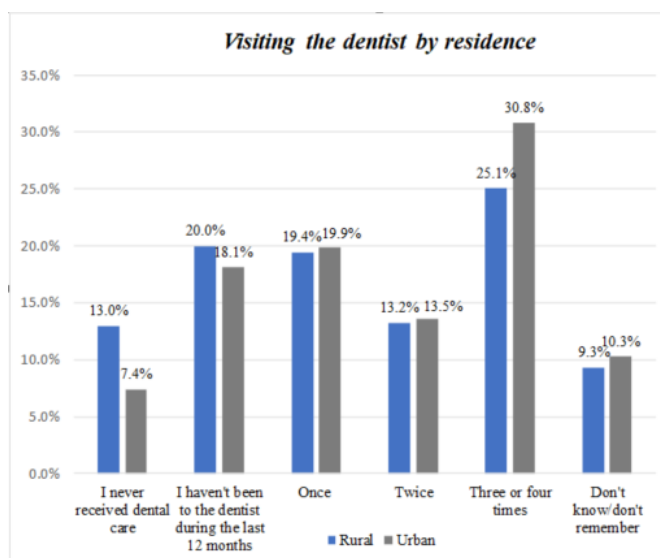


Figure 3. Visiting the dentist by residence

Examining children's eating habits: 34.4% eat fresh fruits multiple times daily, 37.6% enjoy treats like cakes and sweet rolls several times weekly, 33.4% consume sugary drinks several times weekly, 37.2% have jam or honey monthly, 32.2% chew sugary gum weekly. For 6th graders in western Romania: 24.8% eat candies once a day, 23.1% several times daily; 35.9% avoid sweetened milk, 32.8% drink sugary tea monthly. Also, 33.7% enjoy sugar/honey cocoa monthly as shown in Table 3.

Table 3. Description of eating habits

Type of eating habit	Never	A few times a month	A few times a week	Once a day	Several times a day
Fresh fruit	0.60%	4.10%	25.50%	35.30%	34.40%
Biscuits, cakes, creams, sweets, pies, sweets	1.50%	23.5%	37.60%	18.20%	20.80%
Sweetened drinks	4.60%	23%	33.40%	18.20%	20.80%
Jam/ Honey	14%	37.20%	28.70%	14%	6.20%
Chewing gum with sugar	13.90%	26.90%	32.20%	13.40%	13.60%
Sweets/candies	3.70%	13.70%	34.70%	24.80%	23.10%
Milk sweetened with sugar/honey	35.90%	22%	24.20%	12.30%	5.60%
Sweetened tea	15.30%	32.80%	27.80%	14.90%	9.040%
Cocoa sweetened with sugar/honey	31.30%	33.70%	18.90%	9.70%	6.40%

Eating behaviour

Children's eating habits and their correlations with the variables analysed were examined. It was observed that there were significant positive correlations between the consumption of foods with sugar content and the severity index of carious lesions. Parental education also had a significant influence on children's eating behaviour.

Table 4. Correlation between eating behaviour and parental education. *p<0.05, **p<0.001

Type of eating habit	Father's education Rs-rho(p,N)	Mother's education Rs-rho(p,N)
Fresh fruit	-0.01(0.01, 133)	-0.08(0.03, 133)
Biscuits, cakes, creams, sweets, pies, sweets	0.03(0.06, 133)	-0.08(0.03, 133)
Sweetened drinks	-0.02**(0.00, 133)	-0.02**(0.00, 133)
Jam/ Honey	-0.01(0.08, 133)	-0.00(0.09, 133)
Chewing gum with sugar	-0.02(0.07, 133)	-0.01*(0.02, 133)
Sweets/candies	-0.04(0.05, 133)	-0.01(0.00, 133)
Milk sweetened with sugar/honey	-0.01(0.02, 133)	-0.02*(0.00, 133)
Sweetened tea	-0.07(0.03, 133)	-0.02*(0.00, 133)
Cocoa sweetened with sugar/honey	-0.05(0.03, 133)	-0.01*(0.00, 133)

Correlation between eating behaviour and clinical condition

The determined DMFT index value for the sample was 2.93±2.70, within a range of 0 to 20. To better comprehend how the three indices of our study (MT, D3T and RT) are influenced by the dietary behaviour of children in the western region of Romania, a correlation analysis was conducted between these three indices and the variables composing the dimension.

Based on the obtained results, we highlight a statistically significant positive relationship between the consumption of sweetened beverages (0.24**), candies/sweets (0.18*), sweetened tea (0.24**), and D3T among children aged 11 to 14 years.

The relationships between the tested variables are strong and significant due to the significance threshold approaching +1 and the positively significant Spearman rank correlation coefficient. According to the results obtained, statistically negative relationships exist between the consumption of sweetened soft drinks (-0.12**) and the restoration elements in children from the western region of the country, with the significance level nearing -1 and each relationship being significantly negative (Table 5).

Table 5. Correlation between "MT", "D3T" and "RT" index and eating behaviour. *p<0.05, **p<0.001

Type of eating habit	MT rs-sho(p,N)	D3T rs-sho(p,N)	RT rs-sho(p,N)
Fresh fruit	0.07(0.93, 133)	0.08(0.34, 133)	0.01(0.82, 133)
Biscuits, cakes, creams, sweets, pies, sweets	0.08(0.35, 133)	0.14(0.10, 133)	-0.02(0.77, 133)
Sweetened drinks	-0.01(0.24, 133)	0.24**(0.00, 133)	-0.12**(0.11, 133)
Jam/ Honey	-0.07(0.38, 133)	0.06(0.45, 133)	-0.05(0.51, 133)
Chewing gum with sugar	-0.05(0.50, 133)	0.01(0.08, 133)	-0.01(0.19, 133)
Sweets/candies	-0.01(0.90, 133)	0.18*(0.03, 133)	0.05(0.53, 133)
Milk sweetened with sugar/honey	0.08(0.32, 133)	0.15(0.08, 133)	0.06(0.42, 133)
Sweetened tea	-0.02(0.80, 133)	0.24**(00.00, 133)	-0.03(0.68, 133)
Cocoa sweetened with sugar/honey	0.06(0.48, 133)	0.15(0.76, 133)	-0.06(0.43, 133)

DISCUSSIONS

According to a 2014 report from the European Platform for Better Oral Health, Romania lacks an oral health monitoring program involving systematic data collection. Additionally, Romania lacks a comprehensive oral health promotion program and a corresponding strategy. There is also no national scientific research program in the field of oral health in Romania [21].

In Romania, only a few oral epidemiological studies have been conducted in recent years, and systematic data on children's oral health behaviour are limited. This limits the ability to obtain nationally representative data on the prevalence or incidence of caries. Also, differences in diagnostic criteria and the use of non-standardised examiners make it difficult to compare data [21].

The results of two studies on the prevalence of caries in 12-year-old children have been published [22,23], suggesting a national average DMFT of 4.1 for 12-year-old children in 1992 [22] and 2.8 in 2000 [23]. Thus, for this study, the assessment criteria of the "International Caries Detection and Assessment System (ICDAS II)" were used to obtain results comparable to other similar studies worldwide.

Dietary behaviour has been identified as a significant risk factor influencing oral hygiene and caries prevalence among children. Several studies have shown that the consumption of sugar-containing foods and beverages is strongly associated with an increased risk of dental caries (Smith et al., 2017; Jones & Brown, 2019). Our findings are consistent with these studies, as we observed significant positive correlations between the consumption of sweetened drinks and the severity index of carious lesions. Furthermore, the impact of parental education on children's eating behaviour has been documented in previous research (White et al., 2015; Green & Johnson, 2018). Our study also found that parental education level significantly influenced the dietary behaviour of the participants.

It is mentioned that caries is a multifactorial disease, and the results of studies carried out in other European countries have provided valuable information on caries prevalence, reporting significant differences between countries [24]. Numerous comprehensive studies in the field have been conducted in countries such as Italy, Greece, Hungary, Slovenia, and Croatia, providing valuable insights and future considerations, highlighting significant variations between countries (e. g. DMFT of 4 in Croatia, 4.5 in Slovenia, 3.8 in Hungary, 2.05 in Greece, 0.8 in Italy) [25-27]. In contrast, in Romania there is a lack of comprehensive national studies on oral health in children or adults, with the exception of one longitudinal study assessing caries trends in Romanian schoolchildren.

Despite numerous studies conducted in neighbouring countries, comprehensive national studies on oral health in children or adults are lacking in Romania. A previous study

conducted by Petersen and colleagues in the 1990s covered only five major cities, without considering rural areas. Some local studies describe a high prevalence of caries in Romania [24,28,29]. Only one longitudinal study assesses caries trends among Romanian schoolchildren [30].

According to the transnational study HBSC 2001-2002, children's dietary patterns can provide information about their oral health status. A low percentage of daily candy consumption was reported by adolescents in Finland (9%), Denmark (12%), Sweden (13%), Norway and Greece (14%). The highest percentage of 11-13-year-old children reporting consuming sweets once a day or more was in Scotland (46%), Ireland (46%), the Netherlands (45%), Belgium, Flemish-speaking (41%), and Israel (41%). The highest percentages of 11-13-year-old children reporting drinking soft drinks at least once a day were in Israel (52%), Scotland (46%), the USA (42%), and the Netherlands (41%). Soft drink consumption was very uncommon in Finland (7%), Denmark (9%), Lithuania (10%), Sweden (12%), and Latvia (15%) [31].

In a local Romanian study conducted in Cluj-Napoca in 2017, on a sample of 650 schoolchildren with a mean age of 15.3 ± 2.8 years, the research revealed a relatively high caries index associated with sugar consumption. Although 62.2% of the subjects frequently consumed chocolate, the study showed significant gender differences specifically in the consumption of sugar-sweetened beverages ($p < 0.001$). Male adolescents consumed sugar-sweetened beverages more frequently than females (45.2% vs. 32.7%). The results of the DMFT index calculated in this study highlighted a higher prevalence of caries with frequent sweet consumption. Dietary factors and behaviours influence the occurrence of caries. Soda consumption directly influences the DMFT index ($p = 0.028$) [24].

Girls have better knowledge and attitudes regarding dietary habits and sugar consumption. Boys tend to consume more soft drinks than girls, and children with lower parental occupation consume more beverages than those with higher parental occupation [32]. Low parental education is considered one of the predisposing factors leading to poor child health, including oral health. Furthermore, it has been reported that parental educational levels are directly associated with the family's socioeconomic status [33].

One of the limitations of the study lies in interpreting the results, which could be distorted due to the study's design, as cross-sectional studies typically measure both cause and effect simultaneously, introducing temporal ambiguity and an inability to establish causal relationships. Additionally, it is important to consider that the data source from questionnaires may not be sufficiently reliable, especially regarding socioeconomic data.

The validity of the findings is supported by the comprehensive process of sample selection and data analysis, as well as meticulous calibration. This present study addresses caries prevalence and also assesses the roles of key caries risk factors and oral health behaviors, using the STEPS model as recommended by WHO guidelines. This ensures the ability to evaluate national trends and compare them with those of other countries. The STEPS approach advocates for regular and ongoing data collection.

Our study provides information regarding the determinants of oral health status among urban and rural schoolchildren aged between 11 and 14 in the western region of Romania. The data is statistically representative for this area. Considering the significant relevance of oral health issues among schoolchildren, identifying personal parameters that influence caries prevalence is essential for their prevention efforts.

CONCLUSIONS

In Romania, there is a clear lack of an oral health monitoring programme through systematic data collection and a general oral health promotion programme. This lack of

national programmes and strategies has led to few oral epidemiological studies and a lack of systematic data on children's oral health behaviour.

The results of this study indicate a high prevalence of caries in the children evaluated. The risk factors identified were dietary behaviour, especially the consumption of sweetened drinks, sweets and starches, and the level of parental education.

The findings from this study provide a picture of the oral health status of schoolchildren in a specific area of Romania, but comprehensive national studies are needed to obtain representative data and assess trends at national level.

Finally, it is necessary to adopt appropriate strategies and programmes for monitoring and promoting oral health in Romania, taking into account the identified risk factors and involving the education of children and parents in promoting oral hygiene and adopting healthy eating behaviour.

In conclusion, our findings highlight the importance of addressing dietary behaviour, particularly the consumption of sugary foods and beverages, as a key factor in preventing dental caries. To ensure the long-term oral health of Romanian children, educational efforts must be directed towards promoting proper nutrition and oral hygiene practices from an early age.

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[Book Antiqua, 11 point, normal, justified alignment].

MATERIAL AND METHODS [Book Antiqua, 11, bold, left alignment]

Describe the selection of observations or subjects for the experiment (including controls). Identify methods, equipments (with the name and address of the manufacturer in brackets) and give sufficient details on procedures. Give references for the selected methods, including statistical methods; offer details and brief descriptions for previously published methods which are not well known; describe new or substantially modified methods, justify their use and assess their limitations. Precisely identify all used drugs and chemicals, including generic names, dosage and administration ways. Describe statistical methods with sufficient details for reported results to be verified. Whenever possible, quantify discovered aspects and present them with appropriate measurement indicators for the uncertainty or error of measurement (such as confidence intervals). [Book Antiqua, 11 point, normal, justified alignment].

RESULTS [Book Antiqua, 11, bold, left alignment]

Present results in a logical succession as text, tables and illustrations. Emphasize or briefly describe only important observations. [Book Antiqua, 11 point, normal, justified alignment].

DISCUSSIONS [Book Antiqua, 11, bold, left alignment]

Underline new, important aspects of the study. Do not repeat in detail data which have been presented in previous sections. Include implications of revealed aspects and their limitations, including implications for future studies. Connect your observations to other relevant studies. Relate the results to the aim proposed for the study. [Book Antiqua, 11 point, normal, justified alignment].

CONCLUSIONS [Book Antiqua, 11, bold, left alignment]

Organize conclusions which emerge from the study. In the end state: a) contributions to be acknowledged but which do not justify paternity right; b) thanks for technical support;

c) thanks for financial or material support. [Book Antiqua, 11 point, normal, justified alignment].

REFERENCES [Book Antiqua, 11, bold, left alignment]

A numbered list of references must be provided at the end of the paper. The list should be arranged in the order of citation in the text of the publication, assignment or essay, not in alphabetical order(according to the Vancouver rules). List only one reference per reference number. It is very important that you use the correct punctuation and that the order of details in the references is also correct.

- Books - Standard format - #. Author of Part, AA. Title of chapter or part. In: Editor A, Editor B, editors. Title: subtitle of Book. Edition(if not the first). Place of publication: Publisher; Year. p. page numbers.
- Journal Articles - Standard format - #. Author of article AA, Author of article BB, Author of article CC. Title of article. Abbreviated Title of Journal. year; vol(issue): page number(s).
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- [1] _____
- [2] _____
- [3] _____

6.2. CONTENT OF THE PAPER - INDICATIONS FOR CASE REPORTS

Content of the paper for case report will respect indications for original articles.

Themes may be selected from all medical fields. Manuscripts which offer a special gain for daily activity will have priority. The title must be clearly, precisely stated. It may be completed by a subtitle. It is advisable to include in the key words of the title the main message, the special element which may be observed from the case evolution. The content of a case report must be divided into three parts:

Introduction - It must include a maximum of 15 typed rows (half page). Here, the main medical problem is summarized in order to place the case in a specific domain.

Case report - It contains essential specific information on the case. In order to make a logical, chronological and didactical case report the following 5 chapters are needed:

- I. Anamnesis;
- II. Clinical examination data;
- III. Laboratory data;
- IV. Additional paraclinical investigations;
- V. Treatment and evolution.

Discussions - The reason for the case report must be stated. The report must be patient-centered. Occasional deviations from typical (characteristic) evolutions, nosologically important facts must be presented in such a manner to expose the clinical picture as completely as possible. The case report must not appear as an appendix of a general review. Dimensions of a case report: maximum 6-8 typed pages, 30 rows of 60 characters/page.

6.3. MEASUREMENT UNITS, SYMBOLS, ABBREVIATIONS

All measurements must be expressed in International System (IS) units. Abbreviations must be fully explained when first used.

6.4. TABLES

Tables are noted with Roman figures and they will have a brief and concise title, concordant with their content.

6.5. ILLUSTRATIONS

Number all illustrations in Arabic figures in a single succession. Apply a label on the back side of every illustration, containing its number and an arrow indicating the upper side. Coloured illustrations may be accepted but it is the choice of the editors, according to particular technical abilities of each journal issue, or it may involve a fee in special cases.

6.6. EXPLANATIONS FOR DRAWINGS AND GRAPHS

Explanation for drawings and graphs must be clear and in readable dimensions, considering the necessary publishing shrinkage.

6.7. PHOTOGRAPHS

Offer glossy, good quality photographs. Any annotation, inscription, etc. must contrast with the ground. Microphotographs must include a scale marker.

6.8. ILLUSTRATION LEGENDS

Include explanations for each used symbol, etc. Identify the printing method for microphotographs.

7. COPIES FOR PUBLISHING

In order to accelerate publishing, the main author will send a set of printed sheets presenting the final version of the paper, as it will appear in the journal. It is really helpful that texts to be also sent on electronic support, diacritic characters mandatory.

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