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FACULTY OF DENTAL MEDICINE

PHD THESIS
ABSTRACT

THE EVALUATION OF ODONTAL-PERIODONTAL MANIFESTATIONS IN PATIENTS WITH CHEMOTHERAPY IN THE CONTEXT OF TUMOR PATHOLOGY

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SUMMARY

Note: The abstract of the PhD thesis presents the results of personal researches, general conclusions and selective references. The editing of the abstract respected the same countering of tables and pictures used in the PhD thesis.

The PhD thesis contains 68 figures, 43 tables and 411 references. The abstract includes a limited number of tables and figures, maintaining the original numbers from the PhD thesis.

KEY WORDS:
- Chronic periodontitis
- Chemotherapy
- Periodontal parameters
- Subgingival microbiome
- Cancer

PERSONAL CONTRIBUTIONS

CHAPTER 4. MOTIVATION AND GENERAL OBJECTIVES OF THE STUDY. MATERIALS AND METHOD

4.1 Motivation and general objectives of the study

The researched theme is complex, aiming to clarify the relationship between malignant tumour treatment – chemotherapy and the local oral status with a focus on periodontal disease.

A. General objective: verifying the hypothesis of an existing link between oral and periodontal modifications and local and systemic effects of chemotherapy and demonstrating the bidirectional relationship between them by epidemiological, clinical and fundamental research.

B. Specific objectives:

1. The included studies propose an evaluation of clinical parameters specific to periodontal disease in oncology patients treated with chemotherapy, establishing the correlation between the type of chemotherapy administered and periodontal status researched in chapter 5. The results of that study motivated and justified the expansion of this research project towards a clinical and fundamental level.
2. Verifying the hypothesis according to which chemotherapy is a local risk factor for periodontal disease by dosing the chemotherapy drugs in saliva and demonstrating a possible process of direct influence of chemotherapy on periodontal disease alongside the indirect influence that is already known.
3. Analyzing the modifications of
4. We also proposed the clinical evaluation of the efficiency of antiseptic, antimicrobial and antifungal substances on the periodontal status in patients receiving chemotherapy by analyzing the influence of two oral hygiene compounds.
5. Verifying the hypothesis according to which chemotherapy has unwanted effects in the oral cavity of patients during chemotherapy and establishing an individualized periodontal therapy protocol in direct collaboration with the oncologist.

4.2 Materials and method

The study followed the rules stated in the Declaration of Helsinki and was made

according to the basic principles of ethics in clinical and paraclinical research on human subjects. Patients were informed of the implications of the study; each patient's signed consent has been obtained.

In the study were included patients suffering of systemic cancer, receiving chemotherapy and have periodontal disease.

In order to avoid the risk of compromising the validity and relevance of the results, the following exclusion criteria were considered:

- Smokers
- Patients with infectious and/or inflammatory diseases that could affect the periodontal status with the exception of systemic cancer
- Patients that have received periodontal treatment in the last 6 months
- Patients that have taken antibiotic or anti-inflammatory drugs in the last 3 months, with the exception of chemotherapy

Each patient filled in a questionnaire that contained questions about general data of the patient and contact, oncological diagnostic, cancer stage, any previous therapy and

chemotherapy (prescribed drug, chemotherapy history or any other treatments related to the oncological diagnostic), oral hygiene data and periodontal status. The patients were diagnosed with cancer by the oncologist following clinical and paraclinical examinations and the inclusion of oncological data in the present study was made with the consent of the oncologist. This data consisted of information regarding: TNM classification of cancers, whether the tumor was primary or secondary, the degree of histological differentiation and previous therapy.

The data regarding the chemotherapy treatment consisted of the chemotherapy agent administered, dosage, frequency of administration and total number of administrations.

The periodontal examination were made with the help of the WHO periodontal probe by a single examiner. During the vertical probing we evaluated the clinical attachment loss and probing depth; the examination was conducted on Ramfjord teeth in six sites: distal-buccal, central-buccal, mesial-buccal, distal-oral, central-oral and mesial-oral. We considered pathological measurements deeper than 3 mm on teeth without gingival recessions. In the case of recessions, these were included in the clinical attachment loss (CAL) alongside probing depth (PD).

The following periodontal indexes were used in the periodontal evaluation: Sillness & Loe plaque index, calculus index, dental mobility index, Sillness & Loe gingival index, papilla bleeding index PBI and the PDI periodontal index.

Chapter 5. EVALUATION OF THE RELATIONSHIP BETWEEN PERIODONTAL DISEASE AND CHEMOTHERAPY SIDE-EFFECTS IN ONCOLOGY PATIENTS

5.1. STUDY 1. EVALUATION OF THE RELATIONSHIP BETWEEN PERIODONTAL DISEASE AND CHEMOTHERAPY AND BEVACIZUMAB SIDE-EFFECTS IN ONCOLOGY PATIENTS

5.1.1 Aim of the study

Cancer and chemotherapy are a globally widespread concern. Periodontal disease is also characterized by a high incidence rate in the global population and is a inflammatory site with continuous action when the patient does not benefit of periodontal treatment.

The aim of this study was to evaluate the clinical periodontal parameters in oncology patients treated with chemotherapy, with or without the addition of bevacizumab, to establish a correlation between the type of chemotherapy administered and the periodontal status in these conditions.

5.1.2. Materials and method

The study was conducted on a number of 30 subjects between the ages of 44 and 76 in the Oncology Clinic of "Victoria" Hospital, Iasi. The examination and diagnostic of the patients was conducted after the methods previously described.

5.1.3. Results

The patients included in the study suffered from systemic cancer localized in the colon (6 cases – 20%), esophagus (4 caseses – 13.3%), lungs (5 cases – 16.6%), rectum (6 cases – 20%), kidneys (3 cases – 10%) and breast (6 cases – 20%).

The oncological treatment that was administered to the patients were zoledronic acid, cisplatin, docetaxel, folfiri ((leucovorin+5FU+irinotecan) and oxaliplatin. From the total of 30 patients, 15 were treated with the aforementioned chemotherapy and the rest had the addition of bevacizumab. More than half (60%) of subjects were treated with platinum-based chemotherapy (cisplatin and oxaliplatin).

90% of patients had a medium or unsatisfactory level of oral hygiene.

The average value of the plaque index was 1,908 (DS 0.49). Patients that received chemotherapy had an average value of 1,886 (DS 0,42) and patients with chemotherapy and bevacizumab a value of 1,93 (DS 0,56).

The average value of the calculus index was 1,039 (DS 0.47). Patients that received chemotherapy had an average value of 1,04 (DS 0,61) and patients with chemotherapy and bevacizumab a value of 1,03 (DS 0,30).

The average value of the PDI periodontal index was 2,123 (DS 1.27). Patients that received chemotherapy had an average value of 1,74 (DS 0,86) and patients with chemotherapy and bevacizumab a value of 2,50 (DS 1,51).

The average value of PD was 1.625 (DS 0.61). Patients that received chemotherapy had an average value of 1,41 (DS 0,31) and patients with chemotherapy and bevacizumab a value of 1,83 (DS 0,77).

Average values of plaque, calculus and PDI indexes are compared in Table 5.1.I taking into account the differences between the group of patients receiving chemotherapy and the group receiving chemotherapy and bevacizumab.

Table 5.1.I. *Statistical analysis of average values depending on the administration of bevacizumavb*

Paremeter	Non Bevacizumab	Bevacizumab	*p value*
Average value plque index	1,886 (0,420)	1,930 (0,569)	0,741
Average value calculus index	1,046 (0,619)	1,032 (0,300)	0,904
Average value PDI index	1,746 (0,869)	2,500 (1,513)	0,121
Average value PD	1,414 (0,313)	1,837 (0,774)	0,093

The average value of the PDI periodontal index was higher in patients treated with bevacizumab (2.5) compared to the chemotherapy group (1.74). The *p* value was not lower than 0.05, hence this was statistically insignificant.

Moreover, we evaluated the relation between average values of plaque index, calculus index, PDI periodontal index and PD in regards to the chemotherapy administered while maintaining the integrity of the two major groups (chemotherapy with or without bevacizumab) in Table 5.1.II and 5.1.III.

Table 5.1.II. *Average values of periodontal indexes in regards to the chemotherapy agent administered in the non-bevacizumab group*

Non Bevacizumab	Average plaque index	Average calculus index	Average PDI index	Average PD
Cisplatin	1.700 (0.629)	0.600 (0.522)	1.650 (1.118)	1.466 (0.292)
Docetaxel	2.000 (0.200)	1.200 (0.200)	1.067 (0.231)	1.041 (0.115)
Oxaliplatin	2.066 (0.115)	0.867 (0.115)	1.667 (0.115)	1.382 (0.305)
zoledronic acid	1.963 (0.115)	1.963 (0.115)	2.696 (0.115)	1.713 (0.115)

The PDI index was found higher in patients treated with cisplatin and bevacizumab compared to those treated with cisplatin and those treated with oxaliplatin as it is shown in the table above.

A weak statistical significance was found for the values of the PDI periosontal index in patients treated with cisplatin versus cisplatin and bevacizumab.

Table 5.1.III. *Average values of periodontal indexes in relation to the administered chemotherapy agent in the bevacizumab group*

Bevacizumab	Average plaque index	Average calculus index	Average PDI index	Average PD
Cisplatin	1.886 (0.280)	1.000 (0.200)	2.800 (0.200)	1.383 (0.200)
Docetaxel	1.526 (0.115)	1.330 (0.200)	1.200 (0.200)	1.183 (0.200)
Oxaliplatin	2.080 (0.867)	0.767 (0.175)	2.700 (2.235)	2.071 (0.971)
Folfiri	2.267 (0.115)	1.067 (0.115)	2.666 (0.115)	1.716 (0.115)

5.1.4 Discussions

The patients part of this study had a medium or unsatisfactory level of oral hygiene, making up 90% of subjects. Taking into account that 60% of patients declared that they brush twice a day, we can stipulate that the technique they used was not a correct one. Moreover, 40% of patients replaced their toothbrush less often than 3 months or at its deterioration and not every 3 months as is recommended (Sforza et al., 2001).

In the present study we demonstrated that chemotherapy and bevacizumab lead to an increase of bacterial plaque expressed through the plaque index (non-bevacizumab 1,886 vs bevacizumab 1,93). This can have an important impact on periodontal disease, the ladder having the potential to have an accelerated rhythm in patients treated with chemotherapy and bevacizumab. Regarding the quantity of calculus, the average value of this index was comparable between the two groups (1.04 vs 1.03) and making for an insignificant difference.

The average value of PD was different between the two groups. The average value was higher in the bevacizumab group (1,837, deviaţie standard 0,774) compared to the non-bevacizumab group (1,414, deviaţie standard 0,313).

In the present study we found that differences in the manifestation of periodontal disease in patients treated with cisplatin or oxaliplatin compared to patients treated with cisplatin or oxaliplatin and bevacizumab, the average value of the PDI periodontal index being higher in patients who received bevacizumab.

The chemotherapy treatment, especially associated with bevacizumab, leads to various systemic modifications like temporary suppression of the host immune system. This immunosuppression has an impact on the periodontium, altering the local antibacterial efficiency to oppose the aggressors in the bacterial plaque.

The main motivation for tooth loss was caries lesions (50% of cases). Only 20%, a number of 6 cases declared the tooth loss due to periodontal disease in the form of high tooth mobility. Dental loss can be a proxy for chronic dental or periodontal infections and, moreover, Virtanen et al. demonstrated in his cohort studies that tooth loss can be a risk factor for the development of cancerous lesions (Virtanen et al., 2014).

Generally, it is often cited that there are a high number of cases with chemotherapy the develop infections during its course of treatment along with the hematological toxicities (neutropenia and thrombocytopenia) that can affect up to 29% and, respectively, 14% of patients. The addition of bevacizumab to the chemotherapy treatment plan can lead to more rarely cited complications like digestive tract perforations (up to 6% of cases) (Richardson et al., 2008).

This bacterial manifestation was reported by multiple authors that studied the effects of bevacizumab and chemotherapy that have developed various forms of periodontal disease during the time of treatment (Lim et al., 2014; Agranovich et al., 2008).

5.1.5. Conclusions

1. The present study demonstrates the correlation between the administration of bevacizumab with higher bacterial plaque quantities, altering of the periodontal clinical aspect and a rise in probing depth values in chemotherapy patients treated with bevacizumab compared to the non-bevacizumab group.

2. Oncology patients that undergo chemotherapy and, especially, patients with bevacizumab in their treatment plan, require prophylactic treatment and careful monitoring because of the high risk of systemic infectious complications during treatment and accelerated progression of periodontal disease.

5.2. STUDY 2. THE DETERMINATION OF CHEMOTHERAPY DRUGS IN SALIVA THROUGH SPECTROMETRY AND CROMATOGRAPHY METHODS CORRELATED WITH THE PERIODONTAL STATUS IN ONCOLOGY PATIENTS

5.2.1. Aim of the study

The aim of this study was to quantify systemically administered chemotherapy in saliva in the context of oncological treatment in patients with malignant tumors. The oral and periodontal clinical modifications are also investigated in correlation with the quantity of chemotherapy that has reached the oral cavity.

5.2.2. Materials and method

The examination and diagnostic of the patients was conducted after the methods previously described.

The collection of saliva samples took place before the clinical examination. The subjects included were asked to rinse with water, then collect a minimum of 5 ml of total saliva in sterile recipients before commencing the administration of chemotherapy, at 21 days from the previous administration. The procedure was repeated at 30 minutes and 2 hours after the completion of the chemotherapy administration, thus obtaining three samples from each of the patients.

Cisplatin, oxaliplatin and gemcitabine chlorhidrate provided by the European Pharmacopeia were used in the present study. The determinations were done using a mass spectrometry type system (Tripluquadrupol Access Max) and chromatographic separation system type (Transcend TLX 1). The chromatographic separation was done on a chromatographic column Phenomenex Kinetex C18, 50 mm length, internal diameter of 4.6 mm and particle size of 5µm.

5.2.3. Results

This study was done on a number of 29 subjects. 37.9% were females and 62.1% were male, the majority of patients being admitted for chemotherapy originating from the urban areas and almost 38% from rural areas.

The age of the patients varied between 43 and 80 years old, having the lowest minimum and highest maximum among the male population in our study. The global age average was 59.62 years old.

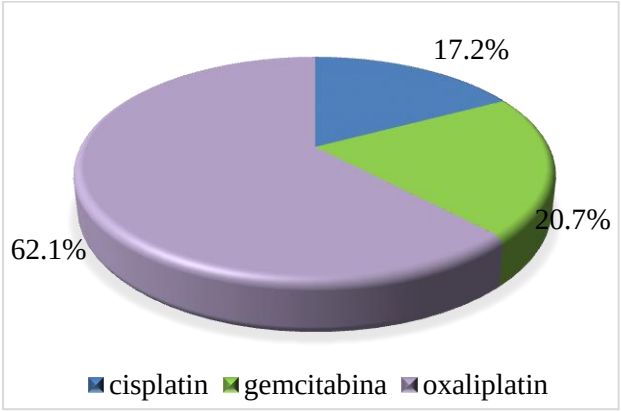

Fig. 5.2.1. *Lot distribution depending on the chemotherapy*

From the oncological standpoint, the highest frequency in diagnostic was colon cancer (n=10 cazuri; 34.2%), followed by lung cancer (n=7; 24.1%), pancreas and bileduct cancer (13.8%), rectum cancer (13.8%), esophagus cancer (6.9%) and mixtoid liposarcoma (6.9%).

The chemotherapy patients received was the following: cisplatin, oxaliplatin or gemcitabine, the highest frequency being those treated with oxaliplatin (62.1%, n=18) (Fig. 5.2.1).

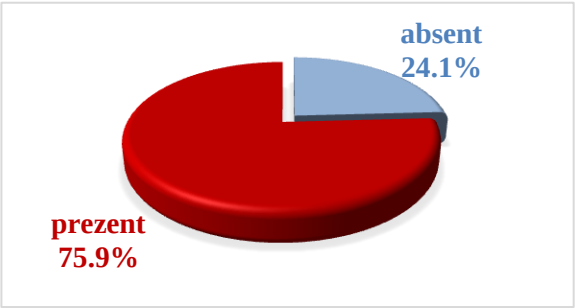

Fig. 5.2.2. *Percentage of patients that experienced side effets*

A higher number of patients experienced nausea (34.5%), loss of appetite (27.6%), alopecia (27.6%), mucositis (13.8%), xerostomia (13.8%), dizziness (13.8%), peripheral sensations (13.8%) and fever (13.8%). The rest of the side effects has a frequency lower than 7%.

The majority of patients had a low level of oral hygiene (65.5%), only 13.8% of them presented with good oral hygiene. The highest values of the gingival index with a score of 3 were at 16 (15.4%), followed by 21 (7.4%) and 41 (3.7%). The most affected areas of the oral cavity of patients were frontal areas and posterior maxillary areas.

In correlation with the previous index, the gingival bleeding index had the highest values at 16 (score 5 – 15.4%), followed by 21 (score 4 – 7.4%) and 41 (score 4 – 3.7%).

Tabel 5.2.I. *Probing depth values on Ramfjord teeth*

Probing depth	N	Average	Mean std. error	Standard deviation	Minimum	Maximum
16	13	1.6115	0.20165	0.72707	1	3.16
21	27	1.3844	0.11746	0.61035	0.58	2.91
24	23	1.3574	0.09628	0.46174	0.83	2.5
36	10	1.464	0.11756	0.37176	1.08	2
41	27	1.2863	0.14588	0.75802	0.58	2.83
44	25	1.2896	0.13483	0.67416	0.83	3.08

The teeth most frequently affected during probing depth were 16 (average=1.6115 mm) and 36 (average=1.464 mm) (Table 5.2.I).
The highest values for CAL were observed at 36. 41 and 44 (average=3.214, 3.308 and respectively 3.163 mm). On the maxillary, the highest mean value was found at 21 of 2.415 mm (Fig. 5.2.10).

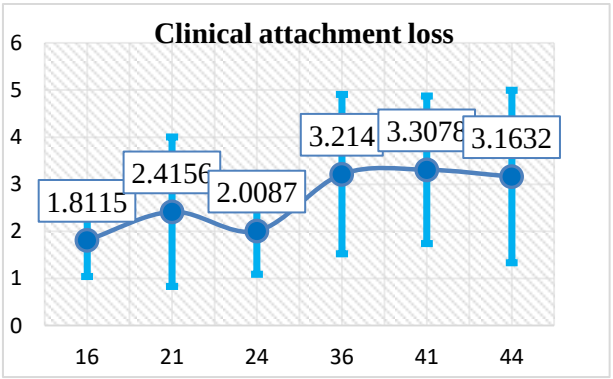

Fig. 5.2.3. *Mean value distribution of CAL*

Correlating the quantity of chemotherapy in saliva in T0, T1 and T2 we obtained that the registered quantity differences were statistically significant ($p<0.05$). The highest values obtained for each of the three chemotherapy agents (CIS, OXA, GEM) in saliva was at T1, followed by a decrease in T2.

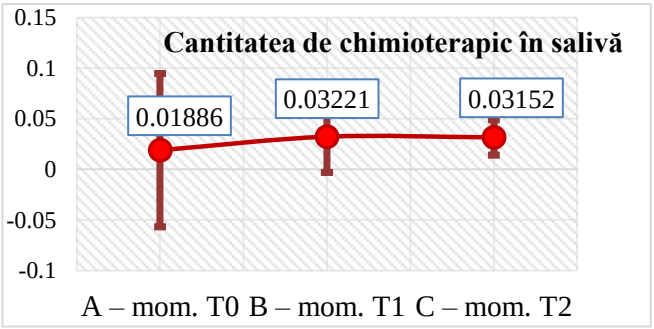

Fig. 5.2.4. *Mean quantity values (µg/ml) of chemotherapy found in saliva at 21 days, 30 min and 2 hours after administration*

The concentration curves for CIS, OXA and GEM followed the same pattern in T0, T1 and T2. The highest concentrations were registered after 30 minutes from the completion of the chemotherapy as follows: CIS T1=0.0016 µg/ml, OXA T1=0.0507 µg/ml şi GEM T1=0.0106 µg/ml.

5.2.4. Discussions

Saliva is an extremely valuable fluid from the diagnostic point of view, used in numerous instances due to its complex and varied composition that is closely related to the general status (Solomon et al., 2015). Because of the multiple secondary effects, dosing various substances in plasma have the potential to put the patients at risk and for this reason,

dosing in saliva with the help of spectrometry can offer useful information but without any additional risks (Gift et al., 2004).

A big part of the administered quantity of cisplatin (around 90%) is inactivated by plasmatic proteins (Wang et al., 2007). Determination in blood samples of this compound is done for both forms – free and bound – (Vermorken et al., 1986; Korst et al., 1998) and also the total quantity of plasmatic platinum (Bonetti et al., 1995). The concentrations in plasma of cisplatin were reported to be undetectable at 24-25 hours after perfusion, according to a study from 2004 (Urien, Lokiec, 2004).

Among the analogues of pirimidine, gemcitabine is one of the more frequently used drugs in oncology, being the third most used drug worldwide. It makes for basic treatment for pancreatic adenocarcinoma (Burris et al., 1997) and various other solid tumors like mammalian, ovarian, lung and bladder cancer (Sandler, Ettinger, 1999; Shelley et al., 2012; Kroep et al., 2000).

The maximum concentration values within the oral cavity for oxaliplatin is, in average, 2.59 µg/ml (Massari et al., 2000) and compared to the maximum values obtained in saliva in the present study which were 0.0507 µg/ml it can be said that only a small fraction of the administered dose reaches this level, the concentration in saliva being aproximately 45 times lower than in plasma.

The secondary effects in the oral cavity seem to be also linked to a series of factors patient specific, like age, nutritional status, cancer type, dental and oral pretreatment, oral treatment, care and monitorizing during chemotherapy and the neutrofil count before commencing chemotherapy (Sonis, 1998; Wilkes, 1998). The oral hygiene status of patients was, in general, unsatisfactory with important bleeding on probing and significant clinical attachment loss. Patients with hematological malignancies, pre-existing poor oral hygiene and periodontal disease, a low nutritional level and low immunity have a higher rate of oral complications following the administration of chemotherapy (Epstein et al., 2012).

Generally, taking into account the mean value of the CPITN index, the need for oral hygiene for these patients is confirmed, a part of these in need of etiological periodontal treatment.

The possibility that chemotherapy can mask the real clinical periodontal aspects can be due to myelosuppression that leads to anemia, leucopenia and thrombocytopenia. This lowering of the immune system can lead to a reduction or even absence of oral and periodontal inflammatory phenomena (Chaveli-López, 2014).

It is possible that the administration of chemotherapy along with poor oral hygiene manifest with predilection in some areas of the oral cavity, in posterior mandible and frontal maxillary areas as shown in the present study.

5.2.5. Conclusions

1. A fraction of systemically intravenously administered chemotherapy can be found in saliva where it can be detected up to 21 days from administration. The maximum saliva concentrations for cisplatin, oxaliplatin and gemcitabine were registered at 30 minutes after the complesion of the perfusion. The correlation between the three determinations was statistically significant.

2. The presence of chemotherapy in saliva, that have the potential to worsen the oral status, is an additional motive that supports the establishing of an oral and periodontal prophylactic treatment protocol before the start of oncological therapy along with a close monitoring of the patient throughout chemotherapy.

Chapter 6. MODIFICATION OF THE SUBGINGIVAL MICROBIOME AFTER CHEMOTHERAPY IN THE CONTEXT OF MALIGNANT TUMOR PATHOLOGY

6.1. Aim of the study

The aim of this study was to analyze and evaluate the microbial modifications in periodontal pockets that can occur once the chemotherapy treatment commences in patients with malignant tumor pathology. The correlation between microbial dynamic modification with the clinical periodontal modifications is investigated in order to observe the influence of periodontal pathogens on periodontal disease manifestations.

6.2. Materials and method

The examination and diagnostic of the patients was conducted after the methods previously described.

The collection of gingival fluid samples was done before the clinical examination. The gingival fluid samples were collected using six sterile paper cones of standard diameter manipulated with a sterile forceps before the commence of chemotherapy treatment (sample A) and at 21 days from the first administration (sample B). After the removal of paper cones from periodontal pockets they were conserved in Eppendorf tubes of 1.5 ml in a RNAlater solution until the moment of analysis.

The laboratory analysis of the samples was done using the Illumina kit Metagenomics with the help of Next Generation Sequencing.

6.3. Results

Of the 30 subjects, 18 were male (60%) and 12 female (40%) (Fig. 6.1), 27 patients residing in urban areas and only 3 originated from rural areas (90% vs 10%) (Fig. 6.2).

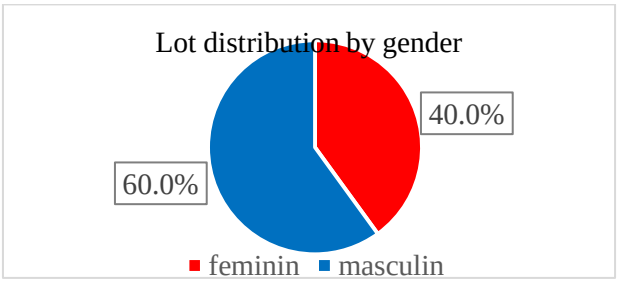

Fig. 6.1. *Lot distribution by gender*

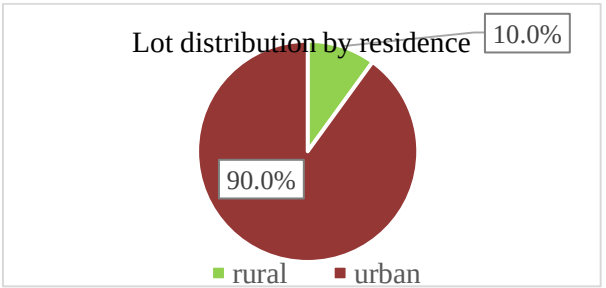

Fig. 6.2. *Lot distribution by residence*

The oncology diagnostics that were most frequent were the malignant tumors of the colon (transverse – 5 cases, descending – 1 case) and liver tumors (5 cases).

Correlated with the type of chemotherapy administered, the plaque index varied significantly. It was also of note that the calculus index was modified after treatment with cisplatin and oxaliplatin.

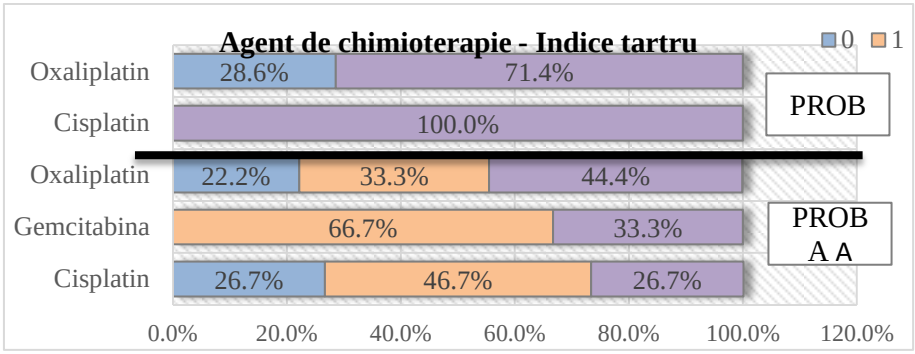

Fig. 6.3. *Calculus index values modification correlated with chemotherapy administered*

The biggest differences in PD values were observed at teeth 16 and 36 (Fig. 6.4.).

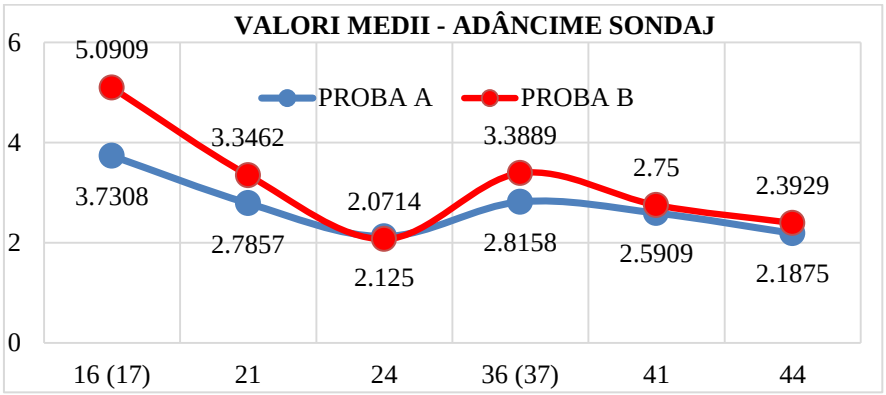

Fig. 6.4. *Mean PD values before (A) and after chemotherapy (B)*

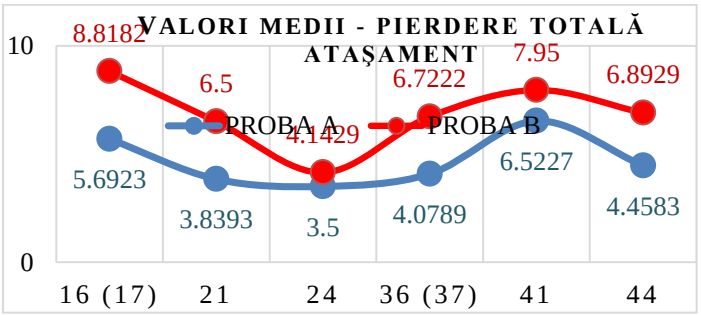

Fig. 6.5. *Mean value modification of CAL before (T0 - Proba A) and after chemotherapy (T1 – Proba B)*

The percentage of PD between 3-6 mm increased after the chemotherapy treatment started (T1). The percentage increase in patients treated with cisplatin was from 26,7% to 57,1% and from 55,6% to 71,4% in patients treated with oxaliplatin.

The mean values of CAL marked an important increase in all the evaluated areas (Fig. 6.5.).

Bacterial phylum results showed the following in the subgingival area in both samples: *Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria, Actinobacteria, Spirochaetes, Verrucomicrobia, Cyanobacteria, Synergistete* and *Tenericutes.* Sample B showed a statistically significant increase of the *Bacteroidete* group.

Within the bacterial order, an important decrease in percentage was observed in *Xanthomonadalelor* (28,188% mean in sample A vs 24,989% mean in sample B), *Rhizobialelor* (15,722% A vs 10,1% B), *Pseudomonadalelor* (32,436% A vs 10% B). Only the decrease in percentage for *Rhizobialelor* was statistically significant (*p* - 0,001).

The bacterial orders that noted an increase in sample B compared to sample A are: *Bacteroidales* (14,409% vs 18,215%), *Lactobacilalle* (5,07% vs 12,39%) şi *Spirochaetale* (8,231% vs 15,65%). The increase of *Bacteroidales* which are frequently seen bacteria within the oral cavity (and closely related to *Porphyromonas spp, Tanerella spp, Prevotella spp*) is a statistically significant increase with a *p* value of 0.026.

Tabel 6.I. *Modification of percentage of bacterial genum before (T0) and after chemotherapy (T1)*

Bacterial genum	Mean % sample A	Mean % sample B
Phyllobacterium	12,553	7,655
Veillonella	6,107	3,646
Porphyromonas	7,603	5,112
Pseudomonas	32,4	9,96
Rhizobium	2,02	undetectable
Ochrobactrum	1,955	undetectable
Mesorhizobium	9,06	undetectable
Staphylococcus	4,85	undetectable

The bacterial genum that showed decrease in quantity after chemotherapy are presented in table 6.I.

We found statistical significance for the decrease of *Phyllobacterium* and *Vellionella* in sample B with *p* values of p=0,00 and, respectively, p=0,016.

Within bacterial families we observed a decrease in percentage for the following: *Xanthomonadaceae* (28,14% vs 24,938%), *Phyllobacteriaceae* (14,7% vs 9,965%), *Porphyromonadaceae* (10,17% vs 6,284%), *Pseudomonadaceae* (32,406% vs 9,98%), *Staphylococcaceae* (4,88% vs undetectable in sample B), *Campylobacteraceae* (3,37% vs undetectable in sample B) and *Leptotrichiaceae* (7,717% vs undetectable in sample B).

The bacterial families that showed increase in quantity in sample B were: *Prevotellaceae* (7,891% vs 10,062%), *Streptococcaceae* (4,74% vs 9,094%), *Fusobacteriaceae* (4,46% vs 6,69), *Pasteurellaceae* (undetectable vs 3,48%), *Flavobacteriaceae* (6,91% vs 14,09%) and *Spirochaetaceae* (8,23% vs 15,65%).

Tabel 6.II. *Modification of possible periodontal pathogen genums before (A) and after chemotherapy (B)*

Bacterial genum	Mean % sample A	Mean % sample B
Prevotella	9,417	13,584
Streptococcus	4,591	6,629
Fusobacterium	4,072	5,63
Neisseria	-	4,01
Treponema	7,762	15,65
Capnocytophaga	2,344	4,29
Chryseobacterium	15,035	18,03

The increase of *Prevotella* genum was statistically significant (p=0,026).

Overall, the bacterial special without any known implication in periodontal disease development had the tendency to decrease in quantity at 21 days from the first dose of chemotherapy (Table 6.III).

Tabel 6.III. *Percentage differences of non-pathogens before (T0 – proba A) and after chemotherapy (T1 – Proba B)*

Bacterial species	**Mean % sample A**	**Mean % sample B**
Stenotrophomonas retroflexus	11,309	7,472
Stenotrophomonas chelatiphaga	3,675	2,766
Stenotrophomonas maltophilia	4,1	3,34
Phyllobacterium brassicacearum	3,126	2,36
Stenotrophomonas pavanii	2,333	1,71
Sphingobacterium bambusae	2,51	2,22
Veillonella dispar	2,43	1,87

Of the bacterial species that could be involved in the development of periodontal disease, an important part noted increase in percentage after chemotherapy, with the exception of *Porphyromonas gingivalis* that noted a slight decrease (7.11% vs 6.44%) and *Prevotella intermedia* (4,49% vs 2,74%). The periodontal pathogens that has an increase in quantity in sample B are presented in table 6.IV.

Tabel 6.IV. *Percentage differences for periodontal pathogens before (T0 – Proba A) and after chemotherapy (T1 – Proba B)*

Bacterial species	**Mean % sample A**	**Mean % sample B**
Prevotella tannerae	2,477	5,425
Streptococcus tigurinus	1,82	3,45
Fusobacterium nucleatum	2,73	2,95
Fusobacterium naviforme	2,19	3,97
Prevotella multiformis	1,62	6,47
Treponema medium	4,22	5,81

The administration of oxaliplatin resulted in a quantity increase of a higher number of periodontal pathogens compared to patients whom were treated with cisplatin.

6.4. Discussions

The clinical modifications we obtained in the present study highlights major differences in periodontium between the two moments when the clinical examination was done, at only 21 days apart. The degree of periodontal degradation was especially highlighted due to the changes in probing depth and clinical attachment loss.

Recently, periodontal disease has been considered a disbiosis of the subgingival bacterial community, the interaction with the host and modifications that can appear in certain cases promoting a holistic vision on inflammatory processes. An imbalance in the subgingival area can be of immune nature, saprophytic bacteria, viral or others (Solomon et al., 2018).

Healthy subjects have been shown to have a higher abundance of a number of eight bacteria (*Lautropia, Corynebacterium, Parvimonas, Streptococcus, Actinomyces, Haemophilus, Capnocytophaga, Paludibacter*), whereas patients with periodontal disease have a number of six that are associated with periodontal distruction (*Filifactor, Tannerella, Treponema, Porphiromonas, Aggregatibacter, Peptostreptococcus*)(Tsai et al., 2016). The increase in *Bacteroidale*, which are frequently identified in the oral cavity and related to *Porphyromonas spp, Tanerella spp, Prevotella spp*)(Dewhirst, 2010) was statistically significant.

The bacterial genum of *Prevotella*, *Fusobacterium* and *Treponema* showed an increase in quantity after chemotherapy in patients with unsatisfactory oral hygiene, as opposed to the *Porphyromonas* genum that marked a decrease.

The bacterial groups without any implication in periodontal disease had the tendency to decrease in favor of periodontal pathogens. *Porphyromonas gingivalis* is a well known pathogen involved in the etiopathogeny of periodontal disease and is associated with a low oral hygiene status and periodontal pockets deeper than 3 mm mm (McNabb et al., 1992). Bleeding on probing and the presence of bacterial plaque is associated not only with high quantities of *P. gingivalis*, but also *P. intermedia* (Cortellini, Pini-Prato şi Tonetti, 1994) which can be associated with the increase of *Prevotella* genum in our study that was correlated to a low level of oral hygiene.

6.5. Conclusions

1. The administration of chemotherapy leads to the modification of the subgingival microbiome characterized by a decrease of nonpathogenic bacterial classes (*Xanthomonada*, *Phyllobacterium*, *Pseudomonas, Stenotrophomonas*) and increase of periodontal pathogens, especially *Treponema*, *Prevotella*, *Fusobacterium* and *Capnocytophaga*. Chemotherapy may have an inhibitory effect on some bacteria that are strongly associated with periodontal disease (*Porphyromonas gingivalis*, *Treponema denticola)* and potentiating the development of others (*Prevotella tannerae*, *Fusobacterium nucleatum*, *Fusobacterium naviforme*, *Prevotella multiformis* şi *Treponema medium*).

2. The modification of the microbiome in favor of periodontal pathogens was greater in patients treated with chemotherapy that had higher values of probing depth, pocket depth playing an important role in the composition of the subgingival bacterial plaque.

3. Understanding the effect of chemotherapy on the periodontal tissues and subgingival bacterial community offers important information useful in developing an individualized, complete and complex periodontal treatment and achieving optimal results during chemotherapy and in long term.

Chapter 7. EVALUATION OF THE ORAL EFFECTS OF TWO COMPOUNDS (BASED ON ANTISEPTIC, ANTIMICROBIAL AND ANTIFUNGAL SUBSTANCES) IN PATIENTS WITH CHEMOTHERAPY IN THE CONTEXT OF ONCOLOGICAL PATHOLOGY

7.1. Aim of the study

The aim of this study was to evaluate the effects that two products based on antiseptic, antibacterial and antifungal substances have on the oral cavity of patients with chemotherapy.

7.2. Materials and method

The examination and diagnostic of the patients was conducted after the methods previously described.

The patients were randomly grouped in three groups:

1. Control, that included patients to which no compound was administered.
2. Group A that included patients who used oral rinse with cetrimid three times a day
3. Group B that included patients who used oral embrocate (containing metronidazole, nistatin and neomicine) and produced in the pharmacy twice a day

7.3. Results

The study consisted of 50 subjects with ages between 48 and 80 years old recruited from the Oncology clinic of Victoria Hospital, Iasi.

The controls represented 24% of total patients included in the present study. Group A and B consisted of 22 and, respectively, 16 patients that used secondary means of oral hygiene (representing 44% and 34% of total number of patients).

The chemotherapy administered were cisplatin, oxaliplatin and gemcitabine, the one with the highest frequency being cisplatin (n=26, 52%), followed by patients treated with oxaliplatin (n=17, 34%) and gemcitabine (n=7, 14%)(Fig. 7.1).

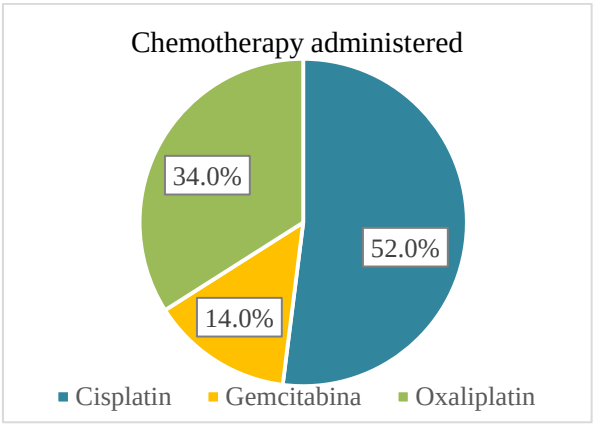

Fig. 7.3. *Subject distribution depending on the chemotherapy administered*

By analyzing the plaque index we obtained that group A which used oral rinses with cetrimid had lower mean values of the plaque index in T1 compared to T0 (Tabel 7.I).

Tabel 7.I. *Sillness & Loe plaque index values in control, group A and group B*

Sillness & Loe plaque index	N	Media	Eroarea standard a mediei	Deviația standard	Min	Max
T0						
Control	12	1.83833	.129228	.447657	1.000	2.400
Group A	22	**1.78864**	.086538	.405900	1.160	2.400
Group B	16	1.99688	.085502	.342008	1.160	2.400
Total	50	1.86720	.056545	.399837	1.000	2.400
T1						
Control	12	2.0025	0.09289	0.32179	1.400	2.500
Group A	22	**1.69682**	0.0861	0.40384	1.000	2.200
Group B	16	2.055	0.08018	0.32073	1.330	2.400
Total	50	1.8848	0.05538	0.3916	1.000	2.500

PBI index showed an important decrease of mean values for group A (T1=2.12 vs T0=2.28).

Moreover, the differences regarding the PDI index showed an increase of mean values for controls (T1=4.241 și T0=3.770) and decrease for group A (T1=3.652 și T0=3.756) and group B (T1=3.860 și T0=4.079).

Mean values of PD were lower in group A after the use of cetrimid oral rinse (T0=2.696 vs T1=2.552) compared to the controls and group B where the values marked an increase.

The results of CAL comparatively between the three groups showed an important increase in value for controls, from 4.660 mm in T0 to 5.844 mm in T1. On the other hand, groups A and B manifested a decrease of CAL values (group A: T0=4.316 and T1=4.155; group B: T0=4.333 and T1=4.239).

Table 7.II. *Statistical significance for group A comparing T0 and T1*

Analyzed indexes	**Group A T0 – T1**		
	Mean difference	t	p
Plaque index	.091818	2.358	.028, SS
Mean PDI index	.104091	2.097	.048, SS
Mean PBI index	.165000	3.578	.002, SS
Mean dental mobility	-.045000	-1.821	.083, NS
PD	.144636	4.661	.000, SS
CAL	.161318	3.409	.003, SS

Paired t test showed statistical significance of the improvements obtained for group A and periodontal disease advancement in controls.

Tabel 7.III. *Statistical significance for controls comparing T0 and T1*

Analyzed indexes	**Controls T0 – T1**		
	Mean difference	t	p
Plaque index	-.164167	-2.680	.021, SS
Mean PDI index	-.471667	-4.513	.001, SS
Mean PBI index	-.300833	-7.473	.000, SS
Mean dental mobility	-.080000	-1.948	.077, NS
PD	-.479167	-4.823	.001, SS
CAL	-1.183250	-3.467	.005, SS

7.4. Discussions

In the present study we analyzed the modifications between T0 and T1 taking into account the type of chemotherapy and usage of oral rinses or pharmacy compound. The controls, indifferent of chemotherapy administered, showed an increase in index values, PD and CAL. The most important improvements of all oral parameters including PD and CAL were noted in the group that used oral rinses with cetrimid (Guerreiro-Tanomaru et al., 2014).

The use of antimicrobial and antiseptic subtances are efficient in reducing bacterial plaque and amelioration of periodontal parameters (DePaola et al., 1989). Cetrimid, the active substance in Citrolin, is an antiseptic composed of ammonia quaternary salts with a bactericidal effect on a wide spectrum of gram positive and gram negative bacteria (Engebretsen et al., 2015).

The highest frequency in the present study was of patients treated with cisplatin whom represented more than half of the total number of patients included and approximately half in each of the study groups. It is important to take into account the high number of adverse effects that cisplatin has quote in literature (Urien, Lokiec, 2004; 384. Vermorken et al., 1984; Breglio et al., 2017), side effects due to cytotoxicity mediated primarily through cross link DNA and production of oxygen reactive species (Breglio et al, 2017).

The modifications of values for the PBI index has considerable importance because during chemotherapy and once the marrow modifications take place (Florea, Büsselberg, 2011) then thrombocytopenia may appear and induce a high incidence of gingival bleeding in the oral cavity, especially on the premises of a pre-existing periodontal condition (Rapone et al., 2016).

Mean probing depth resulted in this study showed considerable differences between the patients in the control group and patients who used secondary means of hygiene discussed. The highest improvements were observed in patients who used the cetrimid oral rinse. One way to explain these results is because cetrimid's saliva concentration holds a higher rate of oral retention than chlorhexidine immediately after the rinse.

In the absence of oral hygiene and secondary means of hygiene, patients with chemotherapy have a predisposition towards periodontal disease advancement by periodontal parameter modification, especially the PDI index, probing depth and clinical attachment loss.

7.5. Conclusions

1. Cetrimid oral rinse was proven to be the most effective secondary means of hygiene in this study, leading to a better oral hygiene degree and preventing periodontal disease advancement in patients treated with chemotherapy.

2. The present study offers an alternative to the frequently used secondary means of hygiene, promoting oral health and preventing the evolution of oral and periodontal manifestations during chemotherapy.

Chapter 8. ORAL CARE RECOMMANDATIONS AND PROTOCOLS FOR ONCOLOGY PATIENTS

The oral care recommendations and protocols have been split into three categories depending on the oncological status of the patient and in relation to the administration of chemotherapy as follows:

1. Pre-chemotherapy periodontal protocol
2. Odontal and periodontal treatment during chemotherapy
3. Post-chemotherapy care protocol

Chapter 10. GENERAL CONCLUSIONS

The studies that were conducted in the present thesis bring new information regarding the bidirectional link between periodontal disease and chemotherapy effects. From the obtained results there is important information extracted, with practical applicability, regarding the periodontal treatment in the oncological context, having the aim to minimize or even lead to the absence of oral and periodontal effects of chemotherapy:

1. The present study demonstrates the correlation between administering chemotherapy with an increase of bacterial plaque, pathological modifications of periodontal clinical aspect and increase of periodontal parameter values in chemotherapy patients. These effects are amplified by administering anti-tumor agents with the effect of bolstering usual chemotherapy drugs.

2. The presence of chemotherapy in saliva has the potential to aggravate the oral condition and represents an additional motive for establishing an oral and periodontal treatment protocol with prophylactic character before and during chemotherapy.

3. The presence of chemotherapy drugs at periodontal level leads to the modification of subgingival microbiome by a decrease of non-pathogenic bacteria and increase of periodontal pathogens, especially *Treponema*, *Prevotella*, *Fusobacterium* and *Capnocytophaga*. Chemotherapy can have an inhibitory effect on certain bacteria that is associated with periodontal disease (*Porphyromonas gingivalis*, *Treponema denticola)* and promoting growth for others (*Prevotella tannerae*, *Fusobacterium nucleatum*, *Fusobacterium naviforme*, *Prevotella multiformis* and *Treponema medium*). Knowing and understanding the subgingival microbial component offers useful information for an individualized, complete and complex periodontal treatment and obtaining optimal long term results.

4. The pathological modification of the subgingival microbiome is accentuated by an advanced form of periodontal disease in patients treated with chemotherapy; deeper periodontal pockets lead to an increase in subgingival periodontal pathogen quantity, fact that stresses the importance of periodontal treatment before the commence of chemotherapy.

5. Cetrmid oral rinses were proven to be efficient by decreasing the amount of bacterial plaque, improving the periodontal clinical aspect and preventing periodontal disease progression. It offers an alternative to more usual secondary methods of hygiene.

6. The recommendations and protocols of oral care for patients treated with chemotherapy developed in chapter 8 has the aim to prevent and treat oral cavity affections and prevent systemic complications that can occur during chemotherapy.

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