UDK: 616.311-002-02:615277.036.8]-085.272:616.15

IMPACT OF ANTIOXIDANTS ON COMPLETE BLOOD COUNT PARAMETERS AND THEIR ASSOCIATION WITH THE SEVERITY OF CHEMOTHERAPY-INDUCED ORAL MUCOSITIS – ANIMAL STUDY

ВЛИЈАНИЕТО НА АНТИОКСИДАНТИТЕ ВРЗ ПАРАМЕТРИТЕ ОД ДИФЕРЕНЦИЈАЛНАТА КРВНА СЛИКА И НИВНАТА ПОВРЗАНОСТ СО СТЕПЕНОТ НА ОРАЛЕН МУКОЗИТИС ИНДУЦИРАН ОД ХЕМОТЕРАПИЈА – АНИМАЛНА СТУДИЈА

Ivanovski K[1], Kokolanski V[1], Mitik K[1], Stefanovska E[1], Ristoska S[1], Risteska N[1], Shopkovski K[1], Poposki B.[1]*

[1]Faculty of Dentistry – Skopje, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia,
[1]*Correspondence Author

Abstract

Aim of the study: To evaluate the impact of antioxidants on complete blood count parameters and to investigate their relationship with the intensity of chemotherapy-induced oral mucositis on an animal model. **Material and methods:** An animal rat model for oral mucositis induced by cancer chemotherapy was used in the study. The study involved 40 healthy male adult 9 weeks-old Wistar rats, divided into 5 groups: negative control group; positive control group (5-FU + acetic acid); treatment group 1: 5-FU + acetic acid + vitamin C in alkaline water + glutathione; treatment group 2: 5-FU + acetic acid + zinc sulfate; treatment group 3: 5-FU + acetic acid + vitamin C in alkaline water + glutathione + zinc sulfate. For each treatment group, prior to 5-FU administration, solutions of antioxidants (individually or combined) were prepared and given each day for 14 days to assess their protective effects. The cytostatic agent 5-fluorouracil (5-FU) was administered intraperitoneally at a dose of 40 mg/kg on days 15, 17, and 19 to induce immunosuppression. On day 20, oral ulcers were induced using 25 μL of a 30% acetic acid solution applied to the left buccal mucosa under ether anesthesia. The complete blood count (CBC) was also done on day 20. The initial size of the lesion was measured on day 21. **Results:** Significant differences between the groups were identified for the following parameters based on the Kruskal-Wallis test: WBC (p=0.020), RBC (p=0.006), HGB (p=0.003), HCT (p=0.005), MCH (p=0.002) and PLT (p=0.004). There was no statistically significant difference in the initial lesion size between the four groups treated with 5-FU [Kruskal-Wallis H (χ^2=1.574; p=0.665)]. No statistically significant association was registered between the blood parameters and the initial lesion size in the four groups treated with 5-FU and acetic acid. **Conclusion:** The modulatory effects of antioxidants may influence oxidative stress and, consequently, impact the hematological profile. While significant differences in WBC, RBC, HGB, HCT, MCH and PLT were observed among the groups treated with antioxidants and the negative control group, no significant differences in lesion size or associations between complete blood count parameters and lesion size were detected. The findings underscore the influence of chemotherapy and antioxidants on hematological parameters, paving the way for further investigations into the interactions between antioxidants, complete blood count parameters and oral mucositis severity. **Key words:** oral mucositis, chemotherapy, antioxidants, oxidative stress.

Апстракт

Цел на трудот: Да се утврди влијанието на антиоксидансите врз параметрите на диференцијалната крвна слика и да се истражи нивната поврзаност со степенот на оралниот мукозитис индуциран од хемотерапија на анимален модел. **Материјал и методи:** Беше употребен анимален модел на стаорци за орален мукозитис индуциран од хемотерапија. Во студијата беа вклучени 40 машки стаорци од сојот Wistar, стари 9 недели, поделени во 5 групи: негативна контролна група, позитивна контролна група (5-FU + оцетна киселина), испитувана група 1: 5-FU + оцетна киселина + витамин Ц во алкална вода + глутатион ; испитувана група 2: 5-FU + оцетна киселина + цинк сулфат; испитувана група 3: 5-FU + оцетна киселина + витамин Ц во алкална вода + глутатион + цинк сулфат. Во секоја третирана група, секојдневно, во времетраење од 14 дена пред администрирањето на 5-FU, беа подготвени и администрирани раствори на антиоксиданти (поединечно и во комбинација), со цел евалуација на нивниот протективен ефект. Цитостатикот 5-флуороурацил (5-FU) беше администриран

интраперитонеално во доза од 40 mg/kgна 15-от, 17-от и 19-от ден, за да се предизвика имуносупресија. На 20-от ден беше индуцирана орална улцерација со помош на 25 μLна 30% раствор на оцетна киселина во левата букална лигавица под анестезија со етер. Диференцијалната крвна слика исто така беше направена на 20-от ден. Големината на иницијалната лезија беше измерена на 21-от ден. **Резултати:** Статистички значајна разлика помеѓу групите беше определена со помош на тестот Kruskal-Wallis за следните параметри: WBC (p=0.020), RBC (p=0.006), HGB (p=0.003), HCT (p=0.005), MCH (p=0.002) иPLT (p=0.004). Не беше регистрирана статистички значајна разлика во големината на иницијалната лезија помеѓу четирите групи третирани со 5-FU [Kruskal-WallisH (χ^2=1.574; p=0.665)]. Не беше регистрирана статистички значајна поврзаност помеѓу хематолошките параметри и големината на иницијалната лезија во четирите групи третирани со 5-FU и оцетна киселина. **Заклучок:** Модулаторниот ефект на антиоксидантите може да влијае на оксидативниот стрес, а последователно и на хематолошкиот профил. Иако беа регистрирани значајни разлики во WBC, RBC, HGB, HCT, MCH и PLT помеѓу групите третирани со антиоксиданти и негативната контролна група, не беше регистрирана значајна разлика во големината на иницијалната лезија помеѓу групите, ниту пак поврзаност на параметрите од диференцијалната крвна слика и големината на лезијата. Овие резултати го нагласуваат влијанието на хемотерапијата и антиоксидантите врз хематолошките параметри, отворајќи го патот за понатамошни истражувања за интеракциите помеѓу антиоксидантите, диференцијалната крвна слика и степенот на оралниот мукозитис. **Клучни зборови:** орален мукозитис, хемотерапија, антиоксиданти, оксидативен стрес.

Introduction

Oral mucositis (OM) is one of the most prevalent and painful side effects experienced by cancer patients undergoing radiotherapy and/or chemotherapy, affecting 20–40% of patients receiving conventional chemotherapy, approximately 80% of those with hematological malignancies undergoing myeloablative conditioning prior to stem cell transplantation, and nearly all patients with head and neck cancer receiving radiotherapy[1].

This complication not only reduces the quality of life for affected patients but also poses significant challenges to the integrity and efficacy of the cancer therapy program, having a profound and adverse impact on the patients' clinical outcomes and overall survival prognosis[2]. Depending on the intensity, OM is clinically characterized by erythema, ulcers, pain and eating difficulties, consequently leading to weight loss[3]. OM patients are often readmitted to the hospital and their hospital stay is prolonged which increases the economic and social costs[3].

Chemo-/radiotherapy-induced reactive oxygen species (ROS) are associated with OM, thus making the oxidative stress pathway a target for potential therapeutic effect and prophylaxis (4). Chemotherapy and oxidative stress can affect the functions of blood cells, leading to anemia, neutropenia and thrombocytopenia[5,6].

Studies[3,7-12] report that certain biochemical parameters, like complete blood count (CBC) parameters, before starting chemoradiotherapy were highly associated with the development of severe OM. Identifying the risk factors for oral mucositis could lead to more accurate clinical evaluation and treatment. However, even though many factors have been associated with OM[13-17], literature still requires additional research to provide a clearer understanding of this topic.

The aim of this study was to evaluate the impact of antioxidants on complete blood count parameters and investigate their relationship with the intensity of chemotherapy-induced oral mucositis on an animal model.

Material and methods

To realize the set goal, we used the animal rat model for oral mucositis induced by cancer chemotherapy by Takeuchi et al[18].

The study was conducted at the Institute of Biology (Faculty of Natural Sciences and Mathematics – Skopje) (Faculty of Veterinary Medicine – Skopje), Ss. Cyril and Methodius University in Skopje, after approval by the Ethical Committee for Medical-Dental Research of the Ss. Cyril and Methodius University in Skopje, Faculty of Dentistry – Skopje (02-284/2), as well as after receiving the approval for conducting an animal study by the Food and Veterinary Agency of the Republic of North Macedonia (10-3338/8). All procedures were conducted in compliance with ethical standards approved by the Animal Ethics Committee of the Ss. Cyril and Methodius University in Skopje, North Macedonia (03-2525/1), adhering to the International Guiding Principles for Biomedical Research Involving Animals as outlined by the Council for International Organizations of Medical Sciences (EEC Directive of 1986; 86/609/EEC).

Animals

In this study, 40 healthy male adult Wistar rats, weighing between 250 and 400g (300.4±38.6g) and 9-weeks-old, were obtained from the Vivarium at the Faculty of Natural Sciences and Mathematics in Skopje, Macedonia. The rats were housed under controlled environmental conditions, including a temperature of 25 ± 2°C, relative humidity of 55 ± 10%, and a 12-hour light/dark cycle. They were provided with a standard pellet diet consisting of 20% protein, 30% carbohydrates, 9% fat, 2.5% cellulose, and 10% water, delivering an energy value of 310 kcal, along with free access to water.

Materials

As a chemotherapeutic agent we used ≥99% (HPLC) 5-fluorouracil (5-FU) (Sigma-Aldrich, Saint Louis, MO

63103, USA). To induce the oral lesion, we used an aqueous solution of glacial acetic acid (Alkaloid AD Skopje, Skopje, Republic of North Macedonia). As non-enzymatic antioxidants we used Vitamin C (Galenika a.d., Belgrade, Serbia) dissolved in alkaline water (Faculty of Natural Sciences and Mathematics – Skopje, Ss. Cyril and Methodius University in Skopje), glutathione (NOW Sports – Nutrition and Wellness, Bloomingdale, Illinois 60108, USA) and zinc sulfate heptahydrate (Sigma-Aldrich, Saint Louis, MO 63103, USA). To measure the oral lesions, the animals were narcotized with ether (Alkaloid AD Skopje, Skopje, Republic of North Macedonia).

Experimental design

The animals were divided into five groups to evaluate the effects of antioxidants on chemotherapy-induced oral mucositis, as follows:

1) Negative control group: received 1 mL of physiological saline intraperitoneally.
2) Positive control group: 5-FU + acetic acid, without antioxidant treatment.
3) Treatment group 1: 5-FU + acetic acid + vitamin C in alkaline water + glutathione
4) Treatment group 2: 5-FU + acetic acid + zinc sulfate
5) Treatment group 3: 5-FU + acetic acid + vitamin C in alkaline water + glutathione + zinc sulfate

For each treatment group, prior to 5-FU administration, solutions of antioxidants (individually or combined) were prepared and given each day for 14 days to assess their protective effects, including:

- vitamin C in alkaline water (8 mg/kg body weight; given intragastrically),
- glutathione (4 mg/kg body weight; given intragastrically) and
- zinc sulfate (40mg/kg body weight; given intragastrically).

The cytostatic agent 5-fluorouracil (5-FU) was administered intraperitoneally at a dose of 40 mg/kg on days 15, 17, and 19 to induce immunosuppression. On day 20, oral ulcers were induced using 25 µL 30% acetic acid solution applied to the left buccal mucosa under ether anesthesia. The complete blood count (CBC) was also done on day 20. The initial size of the lesion was measured on day 21.

The data was processed using IBM SPSS Statistics v24 for Windows.

Results

The complete blood count parameters are presented in Table 1 as Mean±SD. The Kruskal-Wallis H test was conducted to assess the difference in the complete blood count parameters between the 5 groups. Significant differences were identified for the following parameters based on the Kruskal-Wallis test: WBC ($p=0.020$), RBC ($p=0.006$), HGB ($p=0.003$), HCT ($p=0.005$), MCH ($p=0.002$) and PLT ($p=0.004$). Post hoc pairwise comparisons were conducted using the Bonferroni-adjusted Mann-Whitney U test to control for Type I error. Significant differences between groups were marked with

Table 1. Complete blood count parameters (Mean±SD)

	WBC ($x10^3/\mu L$)	RBC ($x10^6/\mu L$)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT ($x10^3/\mu L$)
G1	9.1±1.6[a]	6.6±0.2[a,b]	13.4±0.4[a,b]	38.9±4.5[a,b]	53.2±15.8	27.2±2.9[a,b]	36±11	859±70[a]
G2	11±7	9.3±2.3	18±3.2	53.1±12.9	57.3±1.1	19.8±2.4	34.6±3.8	450±348
G3	5±2.4[a]	10.7±1.4[a]	20±2.3[a]	62.1±7.7[a]	57.9±0.9	18.7±0.4[a]	32.3±0.4	324±155[a]
G4	5.9±1.3	10.5±0.3[b]	19.9±0.8[b]	60.7±2.4[b]	58±1.3	19±0.5	32.7±0.5	391±100
G5	7.2±2.3	10.3±0.4	19.4±1.1	59.9±3.3	58±2.2	18.8±1[b]	32.4±0.8	484±118

Table 2. Initial lesion size

Group	N	Size (mm) / Mean±SD	Minimum	Maximum
G2	8	4.5±2.7	1	9
G3	8	3.6±3	0	8
G4	8	4.4±1.9	1	7
G5	8	3.1±3	0	7

Table 3. Correlations between the blood parameters and lesion size

	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
Lesion size	r=-0.202 p=0.421	r=-0.011 p=0.965	r=0.065 p=0.797	r=-0.085 p=0.738	r=0.133 p=0.600	r=0.140 p=0.579	r=-0.289 p=0.245	r=0.035 p=0.891

superscript letters in Table 1, where groups sharing a letter are significantly different.

Table 2 presents the initial size of the lesion in the groups treated with 5-FU and acetic acid, presented as Mean±SD, minimum and maximum values. There was no statistically significant difference in the initial lesion size between the four groups [Kruskal-Wallis H ($\chi2$=1.574; p=0.665)].

Spearman's rank correlation coefficient was conducted to determine the association between the blood parameters and the initial size of the lesion. No statistically significant association was registered between the blood parameters and the initial lesion size in the four groups treated with 5-FU and acetic acid (Table 3).

Discussion

Oral mucositis is an adverse effect of chemotherapy and/or radiotherapy (head and neck), and in aspect of chemotherapy, its pathogenesis occurs both by direct tissue damage of the chemotherapeutic agent and by formation of reactive oxygen species as a result of direct tissue damage[19]. Different risk factors for oral mucositis are mentioned in the literature, such as: advanced age[20,21], lack of appetite[20], duration of chemotherapy[20], type of chemotherapy[21], disease stage[21], salivary composition[22] and biochemical parameters[3,7-9, 11,23]. According to the literature[7,8,10,11,12,24], hemoglobin, platelets and white blood cells are the most mentioned complete blood count parameters associated with oral mucositis, making them the primary focus of this study.

***Hemoglobin**:* Soutome et al.[11] conducted a retrospective study of 181 patients to examine the risk factor for developing severe oral mucositis in patients with oral cancer undergoing radiotherapy. Their analysis revealed that lower hemoglobin levels receiving concurrent cisplatin or cetuximab and not receiving pilocarpine (low unstimulated salivary flow) correlated with a significantly higher incidence of severe oral mucositis. A similar study by Nishii et al.[7] evaluated 326 patients, who underwent radiotherapy for oral and oropharyngeal cancer, to investigate the factors associated with severe oral mucositis, concluding that low hemoglobin levels, low leukocytes/lymphocytes, concurrent cisplatin or cetuximab treatment, and oral feeding were found to be significantly associated with a higher incidence of severe oral mucositis. Mendonça et al.[24] found no association between oral mucositis severity and hemoglobin levels, while Curra et al.[12] reported lower levels of hemoglobin in patients with severe oral mucositis. In our study, hemoglobin levels increased in the groups treated with 5-FU, withstatistically higher hemoglobin levels being detected in Groups 3 and 4, compared to Group 1 (control group) (Table 1). Finkelstein et al.[25] reported an association between higher plasma vitamin C levels and higher hemoglobin levels. Vitamin C serves a key role in the kinetics of iron metabolism and the utilization of iron for erythropoiesis[25]. Hanson et al.[26] reported a significant improvement in hemoglobin levels in patients with zinc supplementation, which might be due to the importance of zinc as a catalyst, structural element and regulatory ion in the metabolic processes of erythropoiesis. Groups 3 and 4 exhibited smaller initial lesion size compared to the other groups, but the difference was not statistically significant [Kruskal-Wallis H ($\chi2$=1.574; p=0.665)] (Table 2). No statistically significant association was observed between the initial lesion size and hemoglobin (r=0.065; p=0.797) (Table 3). While it is expected for chemotherapy to cause myelosuppression, anemia and lower hemoglobin levels, in our study, the hemoglobin levels increased in the treatment groups, which can be explained by the increase of hemoglobin levels in the plasma, caused by the ROS damage toerythrocytes. Plasma hemoglobin levels can beconsidered a very good indicator of the oxidative damage of the erythrocyte membrane[27]. While it is logical for low hemoglobin levels to be negatively associated with the severity of oral mucositis, the findings in literature are inconsistent.

Platelets: Platelets are a key factor in the injury repair of ulcers and studies have demonstrated a variety of cytokines released by the platelets which are important for wound healing[8,28-32]. Given that the platelet-derived growth factor (PDGF) attracts mesenchymal cells which play a vital role in cell division regulation and growth, angiogenesis, stimulation of neutrophils and macrophages chemotaxis, as well as fibroblast proliferation, indicates that platelet count may be an important sign for wound healing capability[33]. Studies[8,10,12,24] report

that there is an increased risk of occurrence and inverse association between platelet count and mucositis grade, i.e. higher pretreatment platelet count resulted in milder oral mucosal reactions. Contrarily, Damascena et al.[34] reported that an increased platelet count was identified as a risk factor for severe oral mucositis. They elaborate that these findings might be due to platelet concentrate transfusions done during the myelosuppression which can lead to the occurrence of side effects, such as severe oral mucositis. The platelets in our study decreased in all groups treated with 5-FU, compared to the control group (Table 1), with significantly lower platelet levels being registered in Group 3 compared to Group 1 (control group). It is already recognized and reported that 5-FU injections can lead to significant platelet decrease[35]. Lesion size was not statistically significantly different between the treated groups [Kruskal-Wallis H ($\chi 2$=1.574; p=0.665)] (Table 2) and no statistically significant association was registered between platelet count and initial lesion size (r=0.035; p=0.891) (Table 3).

White blood cells: Patients with leukopenia have an impaired immune system and are at higher risk of bacterial colonization of the damaged epithelium, which can lead to an increase of pro-inflammatory cytokines in the oral mucosa, thus aggravating oral mucositis[34,36,37]. Studies demonstrate that patients with lower leukocyte count had severe oral mucositis[12], and that low neutrophil count might be a predictor[38] and risk factor[39] of chemotherapy-induced oral mucositis. In our study, the white blood cells exhibited an increase in Group 2, as well as a decrease in Groups 3-5, compared to the control group (Table 1). The decrease in white blood cells was expected due to the effect of 5-FU to reduce the circulating leukocytes. A statistically significant difference was registered in the white blood cells count between Groups 1 and 3. Controversially, the white blood cells count was highest in Group 2 (Table 1), where the initial size was also largest, but not statistically different than the other treated groups [Kruskal-Wallis H ($\chi 2$=1.574; p=0.665)] (Table 2). No statistically significant association was registered between white blood cells count and initial lesion size (r=-0.202; p=0.421) (Table 3). The adequate inflammatory response to the chemotherapy cytotoxic effects on the oral mucosa might be a result of a decrease in neutrophils[24], which was not the case in Group 2 of our study. Additionally, even minor mucosal toxicity may progress to evident ulceration provided the neutrophil counts are low[40].

The lack of a statistically significant association between the complete blood count parameters and oral mucositis severity may be attributed to the limitations of the animal model, which induces lesions irrespective of the biochemical parameters within the body, due to the injection of acetic acid. However, these results could provide valuable insights into how chemotherapy and treatments influence complete blood count parameters, contributing to a better understanding of the physiological changes induced by these interventions.

The findings reported in this study are only a small fraction of a more extensive research project, which may clarify certain methodological peculiarities in the study

Conclusions

The modulatory effects of antioxidants may influence oxidative stress, and, consequently, may have an impact on the hematological profile. While significant differences in WBC, RBC, HGB, HCT, MCH and PLT were observed among the groups treated with antioxidants and the negative control group, no significant differences in lesion size or associations between complete blood count parameters and lesion size were detected. This lack of correlation might reflect limitations of the experimental model, where lesion induction occurs independently of systemic biochemical factors due to acetic acid injection. Nonetheless, the findings underscore the influence of chemotherapy and antioxidants on hematological parameters, paving the way for further investigations into the interactions between antioxidants, complete blood count parameters and oral mucositis severity.

Reference

1. Yang B, Li W, Shi J. Preventive effect of probiotics on oral mucositis induced by anticancer therapy: a systematic review and meta-analysis of randomized controlled trials. BMC Oral Health. 2024 Sep 29;24(1):1159. doi: 10.1186/s12903-024-04955-7. PMID: 39343876; PMCID: PMC11441129.
2. Chen ZX, Qin YS, Shi BH, Gao BY, Tao RC, Yong XZ. Effects of Curcumin on Radiation/Chemotherapy-Induced Oral Mucositis: Combined Meta-Analysis, Network Pharmacology, Molecular Docking, and Molecular Dynamics Simulation. Curr Issues Mol Biol. 2024 Sep 20;46(9):10545-10569. doi: 10.3390/cimb46090625. PMID: 39329977; PMCID: PMC11431004.
3. Lorini L, Perri F, Vecchio S, Belgioia L, Vinches M, Brana I, Elad S, Bossi P. Confounding factors in the assessment of oral mucositis in head and neck cancer. Support Care Cancer. 2022 Oct;30(10):8455-8463. doi: 10.1007/s00520-022-07128-w. Epub 2022 May 31. PMID: 35639187; PMCID: PMC9512735.
4. Nguyen H, Sangha S, Pan M, Shin DH, Park H, Mohammed AI, Cirillo N. Oxidative Stress and Chemoradiation-Induced Oral Mucositis: A Scoping Review of In Vitro, In Vivo and Clinical Studies. Int J Mol Sci. 2022 Apr 27;23(9):4863. doi: 10.3390/ijms23094863. PMID: 35563254; PMCID: PMC9101413.
5. Danesh H, Ziamajidi N, Mesbah-Namin SA, Nafisi N, Abbasalipourkabir R. Association between Oxidative Stress Parameters and Hematological Indices in Breast Cancer Patients. Int J Breast Cancer. 2022 Oct 3;2022:1459410. doi: 10.1155/2022/1459410. PMID: 36225290; PMCID: PMC9550463.

6. Crawford J, Herndon D, Gmitter K, Weiss J. The impact of myelosuppression on quality of life of patients treated with chemotherapy. Future Oncol. 2024;20(21):1515-1530. doi: 10.2217/fon-2023-0513. Epub 2024 Apr 8. PMID: 38587388; PMCID: PMC11441072.
7. Nishii M, Soutome S, Kawakita A, Yutori H, Iwata E, Akashi M, Hasegawa T, Kojima Y, Funahara M, Umeda M, Komori T. Factors associated with severe oral mucositis and candidiasis in patients undergoing radiotherapy for oral and oropharyngeal carcinomas: a retrospective multicenter study of 326 patients. Support Care Cancer. 2020 Mar;28(3):1069-1075. doi: 10.1007/s00520-019-04885-z. Epub 2019 Jun 8. PMID: 31177394.
8. Tao Z, Gao J, Qian L, Huang Y, Zhou Y, Yang L, He J, Yang J, Wang R, Zhang Y. Factors associated with acute oral mucosal reaction induced by radiotherapy in head and neck squamous cell carcinoma: A retrospective single-center experience. Medicine (Baltimore). 2017 Dec;96(50):e8446. doi: 10.1097/MD.0000000000008446. PMID: 29390253; PMCID: PMC5815665.
9. Mizuno H, Miyai H, Yokoi A, Kobayashi T, Inabu C, Maruyama T, Ekuni D, Mizukawa N, Kariya S, Nishizaki K, Kimata Y, Morita M. Relationship Between Renal Dysfunction and Oral Mucositis in Patients Undergoing Concurrent Chemoradiotherapy for Pharyngeal Cancer: A Retrospective Cohort Study. In Vivo. 2019 Jan-Feb;33(1):183-189. doi: 10.21873/invivo.11457. PMID: 30587621; PMCID: PMC6364078.
10. Ribeiro ILA, Melo ACR, Limão NP, Bonan PRF, Lima Neto EA, Valença AMG. Oral Mucositis in Pediatric Oncology Patients: A Nested Case-Control to a Prospective Cohort. Braz Dent J. 2020 Jan-Feb;31(1):78-88. doi: 10.1590/0103-6440201802881. PMID: 32159710.
11. Soutome S, Yanamoto S, Nishii M, Kojima Y, Hasegawa T, Funahara M, Akashi M, Saito T, Umeda M. Risk factors for severe radiation-induced oral mucositis in patients with oral cancer. J Dent Sci. 2021 Oct;16(4):1241-1246. doi: 10.1016/j.jds.2021.01.009. Epub 2021 Feb 9. PMID: 34484592; PMCID: PMC8403800.
12. Curra M, Gabriel AF, Ferreira MBC, Martins MAT, Brunetto AT, Gregianin LJ, Martins MD. Incidence and risk factors for oral mucositis in pediatric patients receiving chemotherapy. Support Care Cancer. 2021 Nov;29(11):6243-6251. doi: 10.1007/s00520-021-06199-5. Epub 2021 Apr 12. PMID: 33846825.
13. Khaw A, Logan R, Keefe D, Bartold M. Radiation-induced oral mucositis and periodontitis - proposal for an inter-relationship. Oral Dis. 2014 Apr;20(3):e7-18. doi: 10.1111/odi.12199. Epub 2013 Nov 19. PMID: 24147592.
14. Cook RT. Alcohol abuse, alcoholism, and damage to the immune system--a review. Alcohol Clin Exp Res. 1998 Dec;22(9):1927-42. PMID: 9884135.
15. Jensen SB, Vissink A. Salivary gland dysfunction and xerostomia in Sjögren's syndrome. Oral Maxillofac Surg Clin North Am. 2014 Feb;26(1):35-53. doi: 10.1016/j.coms.2013.09.003. PMID: 24287192.
16. Saito N, Imai Y, Muto T, Sairenchi T. Low body mass index as a risk factor of moderate to severe oral mucositis in oral cancer patients with radiotherapy. Support Care Cancer. 2012 Dec;20(12):3373-7. doi: 10.1007/s00520-012-1620-7. Epub 2012 Oct 7. PMID: 23052923.
17. De Sanctis V, Bossi P, Sanguineti G, Trippa F, Ferrari D, Bacigalupo A, Ripamonti CI, Buglione M, Pergolizzi S, Langendjik JA, Murphy B, Raber-Durlacher J, Russi EG, Lalla RV. Mucositis in head and neck cancer patients treated with radiotherapy and systemic therapies: Literature review and consensus statements. Crit Rev Oncol Hematol. 2016 Apr;100:147-66. doi: 10.1016/j.critrevonc.2016.01.010. Epub 2016 Feb 1. PMID: 26947812.
18. Takeuchi I, Kawamata R, Makino K. A Rat Model of Oral Mucositis Induced by Cancer Chemotherapy for Quantitative Experiments. Anticancer Res. 2020 May;40(5):2701-2706. doi: 10.21873/anticanres.14241. PMID: 32366415.
19. Martins JO, Borges MM, Malta CE, Carlos AC, Crispim AA, Moura JF, Fernandes-Lima IJ, Silva PG. Risk factors for oral mucositis during chemotherapy treatment for solid tumors: a retrospective STROBE-guided study. Med Oral Patol Oral Cir Bucal. 2022 Jul 1;27(4):e319-e329. doi: 10.4317/medoral.25253. PMID: 35717621; PMCID: PMC9271342.
20. Çakmak S, Nural N. Incidence of and risk factors for development of oral mucositis in outpatients undergoing cancer chemotherapy. Int J NursPract. 2019 Feb;25(1):e12710. doi: 10.1111/ijn.12710. Epub 2018 Nov 21. PMID: 30461128.
21. Nagatani A, Ogawa Y, Sunaga T, Tomura K, Naito Y, Fujii N, Okabe T, Hashimoto T, Kogo M, Sasaki T. [Analysis of the Risk Factors for Severe Oral Mucositis in Head and Neck Cancer after Chemoradiotherapy with S-1]. YakugakuZasshi. 2017;137(2):221-225. Japanese. doi: 10.1248/yakushi.16-00077. PMID: 28154335.
22. Pels EJ. Oral mucositis and saliva IgA, IgG and IgM concentration during anti-tumor treatment in children suffering from acute lymphoblastic leukemia. Adv Clin Exp Med. 2017 Dec;26(9):1351-1358. doi: 10.17219/acem/64940. PMID: 29442455.
23. Chen X, Yao L, Shan Q, Qian X, Lu X, Tang X, Chen S, Yu W. Risk factors for oral mucositis in patients with malignant tumors: a prospective cohort study. Ann Palliat Med. 2021 Jul;10(7):8180-8189. doi: 10.21037/apm-21-1675. PMID: 34353102.
24. Mendonça RM, Araújo Md, Levy CE, Morari J, Silva RA, Yunes JA, Brandalise SR. Oral Mucositis in Pediatric Acute Lymphoblastic Leukemia Patients: Evaluation of Microbiological and Hematological Factors. PediatrHematol Oncol. 2015;32(5):322-30. doi: 10.3109/08880018.2015.1034819. Epub 2015 Jun 18. PMID: 26086683.
25. Finkelstein FO, Juergensen P, Wang S, Santacroce S, Levine M, Kotanko P, Levin NW, Handelman GJ. Hemoglobin and plasma vitamin C levels in patients on peritoneal dialysis. Perit Dial Int. 2011 Jan-Feb;31(1):74-9. doi: 10.3747/pdi.2009.00154. Epub 2010 Jun 17. PMID: 20558814; PMCID: PMC3487381.
26. Hanson, Z. D., Mirshahidi, H., Brothers, J., Mirshahidi, S., Pham, B., Samaeekia, R., & Akhtari, M. (2023). Hemoglobin response to zinc supplementation in patients with zinc deficiency and chronic anemia. Blood, 142(65th ASH Annual Meeting Abstracts), 5222–5223. https://doi.org/10.1182/blood-2023-191197
27. Racek J, Herynková R, Holecek V, Jerábek Z, Sláma V. Influence of antioxidants on the quality of stored blood. Vox Sang. 1997;72(1):16-9. doi: 10.1046/j.1423-0410.1997.00016.x. PMID: 9031495.
28. Slomiany BL, Slomiany A. Biphasic role of platelet-activating factor in oral mucosal ulcer healing. IUBMB Life. 2003 Aug;55(8):483-90. doi: 10.1080/15216540310001602814. PMID: 14609204.
29. Sadeghi-Ardebili M, Hasannia S, Dabirmanesh B, Khavari-Nejad RA. Functional characterization of the dimeric form of PDGF-derived fusion peptide fabricated based on theoretical arguments. Sci Rep. 2024 Jan 10;14(1):1003. doi: 10.1038/s41598-024-51707-2. PMID: 38200288; PMCID: PMC10781716.
30. Morgan K. Radiotherapy-induced skin reactions: prevention and cure. Br J Nurs. 2014 Sep 11-24;23(16):S24, S26-32. doi: 10.12968/bjon.2014.23.Sup16.S24. PMID: 25203851.
31. Fiedler J, Röderer G, Günther KP, Brenner RE. BMP-2, BMP-4, and PDGF-bb stimulate chemotactic migration of primary human mesenchymal progenitor cells. J Cell Biochem. 2002;87(3):305-12. doi: 10.1002/jcb.10309. PMID: 12397612.
32. Lepistö J, Peltonen J, Vähä-Kreula M, Niinikoski J, Laato M. Platelet-derived growth factor isoforms PDGF-AA, -AB and -BB exert specific effects on collagen gene expression and mitotic

activity of cultured human wound fibroblasts. BiochemBiophys Res Commun. 1995 Apr 17;209(2):393-9. doi: 10.1006/bbrc.1995.1516. PMID: 7733905.
33. Arundina I, Diyatri I, Surboyo MDC, Monica E, Afanda NM. Growth factor stimulation for the healing of traumatic ulcers with liquid rice hull smoke. J Taibah Univ Med Sci. 2021 Feb 4;16(3):431-439. doi: 10.1016/j.jtumed.2021.01.003. PMID: 34140871; PMCID: PMC8178683.
34. Damascena LCL, de Lucena NNN, Ribeiro ILA, Pereira TL, Lima-Filho LMA, Valença AMG. Severe Oral Mucositis in Pediatric Cancer Patients: Survival Analysis and Predictive Factors. Int J Environ Res Public Health. 2020 Feb 14;17(4):1235. doi: 10.3390/ijerph17041235. PMID: 32075075; PMCID: PMC7068385.
35. Chenaille PJ, Steward SA, Ashmun RA, Jackson CW. Prolonged thrombocytosis in mice after 5-fluorouracil results from failure to down-regulate megakaryocyte concentration. An experimental model that dissociates regulation of megakaryocyte size and DNA content from megakaryocyte concentration. Blood. 1990 Aug 1;76(3):508-15. PMID: 2378983.
36. Ip WY, Epstein JB, Lee V, Yuen HL, Li R, Thompson DR, Goggins WB, Cheng KK. Oral mucositis in paediatric patients after chemotherapy for cancer. Hong Kong Med J. 2014 Dec;20 Suppl 7:4-8. PMID: 25647816.
37. Viana Filho JMC, Coêlho MC, Ribeiro ILA, Persuhn DC, Valença AMG, Oliveira NFP. ABCG2 polymorphism, age and leukocyte count may contribute to oral mucositis in oncopediatric patients. Braz Dent J. 2021 Mar-Apr;32(2):14-26. doi: 10.1590/0103-6440202103768. PMID: 34614057.
38. McCarthy GM, Awde JD, Ghandi H, Vincent M, Kocha WI. Risk factors associated with mucositis in cancer patients receiving 5-fluorouracil. Oral Oncol. 1998 Nov;34(6):484-90. doi: 10.1016/s1368-8375(98)00068-2. PMID: 9930359.
39. Cheng KK, Goggins WB, Lee VW, Thompson DR. Risk factors for oral mucositis in children undergoing chemotherapy: a matched case-control study. Oral Oncol. 2008 Nov;44(11):1019-25. doi: 10.1016/j.oraloncology.2008.01.003. Epub 2008 Mar 7. PMID: 18329325.
40. Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: molecules, functions and pathophysiological aspects. Lab Invest. 2000 May;80(5):617-53. doi: 10.1038/labinvest.3780067. PMID: 10830774.