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Concentration of Tumour Necrosis Factor-Alpha in Saliva of Patients with Acute Lymphoblastic Leukaemia in Relation to Oral Mucositis during Chemotherapy

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Abstract

Purpose: Ulceration in the oral cavity caused by stomatotoxic chemotherapy is painful and restricts oral administration of drugs. The study objective was to assess concentration of tumour necrosis factor-alpha (TNF-α) cytokine in saliva of patients with acute lymphoblastic leukaemia and to assess the occurrence of oral mucositis during chemotherapy.

Methods: The study included 78 children with ALL in followed three examinations and a control group - 78 healthy children. In the group of patients with ALL was conducted the clinical study to assess oral mucosa based on the five-grade WHO classification of oral mucositis. Two hours after morning meal unstimulated saliva samples were taken and TNF-α was determined by TNF-α human EIA

Results: Saliva TNF-α concentration determined in the group of children with ALL in examination 1 ranged 4.16–135.01pg/ml. Mean saliva TNF-α concentration was 28.2±20.4pg/ml in examination 2, and 28.9±28.8pg/ml in examination 3. In the group of children with ALL mean saliva TNF-α values were lower compared to the control group. Lesions of the mucositis type were observed in ALL children in the period from 48 hours to 6 months, having various intensity and with periods without pathological lesions, which was related to the intensity of the chemotherapy – examination 2.

Conclusions: Disorders in the immune system during chemotherapy may cause increase in pathological changes of oral mucosa. Early assessment of proinflammatory cytokines may prevent complications of standard treatment and prolongation of anti-tumour treatment, which will allow fast recovery of patients with ALL.

Keywords: Oral; Mucositis; Saliva TNF-α concentration; Children; ALL

Introduction

Oral mucositis induced by anti-tumour agents is a serious problem and often causes dose reduction and increase in cost of treatment of the neoplastic disease. Ulceration in the oral cavity caused by stomatotoxic chemotherapy is painful and restricts oral administration of drugs, also increasing the risk of infection of the intrinsic oral cavity flora. In a five-phase model of mucositis pathogenesis, the primary cause and trigger of the inflammatory process is microvascular injury to quickly dividing basal epithelial cells during radiation and chemotherapy, which results in production and release of free oxygen radicals, which in turn activates cytokines, including tumour necrosis factor-alpha (TNF-α), produced mainly by macrophages and interleukin-1 and -6 (IL-1, IL-6). Developing ulcers in the mucosa are a good base for development of bacterial microflora leading to secondary infections. The fifth and last phase is healing, which is characterised by epithelial cell proliferation, tissue differentiation and recovery of epithelial integrity [1-3].

During intensive multidrug chemotherapy, oral mucositis is a hugely important stomatological problem [4]. Early diagnosis and prompt treatment of oral mucositis, which leads to patient deterioration, are of crucial importance for multidisciplinary treatment of patients. Despite discovery of a pathomechanism of chemo- and radiotherapy-induced lesions [1,2], there is no effective method of treatment and elimination of pain related to oral mucosa lesions [5].

Purpose

The study objective was to assess concentration of tumour necrosis factor-alpha cytokine in saliva of patients with acute lymphoblastic leukaemia and to assess the occurrence of oral mucositis during chemotherapy.

Material and Methods

The investigation was carried out in the group of 78 children with ALL aged 2-18 y and analogical in terms of age and gender group of healthy controls. In the group of examined children 5 had recurrent neoplasms located in the brain and spinal cord, 2 children had recurrent bone marrow cancer, 7 children had their CNS affected and 3 children had Down's syndrome. The examination of children with ALL followed three stages: examination 1 performed prior to chemotherapy, examination 2 - a few days to five months following the onset of chemotherapy, examination 3 carried out after 0.5 to 1.5 y of anticancer therapy. The treatment followed the protocol of ALLIC BFM 2002 Program and were qualified for three risk groups: standard risk (SR), intermediate risk (IR) and high risk (HR). Verification of

risk groups took place on 8, 15 and 33 day of treatment by means of bone marrow examination and assessment of leukaemia blast count: M1<5%, M2 5-25%, M3>25%, assessment of response to steroid treatment and assessment of leukocytosis. The children were treated in the Department of Pediatric Hematology and Oncology Medical University of Lublin, with strict adherence to the appropriate protocols for risk groups, in which the successive days of treatment with certain medicines are administered. Dental examinations were performed with basic diagnostic kits in artificial light.

Two hours after morning meal unstimulated saliva samples were taken and tumor necrosis factor-alpha was determined by TNF-α human EIA. The saliva samples were centrifuged for 15min at 5 000rpm. Centrifuged saliva was frozen at -80°C and stored until laboratory tests. In the group of patients with ALL was conducted the clinical study to assess oral mucosa based on the five-grade WHO classification of oral mucositis. Changes in oral mucosa were monitored every day.

The results were analyzed statistically. Measurable parameters were presented as means, medians, minimum, maximum and SD. Chi^2 test was used to compare two independent groups and Wilcoxon's test to compare dependent groups. Statistical analysis was done by STATISTICA 10.0, $p<0.05$ was assumed statistically significant.

Results

The results of examinations in both groups and statistical analysis were listed in Table 1 and graphically presented on (Figure 1). Saliva TNF-α concentration determined in the group of children with ALL in examination 1 ranged 4.16–135.01 pg/ml. In that group in examination 1, mean saliva TNF-α concentration was 36.9 ± 32.6 pg/ml. In the group of healthy children mean saliva TNF-α concentration was 52.1 ± 107.64 pg/ml. Mean saliva TNF-α concentrations (examination 1) were lower in the group of children with ALL in comparison to the healthy controls, however the differences were not statistically significant ($p>0.05$; Table 1). In examination 2, saliva TNF-α concentrations ranged 2.43–92.36 pg/ml, mean saliva TNF-α concentration was 28.2 ± 20.4 pg/ml. In the group of children with ALL mean saliva TNF-α values were lower compared to the control group; however the differences were statistically insignificant ($p>0.05$; Table 1). In examination 3, saliva TNF-α concentrations ranged 2.95 – 171.8 pg/ml, mean saliva TNF-α concentration was 28.9 ± 28.8 pg/ml. In the group of children with ALL mean saliva TNF-α values were lower compared to the control group; however the differences were close to statistically significant ($Chi^2=2.8949$, $p=0.089$; Table 1).

Studied Parameter	Studied group of Children		Mean Value	Me	Min.	Max	SD	Chi2 Test	Significance Level
Saliva TNF-α	ALL	examination 1	36.9	35.5	4.16	135.01	32.6	0.4162	0.5188
		examination 2	28.2	22.4	2.43	92.36	20.4	0.6363	0.425
		examination 3	28.9	18.9	2.95	171.8	28.8	2.8949	0.089
	Healthy		52.1	26.6	3.77	602.49	107.6	The above values refer to healthy children	

Table 1: Saliva TNF-α concentration (pg/ml) in children with ALL and healthy controls.

Wilcoxon's test revealed no significant differences in patient's saliva TNF-α concentrations between subsequent examinations 1 and 2 ($Z=0.4703$; $p=0.6381$) and examination 3 ($Z=1.6265$; $p=0.1038$) over the period of anticancer therapy. Prior to anti-tumour therapy, 4.84% of children with ALL had mild oral inflammation unrelated to chemotherapy, which probably resulted from decreased immunity of the affected children.

Lesions of the mucositis type were observed in ALL children in the period from 48 hours to 6 months, having various intensity and with periods without pathological lesions, which was related to the intensity of the chemotherapy – examination 2. Mucosa opacity followed by redness usually occurred within 2-4 days from the Methotrexat infusion. The most severe lesions of the oral mucosa were observed after the first month of chemotherapy. Wounds and ulcers difficult to heal were related to blood morphology parameters. Changes in the oral mucosa were observed in different severity. Localized erythema of the mucosa (Grade 1) in 35% of children, pseudomembranes mucosa (Grade 2) in 18% of children, ulcers with extensive erythema (Grade 3) in 40% of children, the massive ulcers mucosal and tissue necrosis (Grade 4) in 4% respondents were observed (Figure 1). In the periods between protocols, there were usually no lesions.

After 6 months of chemotherapy – examination 3, lesions in oral mucosa were less intense and were observed mucositis - Grade 1 in 3.17% of the study children. The lesions were usually redness and erosion. No ulcers in the oral cavity were observed (Figure 1).

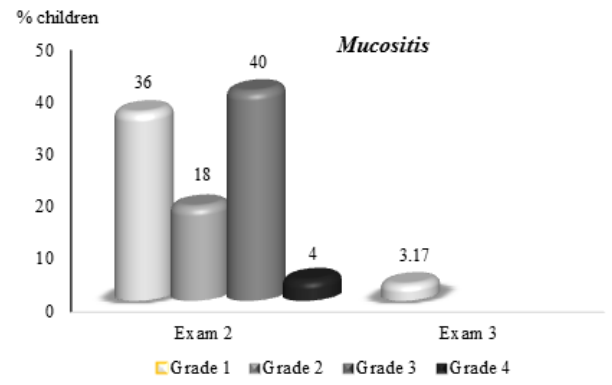

Figure 1: Oral mucositis in children (%) with ALL in examination 2 and examination 3.

When lesions appeared in the oral mucosa, children were administered with a mixture for oral swabbing containing

bicarbonatum, gentamicin, colimycin and nystatin. When massive ulceration in the oral cavity occurred, children were receiving solcoseryl ampoules i.v. and solcoseryl adhesive paste on the oral mucosa. In case of massive milky white opacities, the treatment included antifungal preparations of the azole group, e.g. fluconazole 10 mg/kg/daily.

Inflammatory changes in the oral mucosa usually regressed after a few days up to three weeks from the implementation of treatment, and were mostly dependent on blood morphology and haematological therapy, as well as on the oral cavity hygiene prior to treatment. Lesions in oral mucosa were the most persistent in children with bone marrow aplasia (up to 3 weeks) and in children with neutropenia. Difficult healing was also observed following Methrotexat infusion.

Discussion

TNF-α activates transcription of NFκB factor, which stimulates cell proliferation, inhibits apoptosis and also increases secretion of proinflammatory cytokines. A correlation between chronic inflammation and carcinogenesis is well known and cytokines and other inflammatory mediators are thought to play an important role in pathogenesis of neoplastic transformation [5]. It is claimed that these cytokines may be secreted from two local sources, i.e. from a damaged epithelium and from lymphocytes of tissues affected by leukoplakia with symptoms of chronic inflammation. Oral epithelial cells may secrete IL-6 and TNF-α as a response to various microbial and chemical stimuli [6].

Studies confirmed that some saliva biomarkers such as IFN-γ, IL-1-β1, TNF-α, IL-6, IL-4, IL-8 might be useful to predict future course of oral diseases [7,8]. Cytokine immunoregulatory mechanisms involved in inflammatory processes, infectious and immune diseases affecting the oral cavity play a specific role in host defense and maintaining oral homeostasis [9,10]. Studies conducted by Webb at al. which analysed production of cytokines: IL-2, IL-3, IL-4, IL-6, IL-10, IL-12, TNF-α and IFN-γ in serum of three patient groups: with AML, ALL and control group revealed significant differences in cytokine levels between the groups, except for IL-2 in ALL patients compared to healthy subjects. These findings have confirmed the hypothesis of disturbed functioning of the immune system in various haematological neoplastic diseases, which may be used in the future to describe and treat the disease [11].

A lower TNF-α level in saliva may be related to a significantly higher TNF-α level in serum in a similar group of ALL children given by Drabko et al. The authors revealed that median of TNF-α level in serum was significantly higher in patients newly diagnosed with ALL than in the same subjects in the phase of remission and the controls [12]. It would be advisable to study TNF-α level in both blood and saliva at the same time, which would allow assessment of the influence of this cytokine on mucositis development and possibly a new direction of therapeutic behaviour.

Anti-tumour therapy may lead to changes in oral mucosa and changes in salivary cytokine levels. Gastrointestinal complications, including oral cavity complications, may necessitate modification of therapeutic protocol, which has a negative influence on the final therapeutic effect. Changes in oral mucosa make patients hurt. Oral mucositis may hinder and possibly prolong anti-tumour therapy and also increase treatment cost [6,13,14].

According to studies conducted by Brito Costa et al., mucositis lesions occurred much more often in children who did not use 0.12% chlorhexidine, and they developed between 2 and 4 days after the use of intravenous dose of methotrexate and most frequently occurred on lips and buccal mucosa. The average time of development of mucositis-like lesions and ulceration was about 10-16 days of chemotherapy [15].

Both chlorhexidine and benzydamine have positive effect on reduction of oral mucositis during chemotherapy, but only in children over 6 years of age. Therefore, chlorhexidine has not been recently recommended for use in advanced mucositis. All physicians agree that the most important factor lowering the risk of oral complications is regular, at least twice brushing of teeth, mouth washing and effective motivation of the patient to clean dental surfaces and soft tissues of the oral cavity [16,17].

Pain related to oral lesions of the mucositis type in patients subject to haeematopoietic stem cell transplantation, despite the use of opioids, was significantly correlated with increased expression of the TNF-α gene in buccal cells on the 9th day of therapy in comparison with baseline [18]. However, Sprinzl et al. did not show significantly better results after the use of GM-CSF mouthwash (Leukomax®) compared to conventional mouthwashes (Hydrocortisone, Pantocain) which were used in patients undergoing chemo- and radiotherapy due to a neoplastic disease located in the head or neck region [19]. Moreover, it was revealed that mouthwashes with granulocyte-macrophage colony stimulating factor should not be used in prevention of oral mucositis in patients after transplantation [17]. Numerous authors revealed very good therapeutic effects of preparations containing the following factors: granulocyte-macrophage colony stimulating factor (GM-CSF), transforming growth factor (TGF-β1 and -β3), interleukin 1(IL-1), interleukin 11(IL-11) or epidermal growth factor (EGF) in the form of mouthwashes in patients undergoing chemotherapy [3,16,18,20].

Summary

Mean level of tumour necrosis factor-α in saliva of children with acute lymphoblastic leukaemia was lower and was decreasing during chemotherapy. In the final stage of treatment, the difference with the control group was near statistical significance. After initiation of chemotherapy, a large percentage of ALL children had oral mucositis of various degree, especially in the form of redness, erosions and ulcerations.

Conclusions

Disorders in the immune system during chemotherapy may cause increase in pathological changes of oral mucosa. Early assessment of proinflammatory cytokines may prevent complications of standard treatment and prolongation of anti-tumour treatment, which will allow fast recovery of patients with ALL. Cooperation between paediatric haematologists and dentists with affected children and their parents seems to be of great importance for maintenance of oral health in children with neoplastic diseases.

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2. Ethical standard All patients and their parents consented to clinical examination and to the collection of saliva, in accordance

with the Declaration of Helsinki. The study was approved by the local Ethics Committee.

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