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Determination of Salivary Myeloperoxidase, Immunoglobulin E and Tumor Necrosis Factor Alpha after Complete Denture Insertion

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Short title: Factors of Inflammation after Complete Denture Insertion

Key words: Complete Dentures · Proinflammatory cytokines · Immunoglobulin E

Significance of the Study

- Wearing complete dentures may cause allergic and traumatic reactions of the oral tissue, which could result in an increase in proinflammatory cytokines and salivary immunoglobulins in the saliva of the wearer. Increase in indicators of inflammation directly links the acrylic dentures and oral inflammation.

Accepted manuscript

Abstract

Objective: To detect activities of salivary myeloperoxidase and concentrations of salivary tumor necrosis factor alpha (TNF α) as indicators of inflammatory reaction and salivary immunoglobulin E as an indicator of allergic reaction, after complete insertion of acrylic denture. **Subjects and Methods:** Complete dentures were made for a uniform group of elderly patients, and saliva samples were taken immediately before they were given to the patients, as well as one, three, seven and thirty days after insertion of denture, with simultaneous monitoring of changes on the oral mucosa. **Results:** After seven and thirty days of wearing upper and lower complete dentures, a non-significant increase in salivary myeloperoxidase and TNF α were proven as indicators of inflammation, No changes were observed in the values of salivary immunoglobulin E during a thirty-day observational period, which excluded the appearance of allergic reactions to acrylic materials in the tested group of patients. **Conclusion:** A non-significant increase in levels of MPO was observed on day 7 decreasing at the end of 30 days. TNF α also tended to increase in a non-significant manner.

Introduction

Total teeth loss, as a consequence of hard dental tissue disease and periodontal disease, is especially common in elderly patients. According to the literature, complete denture insertion may be followed by allergic reactions to acrylic materials that they are made of, and inflammation of the oral tissue [1,2]. Wearing dentures may result in the appearance of different oral lesions, especially if their surface which touches the mucous tissue is large, which is the case with complete dentures [3]. Dental-related mucosal lesions are associated with denture plaque, weak retention and stability of dentures, candida-associated denture stomatitis and mechanical trauma of the tissue [4-6].

Candida albicans is a typical commensal microorganism of the oral cavity, and can become virulent in certain conditions and cause denture stomatitis, especially if there are local alterations in the mouth after the insertion of dentures. Denture stomatitis interrupts or completely disables the usage of dentures, inflammation of the mucous membrane is prolonged and is followed by a burning feeling as well as a change in the taste of food, so extensive local and systemic therapy is proposed [7].

Apart from denture stomatitis, which usually appears after prolonged wearing of dentures, all other complications are associated with the adaptation period, immediately after insertion of dentures [3,8]. An analysis of changes in the oral tissue can be done by clinical observation and by methods such as real-time microscopy which represents a non-invasive way for highlighting structural features of oral alterations at a microscopic level [9]. A possible allergic or traumatic reaction of the oral tissue against the presence of dentures in the mouth may be followed by an increase in proinflammatory cytokines and salivary immunoglobins in the saliva. Levels of these factors in the saliva of patients with complete dentures have not been reported.

Myeloperoxidase (MPO) is a heme-containing peroxidase expressed mainly in azurophilic granules of neutrophils, and in the primary lysosomes of monocytes [10]. In the presence of hydrogen peroxide or a halide, MPO catalyses the formation of reactive oxygen intermediates, thus significantly contributing to the antimicrobial effect of neutrophils [11,12]. In addition, MPO has been shown to be a mediator of local tissue damage and subsequent inflammation in different inflammatory diseases [13,14].

Tumor necrosis factor alpha (TNF α) is an important mediator of inflammatory reactions, and plays a key role in the pathogenesis of several serious inflammatory and autoimmune diseases. TNF α is produced by monocytes and macrophages. The local cellular effect of TNF α includes the adhesion of polymorphonuclear leukocytes (PMNs) to endothelial cells, degranulation of PMNs, activation of phagocytes and catabolic reactions of the extracellular matrix [15-18].

Immunoglobulin E (IgE) triggers reactions mediated by mastocytes and eosinophils. It is crucial in defence against parasites and is an indicator of allergic reactions [19]. Early hypersensitivity is characterised by rapid release of fluid from blood vessels, mucus secretion, and is often accompanied by inflammation [20]. An increase in the amount of salivary IgE could indicate acrylate hypersensitivity, therefore, allergic reactions could be the cause of inflammation of the oral tissue after complete denture insertion [21,22].

The aim of this study was to measure activities of salivary myeloperoxidase and concentrations of salivary TNF α as indicators of inflammation, and salivary immunoglobulin E as an indicator of allergy, after complete acrylic denture insertion.

Subjects and Methods

This study was conducted in accordance with the Declaration of Helsinki, and was granted ethical approval by the Faculty of Medicine, University of Nis. Each patient was informed about the aims and method of the study, for which they gave their written consent.

The study included 6 male patients, aged 70-75 (mean 72.33), who were not diagnosed with untreated systemic diseases, current infectious diseases, or changes in the oral mucosa. All patients were non-smokers. The patients were inmates of the elderly care center, which implied they had the same lifestyle and diet, as well as similar health care. Two complete acrylic dentures (Triplex Hot, Ivoclar Vivadent, Lichtenstein), upper and lower, were indicated in all patients. All dentures were made and later corrected by the same dental therapist and dental technician. The patients were asked not to use their old dentures 48 hours prior to the insertion of new dentures. After the application and adaptation to dentures in the mouth, the patients were advised on the use and maintenance of dentures, monitored by medical personnel. Control check-ups were scheduled one, seven and thirty days after insertion of dentures in the patients. Oral lesions (erythema, traumatic irritations or ulcers) were noted at each control check-up as recorded in the patients' files. Dentures were corrected in order not to prolong mechanical trauma.

3 ml of saliva was collected between 8 and 10 am. immediately before insertion of dentures and then 1 day, 3 days and 30 days after dentures were inserted.

The samples were immediately centrifuged at 12,000 g for 10 min, in order to remove cells and microparticulate material. Clear supernatants were stored at -20° C.

Determination of Enzymatic Activity of MPO

Briefly, individual saliva samples were plated in 96-well flat-bottomed tissue culture plates (NUNC, Roskilde, Denmark) previously filled with test substances at final concentrations of 0.5, 1, 5, 10 and 50 μ M. The plates were incubated for 60 minutes at room

temperature. MPO activity was determined using 1,2-diaminobenzene (o-phenylenediamine) in citric buffer activated with H₂O₂ and Triton X-100. The reaction was stopped with H₂SO₄ solution (1 M). Optical densities (ODs) were determined in a Multiscan Ascent (Labsystems, Finland) at 540 nm. The results are expressed as ODs (540 nm). Each experiment was repeated three times.

Quantification of IgE

Salivary IgE levels were quantitated in duplicate by sandwich ELISA, by using commercial kits (Radim, Pomezia, Italy). Salivary IgE levels were measured by using standard samples with known levels of IgE provided by the manufacturer and expressed as IU/dL.

Quantification of TNF α

The concentrations of TNF α were determined using human TNF α ELISA kits (R&D Systems, Minneapolis, USA). The assay was performed according to the manufacturer's instructions, and the results expressed in pg/ml. The detection limit for TNF α was 4.4 pg/ml.

Statistical Analyses

The normality of the distribution of parameters was determined by using the Shapiro/Wilk test; comparison of values during the study period and values prior to denture insertion was determined by using a t-test of paired samples and the Wilcoxon Signed Ranks test (if the data were not normally distributed). *p* values less than 0.05 were considered significant.

Results

The concentrations of MPO, TNF α and IgE are shown in Table 1. No statistically significant differences in values of the tested parameters during the study period and prior to

denture insertion were recorded. Mean values of MPO were higher after seven days, and mean levels of TNF α were higher after seven and thirty days (Table 1).

After a day of wearing complete dentures, mild erythemas were observed in the sublingual space. After a 30-day-long observational period, no oral mucosal lesions were noted in the mouth of the patients (Table 2).

Discussion

Wearing complete dentures may be the cause of mechanical oral lesions and an inflammatory reaction of oral mucosa after insertion of dentures. The study started with the assumption that possible inflammatory changes on oral tissues after the application of complete dentures are followed by an increase in the concentrations of salivary myeloperoxidase, TNF α and IgE. Combining information on possible oral lesions in the mouth of patients with variations in the levels of these parameters was expected to shed light on the state of the oral tissue at the start of using dentures.

The age of patients is associated with systemic diseases, nutritional deficiencies, polypharmacy, changes in the quality and quantity of saliva, and weak oral hygiene; this undoubtedly affects the outcome of treatment with complete dentures. Given that decreased flow of saliva is common in elderly patients, mucosal ulcers and fungal infections are more frequent; thus, it is necessary for dentures to be fitted following principles of good clinical practice and to be kept immaculately clean [23].

The group of patients included in this study was small (n=6) but it was homogenous. The patients were of the same age, lived in the same conditions, had the same diets, medical care and surveillance. The study excluded female patients because of the possibility of osteoporotic changes in the bones and untreated systemic diseases. Dentures were made by a dentist and a dental technician, using materials from the same manufacturer. Regardless of

the objective differences in each clinical case, the dentures had satisfactory retention and stabilization.

An accurate calculation of the sample size in the design of the study was not made, thus the results can be considered preliminary, but at the same time relevant as they were obtained from a homogeneous sample. Analyzing the biomolecular patterns of inflammation through a non-invasive examination such as saliva analysis can provide a new approach for clinical and molecular diagnosis. Further studies will be needed to validate these preliminary results.

Uslu et al. reported an increase in the level of MPO in the inflamed gingival tissue of a rat [24]. TNF α has been shown to be elevated in patients with oral leucoplakia [25], periodontitis [15] and recurrent aphthous stomatitis [26].

No statistically significant differences in the values of the examined parameters during the tested period and prior to denture insertion were observed. A slight increase was recorded in the level of MPO after seven days of wearing dentures, followed by a gradual decrease until the end of the 30-day period. Levels of TNF α exhibited a tendency to increase with the length of wearing dentures.

The appearance of oral mucosal lesions in the mouth of the patients was also monitored during the observational period. Erythema and traumatic irritations of the oral mucosa were recorded in the first three days of wearing complete dentures, which is considered common in clinical practice [27,28]. One of the patients had traumatic ulcers in the sublingual part of the lower jaw. The analysis of the patient's saliva did not show an increase in inflammatory parameters.

Traumatic irritations and ulcers were observed in all patients after 7 days of wearing dentures. Regardless of differences in the degree of severity and the localization of the

resulting changes, the amount of MPO and TNF α in the patients' saliva increased after seven days, which could be associated with the intensity of the resulting changes in the oral cavity. Prolonged wearing of dentures emphasized their shortcomings; therefore, the correction was more extensive. It resulted in the absence of changes on the oral mucosa after thirty days, followed by a decrease in the level of MPO to initial values. A slight increase of salivary TNF α could indicate prolonged oral inflammation which might be associated with wearing dentures. Our results are similar to those from other studies, given the increase of salivary TNF α in the first month after giving complete dentures to patients [15-17].

The absence of changes in the level of salivary IgE is related to the absence of early hypersensitivity to acrylic materials in the examined group of patients. However, some authors have reported allergic reactions to an unbound monomer of acrylic materials [29-31].

Given the paucity of recent studies on inflammatory parameters in denture-wearers in clinical conditions, the comparison of the obtained results with published literature was not possible. On the other hand, a broad range of possibilities has been opened up for further research in this field, which will add to the conclusions reached in this study. Biochemical parameters are excellent markers of changes in oral tissues, so the study of the biochemistry of inflammatory patterns in the lesions of the oral cavity is of considerable importance for monitoring changes in oral mucosa in malignant transformation which is of significance for clinical practice [32].

Conclusion

After seven and thirty days of wearing upper and lower complete dentures, in a uniform group of patients, a non-significant increase in levels of salivary myeloperoxidase and TNF α were seen as indicators of inflammation. No changes were seen in the levels of

salivary immunoglobulin E during a thirty-day observational period, which excludes the appearance of allergic reactions to acrylic materials in the tested group of patients.

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Conflict of Interest: The authors declare no conflict of interest.

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Table 1. The values of the parameters examined during the study period

Period	MPO (U/mcg) proteins in saliva	IgE (U/ml)	TNF α (pg/ml)
Before insertion	3.452 $\pm$ 1.886(2.975)	0.301208 $\pm$ 0.000029(0.301211)	4.821 $\pm$ 2.049(5.231)
1 day	3.485 $\pm$ 2.383(2.205)	0.301222 $\pm$ 0.000038(0.301208)	4.797 $\pm$ 3.337(3.030)
3 days	3.405 $\pm$ 1.592(3.075)	0.301214 $\pm$ 0.000034(0.301214)	6.312 $\pm$ 9.516(3.030)
7 days	5.238 $\pm$ 5.468(3.405)	0.301251 $\pm$ 0.000068(0.301246)	7.726 $\pm$ 4.006(7.879)
30 days	3.270 $\pm$ 0.873(3.515)	0.301200 $\pm$ 0.000028(0.301202)	9.166 $\pm$ 4.838(7.877)

The values are reported as X $\pm$ SD (median)

Table 2. Clinical observation of oral lesions as a consequence of wearing complete dentures

		After three days						After seven days					
		P 1	P 2	P 3	P 4	P 5	P 6	P 1	P 2	P 3	P 4	P 5	P 6
Upper dentures	Erythema	+	-	-	-	+	+	-	+	-	-	-	-
	Traumatic Irritation	-	+	-	+	-	-	+	-	+	-	+	+
	Traumatic Ulceration	-	-	-	-	-	-	-	-	-	+	-	-
Lower dentures	Erythema	-	+	+	+	-	+	-	-	-	-	-	-
	Traumatic Irritation	+	-	-	-	-	-	-	+	+	+	+	-
	Traumatic Ulceration	-	-	-	-	+	-	+	-	-	-	-	-