Comparative Study for Oral Reaction Produced by Polymethylmethacrylate

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Gingival enlargement is also known as gingival hyperplasia or hypertrophy and it is an abnormal overgrowth of gingival tissues. This type of lesions can be produced by a large group of pathologic processes. The most common are the reactive hyperplasia which develops in response to a chronic tissue injury that stimulates an excessive tissue repair response. Often, dental prosthesis have been reported to cause this type of lesions due to a mechanical chronic irritation or by a reaction of the oral tissue to substances released into the oral environment by the materials used in manufacturing this type of prosthesis. One of these substances is the methylmethacrylate (PMMA), a widely used in dentistry. The first step in treatment of these lesions is the removal of the irritative factor which sometimes can lead to healing depending on the type of lesion. The challenge is to differentiate a lesion caused by a mechanical irritation from a lesion in which the cytotoxicity of PMMA may be the cause. Often, surgery is the only treatment option for the first situation. When we have an irritative reaction and a cytotoxicity reaction, we must treat them both.

Keywords: methylmethacrylate, acrylic resin, TNF alfa, citotoxicity

The aim of this article is to report a two lesions caused by a mechanical chronic irritation due to an incorrect adapted dental prosthesis and to focus on the potential local toxic effects of dental materials used in manufacturing dental prosthesis.

The materials used nowadays in dental medicine are ranging from polymers to metals and all have different applications. Their development led to a significant improvement of the quality of the oral rehabilitation. Beside this important esthetical and functional role, dental materials and the prosthesis manufactured from them may show side effects which can lead to severe oral tissue lesions. These harmful side effects can be caused by the reaction of the oral tissues to substances released into the oral environment by the dental materials.[1] A mechanical chronic irritation of the oral soft tissue by an incorrect adapted prosthesis can also lead to local lesions.

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When talking about a dental material we need to take in consideration the physical, mechanical and biological properties. Many studies have been performed to improve the physical and mechanical properties of dental materials, but fewer attempts have been made to assess the biocompatibility of these materials[2-4]. The interaction between the material and the oral environment forms the subject of biocompatibility [5]. Biocompatibility should be taken in consideration when a material is selected and used in the oral cavity. A dental material is considered biological compatible when it has the property of being non-destructive in the oral cavity. This interaction needs to work in both ways such as the effect of the material on the biologic environment and the effects of the biologic environment on the material.[6] All dental materials release substances into the oral environment to varying degree with local or systemic reactions. Methyl methacrylate (MMA), a widely used monomer in dentistry and medicine, has been reported to cause abnormalities or lesions in several organs. Experimental and clinical studies have documented that monomers may cause a wide range of adverse effects. Dental materials that are to be used in the oral cavity should contain no toxic diffusible substance and should be harmless to the oral tissues. The material should not contain potential sensitizing agents and should have no carcinogenic potential [7].

The possible side effects of dental materials on oral mucosa are the following: stomatitis, lichenoid reactions, geographic lesions, recurrent aphthous stomatitis, erythema multiforme, gingivitis, staining, hypersensitivity reactions or discoloration of the mucosa [8, 9].

**Experimental part**

**Material and methods**

Our study was made for 3 cases of oral disorders: two subjects with total denture reactions and one subject with fixed dentures according to with European Directive[11, 13].

In this case, the histological exam showed multinucleated giant cells with additionally mesenchymal cells and deposits of hemosiderin and hemorrhage. These aspects are typical for peripheral giant-cell granuloma (fig.1).

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Fig.1. Palatinal hyperplasia caused by a fixed maxillary prosthesis.(left). Aspect 4 weeks after removing the fixed dentures (center). Aspect after surgery (right)
The hystological findings showed a mass of collagen and fibroblasts with a chronic inflammatory infiltrates. These aspects are typical for epulis fissuratum.

The difference between cytotoxicity detection on acrylic prosthesis components may be possible by Eliza (Enzyme linked immunosorbent assay) method applied on the patient’s serum [12].

There will be 500 microliters harvest from the patient having a duplicate sample. Out of these samples the TNF-alpha concentration will be determined.

All these procedures have used Quantikine Human TNF alpha Immunoassay which allow the quantitatively determination of cytokines on patient serum.

All reagents from the kit will be used to room temperature. The washing solution will be heated to room temperature and will be slightly stirred until the possible crystals completely dissolve. There will be 500 mL of washing solution prepared. The substrate solution is being reconstructed by mixing equal volumes of A and B reactivies from the kit maximum 15 min before usage. The supply solution will be prepared by reconstructing the cytokine standard using calibrator diluent RD6-21. This solution will rest for minimum 15 min , while it will slightly stir. For standard preparation, 500 μL of calibrator diluent RD6-21 will be pipetted in a polypropylene tube. 500 μL of solution will be pipetted in the first tube and the mixing concentration is then calculated. After the solution is well stirred, 500 μL will be transferred into the next tube and the concentration will be calculated again. Standards for different concentrations are prepared in the same way (500 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, 15.6 pg/mL). The supply solution (the undiluted standard) is used as high standard (1000 pg/mL) The calibrator diluent RD6-21 is used like zero standard (0 pg/mL).

The optical densities are determined 30 min after the reaction stops using an automatic reader set for 450 nanometers, with a reference filter of 540nm or 570nm (for correction). Reading the probes only at 450nm without correction can influence the accuracy.

**Results and discussions**

The results are obtained depending on the logarithmic calibration curve of each cytokine, build on absorption (oy) and ox concentration (0-1000 pg/mL) of standards.

The results are shown as optical densities units automatically converted in pg/mL for each of the probe tested. If the probes are diluted, the concentration read will be multiplied with the dilution factor. Determination of TNF-alpha concentration in first case with total denture, serum revealed slightly increased values of this cytokine (50.48-50.7 pg/mL) in second case with the total denture the values were more increased (90.3-90.9 pg/mL) and in sealed. 100 μL of diluent RD1-51 will be pipetted in each hole of the plate. Then, 100 μL of each standard will be pipetted in the first strip in the following order: 0 pg/mL; 15.6 pg/mL; 31.2 pg/mL; 62.5 pg/mL; 125 pg/mL; 250 pg/mL; 500 pg/mL, 1000 pg/mL. Starting with the second strip,100 μL of the unfreeze probes will be pipetted in 15 min. The plate will be covered with an adhesive film and will be incubated for 2 h at room temperature. After that the plate is washed four automatically times with 400 μL of washing solution. At the end of the washing stage, the plate will be put on an absorbent paper for cleaning without damaging the probes. 200 μL of cytokine conjugate will be added in each hole of the plate and the plate will be covered with an adhesive film and incubated for 2 h at room temperature. After the incubation period the adhesive film will be removed and the plate will be washed using the same protocol. 200 μL of substrate solution is then added in hole of the plate and the plate will be covered again with an adhesive film and incubated for 30 min in dark room at room temperature.

After incubation time is over 50μL of stopping solution will be added in each hole of the plate. When the reaction stops, the colour turns from blue to yellow. If the colour is green or does not modify uniformly, the plate will be slightly stirred for a complete homogenization.

The optical densities are determined 30 min after the reaction stops using an automatic reader set for 450 nanometers, with a reference filter of 540nm or 570nm (for correction). Reading the probes only at 450nm without correction can influence the accuracy.

**Table 1**

<table>
<thead>
<tr>
<th>Subject 1 with complete denture</th>
<th>Subject 2 with complete denture</th>
<th>Subject with fixes dentures</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF alpha</td>
<td>50.48</td>
<td>50.6</td>
</tr>
<tr>
<td></td>
<td>90.3</td>
<td>90.9</td>
</tr>
<tr>
<td></td>
<td>100.4</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>50.6</td>
<td>90.9</td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>105</td>
</tr>
</tbody>
</table>

**Fig.2.** Left vestibular hyperplasia caused by an acrylic complete maxillary prosthesis

**Fig.3.** Vestibular hyperplasia caused by an acrylic complete maxillary prosthesis (left). The hystological findings were typical for epulis fissuratum. Alergic reaction of the palatinal mucosa to the maxillar prosthesis in the same patient (right).
the case of the patient with fixises dentures was biggest (100,4-105 pg/mL). These values were compared with normal reference values range between 2-5 pg/mL.

TNF alfa is an eloquent test for cytotoxic detection that can early detect signs of allergy and inflammation by measuring the endotoxine from patient serum.

It is recommended to associate this type of test with another clinical test (Patch Test) and the confirmation should always be histological.

Conclusions

Dental materials, specialty resin based restorative materials, have a wide range of applications nowadays. But, despite the efforts made to continuously improve their physical, mechanical and esthetic properties, they may cause some side effects regarding their biocompatibility. This situations may led to severe local lesions which could prove extremely unpleasant for the patient.

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References

13. *** EUROPEAN GUIDELINE FOR MEDICAL DEVICES 93/42/EEG.

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