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Original Research

Salivary Cells in Patients with Dental Amalgam and Composite Resin Material Restorations – a Morphological Investigation

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Abstract

Dental restorative materials have long-lasting contact with the oral cavity environment and may affect saliva composition. The aim of our study was to compare the composition and morphological activity of salivary cells in patients with amalgam and composite material restorations. Salivary smears of mixed non-stimulated saliva were prepared using typical histological methods. Oral epithelial and inflow cells were investigated. Significant morphological changes were observed in the salivary smears in patients with amalgam restorations. There was a slight difference in salivary cells in patients with composite restorations in comparison to the control group.

Keywords: dental amalgam, composite resin material, saliva

Introduction

Dental amalgam has been widely used as restorative material for over two centuries, especially in premolar and molar cavities. The years of clinical experience, technical and biological tests have led to the production of high-silver and high-copper amalgams, which have better quality and corrode less than the conventional ones. All amalgam alloys, however, have to be mixed with mercury in the proportion of 50:50 [1]. The most controversial are side effects of amalgam application as a dental restorative material, associated particularly with mercury leakage. The amount of mercury escape during everyday amalgam utilization is still under consideration. Some researchers believe that it is minimal, much less than daily mercury intake with water, food and air [2], others are convinced that temperature changes, dental plaque acids, salivary proteins, enzymes, brushing, and chewing cause its significant increase [3]. Higher levels of mercury have been observed in saliva, blood, urine, and faeces in patients with amalgam restorations, being most elevated for a few days after the restorations have been placed or removed [4]. The presence of dental amalgam causes discoloration and higher mercury concentration in restored and neighbouring teeth [5]. It can also affect oral mucosa and induce such changes as discoloration, amalgam tattoo, lichen planus and leucoplakia [6, 7].

Although composite resin materials have a much shorter history, many disadvantages of using them have been noticed. Especially their influence on dentin and dental pulp has been extensively studied [8-10]. Chemical irritation and various degrees of inflammatory pulp response have been detected. However, some authors prove that polymerization shrinkage and bacterial micro-leakage play the most important role in inflammatory pulp reaction in teeth restored with composite materials [11]. Allergic reactions to composite material components have been observed, of which the most aggressive is unpolymerized monomer [12].

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The release of resin factors, including Bis-GMA, TEGDMA, HEMA, and UDMA under the influence of temperature changes, ethanol, enzymes, acids, and water has been proved [13]. These elements demonstrate cytotoxic, genotoxic, and mutagenic effects on epithelial cells, fibroblasts, and monocytes [14]. Bis-GMA may be converted into Bis fenol-A, which has estrogenic activity and can disturb hormonal balance [15]. The liquid environment of the oral cavity is created by the saliva that contains various biologically active substances (enzymes, proteins, and hormones), epithelial cells, numerous blood formed elements, and bacteria. The mouth is always moist and is continuously subjected to fluctuations in temperature and wide ranges of pH. All of these environmental factors contribute to the degrading process of dental restorative materials.

Saliva as a diagnostic material is easily available but frequently underestimated. Given the above, the detection of certain substances in the saliva can be a marker of pathological changes, not only in the mouth but also in the whole body.

The aim of the study was to investigate the composition and the morphological activity of salivary cells in patients with silver amalgam and composite resin material restorations.

Material and Methods

The study was performed on smears of non-stimulated saliva collected from 30 generally healthy patients, aged 16-30, who were divided into three groups.

The control group consisted of 10, neither orthodontically nor prosthodontically treated students of the Catholic Secondary School, aged 16-20, having neither amalgam nor composite resin fillings.

The Study Groups

Group I: 10 people with amalgam restorations, average DMF=16 (mean 7 dental amalgam fillings),

Group II: 10 people with composite resin fillings, average DMF=13.

The study was approved by the Local Bioethics Committee for Research on Humans, Medical University of Białystok, No. R-I-002/90/2007.

Each patient gave a detailed history of accompanying systemic diseases, allergies, and other ailments, and medications used. Then, general oral examination was performed, in artificial light, using a mirror and a periodontically calibrated probe. Patients with any general or local abnormalities were disqualified. Samples of mixed non-stimulated saliva were received from the study subjects each morning between 8-9 am. Saliva smears were done on cleansed and defatted glass slides. After fixation in 96% ethanol, staining was performed with H+E, and using the methods of May-Grűnwald-Giemsa.

Results

Oral epithelial cells and inflow leucocytes were investigated. The examination focused on the number, size, shape, and nucleus-to-cytoplasm ratio of epithelial cells. The presence and number of inflow cells were also taken into consideration.

In the control group, the salivary samples contained several epithelial cells and a few inflow cells, mainly neutrophils and single lymphocytes. In most of the samples, epithelial cells had a relatively homogeneous appearance in terms of size and shape, and their nucleus-to-cytoplasm ratio was normal and levelled. These cells were uniform and larger than inflow cells. They are seen as isolated cells with lightly stained cytoplasm and rounded or oval nuclei, usually centrally placed (Fig. 1).

The salivary smears of study group I (patients with amalgam restorations) showed significant differences in comparison to the control. All cell types (epithelial and inflow) were numerous. Oral epithelial cells demonstrated various morphological alterations, such as: larger size, shape irregularity, unequal intensity of cytoplasm staining, and changes in the nucleus-to-cytoplasm ratio. Cell nuclei presented a different picture: they were bigger or smaller, of the pycnotic type. Some cells had slightly stained oedematous eosinophilic cytoplasm, the cytoplasm of others was dark stained, with a small pycnotic nucleus (Fig. 2A, 2B).

In study group II (patients with composite resin fillings), the changes were less pronounced than in study group I. The numbers of epithelial cells and leucocytes, mainly neutrophils, were similar to those observed in the control group. The detected epithelial cells were rather equal in size, with quite regular oval shape. A few salivary smears presented changes in cytoplasm staining and nucleus image (Fig. 3).

Discussion

The oral cavity is the first part of the digestive and respiratory systems. Its environment is complex. Human saliva has many functions and its composition is influenced by various external and internal parameters. The long-lasting existence of dental restorative materials in extremely tough conditions of the oral cavity affects their functionality and biocompatibility. Dental amalgam and composite resin materials degrade under the influence of saliva and other elements of the oral environment, and release harmful agents whose general and local health risks are still under consideration [3, 13, 14]. *In vitro* studies can be carried out in highly controlled conditions, but their results are not always adequate to the *in vivo* situation.

Our study was performed on generally healthy, young adults to eliminate the other possible causes of saliva smear changes. The most pronounced alterations were noticed in patients with amalgam restorations. We observed a distinctly elevated number of all cell types in saliva smears in patients without general or local complaints or pathological symptoms. The inflow cell counts, mainly neutrophils and lymphocytes, were elevated.

We also noted a higher number of oral epithelial cells than in the control group. There is abundant evidence indicating a correlation of dental amalgam with lichen planus and lichenoid reactions on the oral mucosa [7, 16, 17].

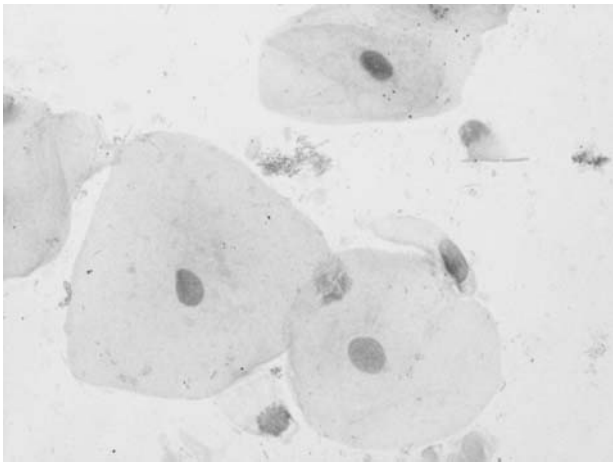

Fig. 1. Control salivary smear. Oral epithelial cells are uniform in size and shape, a few inflow leucocytes are also present. H+E staining, Mag. 400x.

Little et al. [17] have noticed that subcytotoxic concentrations of $HgCl_2$ induce a concentration-related increase in ICAM-1 expression and consequent T-cell binding on oral keratinocytes. They have also suggested that $HgCl_2$ stimulate the release of low levels of tumour necrosis factor-α and interleukin-8, which is a potent chemokine for neutrophils and may have some chemotactic activity for lymphocytes. Although the patients examined in the current study did not show any clinical changes in the oral mucosa, the higher number of epithelial cells in salivary smear can be a symptom of hyperkeratosis characteristic of lichenoid reactions.

We noted various morphological alterations of epithelial cells in the salivary smears of dental amalgam patients, including larger size, irregular shape, unequal intensity of cytoplasm staining and changes in the nucleus-to-cytoplasm ratio. It could be caused by altered metabolism of oral epithelial cells. Similar changes in the picture of oral epithelial cells, also connected with hyperkeratosis, were found in relation to chemical, thermal, or physical irritants.

The changes observed in cell nuclei presented a different picture: the nuclei were bigger or smaller, and of the pycnotic type. The effects of dental materials on the cell nucleus has been widely investigated in *in vitro* studies. Schmid et al. [18], who studied cytotoxicity of mercuric dichloride ($HgCl_2$) in human salivary glands and lymphocytes and noted increasing dose-dependent DNA migration and genotoxicity, found the effects to be harmful. Also Lin et al. [19] observed that amalgam alloy induced breakdown of the cell membrane, which is characteristic of necrosis, inhibition of DNA synthesis, and caused inflammatory and stress responses. In the patients with composite resin restorations, we detected minimal morphological alterations in the picture of salivary cells. The epithelial and inflow cell counts were similar to those in the control group. The detected epithelial cells were equal in size and of rather regular oval shape. Our results are diverse from the findings reported by Tai et al. [20], who compared cytocompatibility of various materials (amalgam, resin, and glass ionomer) using cultured human periodontal ligament (PDL). The authors focused on cell viability and proliferation assays. Both the type of material and exposure time were found to affect cell viability and proliferation. Resin exhibited the most cytotoxic effects, followed by glass ionomer and amalgam, during a 14-day incubation period. Amalgam and glass ionomer slightly inhibited cell viability and growth in the first 24 h, compared with the control. Toxic effects of composite resin materials have been widely investigated also by other authors [8-12]. The main interest of research, however, is to find out whether these harmful substances can reach cytotoxic levels in mammalian tissues. Based on our data, we agree that harmful substances released from dental composite resins hardly ever achieve toxic concentrations.

We observed only a few salivary smears of patients with composite resin presenting changes in cytoplasm staining

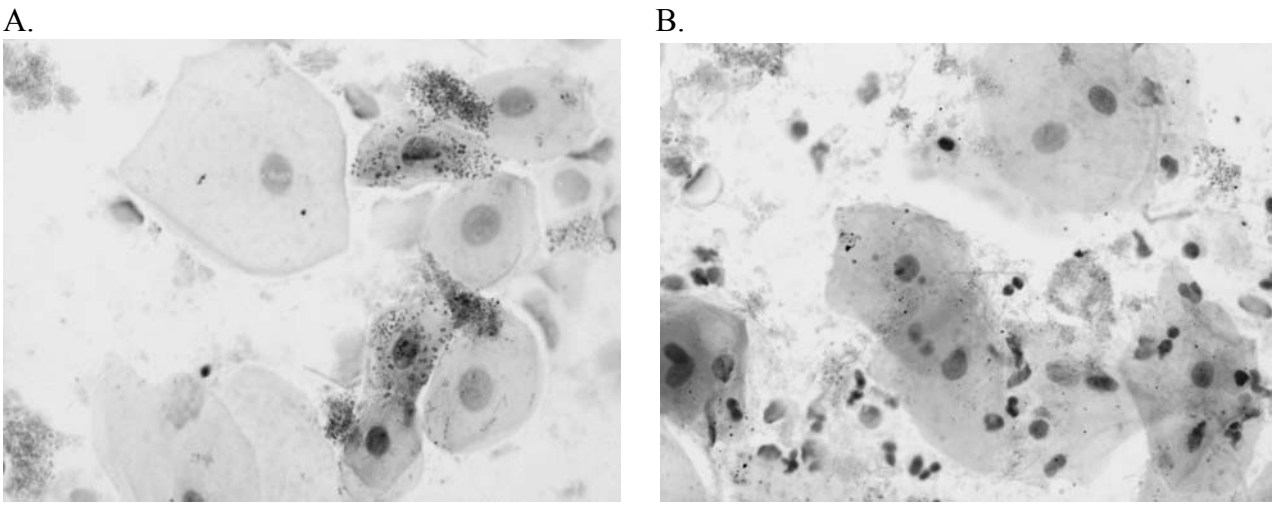

Fig. 2. Smears of saliva of patients from study group I. A) Oral epithelial cells show morphological alterations, such as: differences in size and shape, unequal intensity of cytoplasm staining, and changes in the nucleus-to-cytoplasm ratio. H+E staining, Mag. 400x. B) High number of all types of cells: oral epithelial cells and inflow leucocytes (mainly neutrophiles and lymphocytes) can be observed. H+E staining, Mag. 200x.

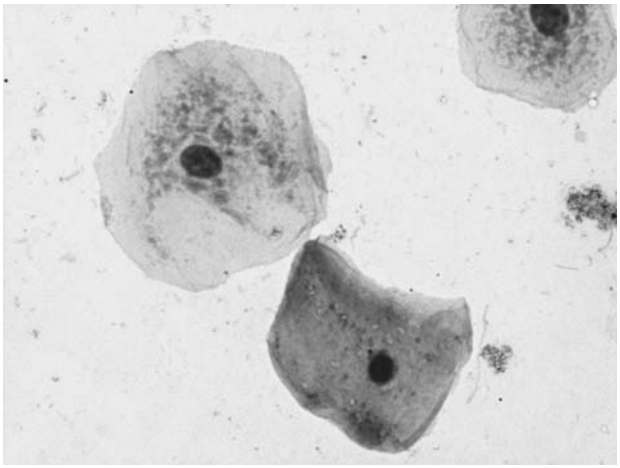

Fig. 3. Salivary smear of patient from study group II. Oral epithelial cells are mostly related in size, with rather a regular oval shape. Changes in cytoplasm and nucleus staining are seen. H+E, Mag. 400x.

and nucleus image. Many researchers have noticed that composite resins have genotoxic potential and affect chromosomal integrity, cell-cycle progression and DNA replication [21, 22]. It has been well-established that the comonomer triethylene glycol dimethacrylate (TEGDMA) causes gene mutations *in vitro*. As a consequence of DNA damage, the mammalian cell cycle is delayed in both G1 and G2/M phases, depending on the concentrations of the monomers.

Nocca et al. [23] have reported that methacrylic monomers present in dental composite resins alter the functionality of peripheral blood monocytes (PBMs) and polymorphonucleate cells (PMNs), which are involved in the biological response to materials and the host ability to respond to bacteria. The authors suggest that methacrylates induce a relevant decrease in PBM oxidative burst, stabilize PMNs in resting state and maximize their stimulated activity. It has been demonstrated that monomers reduce the levels of the natural radical scavenger glutathione (GSH). Depletion of the intracellular GSH pool may then significantly contribute to cytotoxicity, since a related increase in ROS levels can activate pathways leading to mutagenicity and apoptosis [24].

No such severe alterations were observed in the current study, which may be due to substantially higher levels of the components used *in vitro* than could ever be reached *in vivo*.

Conclusions

The long-lasting existence of dental restorative materials in the oral cavity may affect saliva composition and cause morphological and functional alterations in its cells. The most enhanced changes in salivary smears were observed in patients with dental amalgam fillings. The modifications of salivary cells in patients with composite restorations were much slighter.

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