BRIEF COMMUNICATION

Clinicopathological and Immunohistochemical Study of Oral Amalgam Pigmentation

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KEYWORDS Amalgam; Immunohistochemistry; HLA-DR; Metallothionein

Abstract Amalgam tattoo, the most common exogenous oral pigmentation, can sometimes be confused with melanotic lesions, being then biopsied. We present the clinicopathological characteristics of 6 biopsied cases (5 females and 1 male) of oral amalgam pigmentation. The most common location was the gingival mucosa, followed by the buccal and palatal mucosa. Morphology and distribution (stromal, perivascular, perineural, and endomysial) of pigmentation were variable; there was only 1 case with fibrous capsular reaction and likewise only a single case of granulomatous foreign body reaction. Morphological variability is conditioned by the timing and amount of the pigment deposit, which is often associated with infiltration by mast cells (CD117+), as well as overexpression of metallothionein and HLA-DR at different tissue levels.
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PALABRAS CLAVE Amalgama; Inmunohistoquímica; HLA-DR; Metalotionina

Estudio clinicopatológico e inmunohistoquímico de la pigmentación oral por amalgama

Resumen El tatuaje por amalgama, la pigmentación exógena oral más frecuente, puede en ocasiones simular lesiones melanicas y ser motivo de estudio biopsico. Se presentan las características clinicopatológicas de 6 pacientes (5 mujeres y un varón) biopsiados por pigmentación oral por amalgama. La localización más frecuente fue la mucosa gingival, seguida de la yugal y palatina. La morfología y distribución (estromal, perivascular, perineural y endomisial) de la pigmentación fue variable, apreciando solo en un caso reacción capsular fibrosa e igualmente, solo en un caso, reacción granulomatosa tipo cuerpo extraño. Esta variabilidad morfológica esta condicionada por la cuantía y cronología del depósito pigmentario, que a menudo esta asociado

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Introduction

The term amalgam describes metal alloys used in tooth filling/restoration. Historically, this is the most widely used restorative element, due to its low cost and simple management.1 Its use has declined since the 1990s, among other causes due to studies, sometimes contradictory, about the toxic effect of the mercurial component.2-5 Its use has been banned in some countries, although the FDA only considers it as a Class II product (subject to special controls)6 and the American Dental Association (ADA) estimates that over 50.1%7 of dental restorations taking place in the USA in 2009 employed silver amalgam.6

Amalgam may produce local adverse effects,4,7 including mucosal pigmentation due to its metal components (silver, mercury, and tin). This is the most prevalent exogenous oral pigmentation8,9 and can be confused with melanin pigmentation, in which case biopsy studies are indicated. In this sense, we studied the clinical, pathological, and immunohistochemical characteristics of a series of cases of oral pigmentation due to amalgam, all of which had undergone biopsy.

Materials and Methods

We analysed all oral biopsies performed at Hospital Universitario La Fe, in Valencia, Spain, during the period 2000–2010, through the software application PAT-Win® v.3.4.1. We selected those which reported the existence of pigmentation due to amalgam, excluding those from periapical lesions. We obtained the clinical data of these biopsy cases through the MIZAR 2.0 application, noting age, gender, location, duration, reason for biopsy, and service which conducted it. In addition, we also obtained the imaging studies prior to biopsy, visualised with the IMPAX 6.4.0 platform.

For each biopsy we carried out an observation with conventional and polarised light microscopy, as well as staining for iron pigment (Perls staining) and melanin (Masson-Fontana staining). In addition, we observed a histological section without any staining and subsequently conducted immunohistochemistry techniques for CD68, CD117, HLA-DR-α-chain, and metallothionein. Table 1 shows the characteristics of the antibodies employed. All techniques were performed using the Dako® Envision-Plus™ system, and we conducted thermal antigen retrieval with EnVision Flex Target Retrieval solution (Dako®). We introduced positive and negative controls for evaluation in all the techniques.

Results

We collected 6 observations (5 females and 1 male, with a mean age of 56.5 years) of oral pigmentation due to amalgam. Their clinical and anamnestic data are listed in Table 2. All presented blackish blue oral regions, with a history of dental treatment. None of the imaging studies prior to biopsy showed radiopacity in the pigmented areas. In 4 patients, the biopsy was conducted in order to rule out melanin lesion; in 1 case the study was conducted due to marked cancerophobia and in 1 case the resection was carried out for aesthetic reasons. Pigmentation areas were measured between 0.3 and 1 cm (with a mean diameter of 0.45 cm), and were present for a period between 2 months and 5 years, although 5 patients reported an enlargement in the 2–3 months prior to biopsy. The most common location was gingival mucosa (Fig. 1), followed by the jugal and palatal. All biopsies were obtained by oral surgeons, except for 1 case which was conducted by a dermatologist.

In all biopsies (Fig. 2) some unstained sections contained the black colour of the pigment, with negative Perls and Masson-Fontana stains, thus excluding iron and melanin pigmentation. This pigmentation was observed as large deposits or in a fine granular form (0.1–0.5 μm) at the stromal, vascular, perineural, and muscular levels or in the submucosal salivary glands. The black, granular deposits located on elastic or collagen fibres were present in all observations, forming granular chains or in a disorderly manner. In 3 observations we also noted large and coarse deposits in the submucosal or muscular corium. Only the large deposits were surrounded by a fibrous capsule, usually free of inflammatory reaction. In order of frequency, fine stromal deposits were followed by adventitial vascular pigmentation (5 of 6 observations) and in 3 cases there was muscle fibre pigmentation in the perimysium and endomysium, often distant from other deposits, suggesting that this had contributed to increased pigmentation, as reported by 3 patients. Only in 1 case, with presence of large deposits, was there a foreign-body type granulomatous reaction, with giant cells which phagocytosed pigment.

Table 1 Antibodies Employed in the Study.

<table>
<thead>
<tr>
<th>Denomination</th>
<th>Type of antibody</th>
<th>Clone</th>
<th>Source</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD-68</td>
<td>Mouse monoclonal Ab</td>
<td>KP1</td>
<td>Dakoppats®</td>
<td>Rtu</td>
</tr>
<tr>
<td>CD-117 (c-kit)</td>
<td>Rabbit polyclonal Ab</td>
<td>Ab Poly</td>
<td>Dakoppats®</td>
<td>1/400</td>
</tr>
<tr>
<td>HLA-DR α-chain</td>
<td>Mouse monoclonal Ab</td>
<td>TAL 1B5</td>
<td>Dakoppats®</td>
<td>1/30</td>
</tr>
<tr>
<td>Metallothionein</td>
<td>Mouse monoclonal Ab</td>
<td>E-9</td>
<td>Dakoppats®</td>
<td>1/100</td>
</tr>
</tbody>
</table>

Ab, antibody; Poly, polyclonal; RTU, ready to use.

a una infiltración por mastocitos (CD117+), así como a una sobreexpresión de metalotimina y HLA-DR a diferentes niveles tisulares.

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Polarised vision showed whitish or reddish, dual colouring refraction patterns. Large deposits showed no refractive character; this was only observable in the fine granular deposits, which were more evident with polarisation.

Immunohistochemically (Fig. 3), we found CD68+ macrophage involvement in the case suffering a granulomatous, foreign-body type reaction. In the remaining cases, the presence of macrophages was low. However, the presence of CD117+ cells, identifiable as mast cells, was marked. Mast cells appeared at the subepithelial level and in the submucosa suffering pigmentation, as well as alongside large, coarse deposits. The expression of metallothionein was found in the mucosa, at the basal and suprabasal levels, coinciding with the presence of pigment in the corium. A weaker expression of metallothionein was observed perivascularly and in stromal elements. Moreover, an increase in the number of HLA-DR+ cells, often with dendritic morphology, was observed in the mucosal epithelium and in stromal elements of the corium, coinciding with areas of pigment deposit.

**Discussion**

Oral pigmentation caused by amalgam is an adverse local effect reported in patients undergoing dental treatment. It is caused by different mechanisms, including mechanical penetration into soft tissues, corrosion phenomena, and release of metallic components. Pigmentation itself is harmless and relatively frequent, mainly affecting the mandibular gingival mucosa, followed by the buccal mucosa, floor of mouth, tongue, retromolar mandibular area, lips, and palate. It can also be observed in the maxilla and edentulous areas. It may be a cause for biopsy when mistaken with melanosis, especially when there is no radiopacity. Metallic particles are radiopaque, thus facilitating the recognition of pigmentation. However, less than 25% of amalgam pigmentation are radiopaque since their metallic particles are very small or too dispersed to be visible in a radiographic study. In biopsy series, pigmentation is found in about 1.5% of all oral biopsies, giving an idea of the small number of cases in which they are biopsied. In population groups without biopsy studies, its presence is noted in 8.22% of the subjects, reaching 11.2% among women. As in our cases, this increased female incidence is attributed to the fact that the female population has a greater tendency to seek dental care and, therefore, a greater likelihood of exposure to dental restorative materials.

Amalgam consists of 3 phases: phase γ (SnAg₂), phase γ₁ (Hg₂Ag₂), and phase γ₂ (Sn₃Hg), with phase γ₂ being the most corrodbible. These phases have different degradation

**Figure 1** (A) Image of pigmentation in the gingival mucosa (lower left canine), of 2 months evolution. (B) Pigmentation in edentulous gum, of 5 months of evolution, near to 2 molars with amalgam restoration.
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Figure 2  (A and B) Overview of oral biopsies showing pigment deposits in the corium (HE, 15×). (C) Fine granular deposits arranged in rows on the mucosal corium (HE, 400×). (D) Large, deep submucosal pigment deposit, surrounded by a dense fibrous capsule, also pigmented (HE, 200×). (E) Two granulomas with multinucleated giant cells in proximity to a muscular plane, with presence of large, black deposits (HE, 200×). (F) Striated muscle fibres with pigmented contours of fibrous sheaths (HE, 200×).

patterns,19 so they do not contribute equally to pigmentation. Phase γ2 is degraded rapidly and does not participate in pigmentation. Phase γ1 degrades slowly, with loss of Hg. Finally, phase γ degrades slowest and is responsible for the pigmentation, persisting as Ag and sulphur particles, associated with basal membranes or conjunctive tissue.19 There is no Hg in the pigment and Sn is lost by corrosion. Therefore, only Ag18-20 in granular form, remains in the tissues and is responsible for pigmentation, as observed in our cases. As a cation, Ag becomes fixed to basal membranes, in collagen and elastic fibres and the perineurium,21 or may be phagocytosed by macrophages, being the only remaining component.11,18 Although all 3 phases are radiopaque, in the case of phase γ this radiopacity may disappear by fragmentation and dispersion after 1 year evolution.19 This explains the lack of radiopacity in many pigmentationsin8,13,14 and their possible confusion with melanosis.11-13

In our cases, the fine granular deposits were the most frequent, as has also been reported by other authors.20 Foreign-body type granulomas were observed in 1 case, with a similar incidence to that reported in the largest published series,8 specifically 13.4% granulomas. The presence of multinucleated cells was related to particle size, so only particles measuring 2.5–35 μm were phagocytosed by giant
Figure 3  (A) Discontinuous expression of metallothionein at the level of the basal layers of the mucosal epithelium, coinciding with granular deposits and in small, dense lumps of pigment material (metallothionein, 100×). (B) Expression of metallothionein at the level of the adventitial layer of small vessels (metallothionein, 400×). (C) Expression of HLA-DR marking intraepithelial dendritic cells, as well as subepithelial stromal elements and at the perivascular level (HLA-DR, 250×). (D) Immunoreactive stromal elements against CD117, identified as mast cells, at the level of the mucosal corium (CD117, 150×).

cells. This demonstrates a relationship between tissue response and particle size. We only observed a fibrous capsule in large deposits and this was established at 4 weeks of metal implantation, in relation to corrosion of phase γ2. It is interesting to note that, in patients with increased pigmentation diameter, we observed an event reported by other authors, consisting in perimysium pigmentation distant from other deposits.

Immunohistochemically, we verified an elevated number of CD117+ mast cells. Only 1 previous study noted this fact, indicating that this was independent of the size of pigment deposits. This finding is relevant, as certain heavy metals (Ag, Hg, Au) can influence the function and survival of mast cells, and can cause autoimmune reactions in predisposed individuals, which would explain the appearance of lichenoid reactions.

Other data included the overexpression of metallothionein and HLA-DR. Metallothionein is a ubiquitous protein involved in the detoxification of metals and free radicals. Our study, as well as others, found overexpression at the mucosal level, above the pigment deposits, and at the perivascular and stromal levels. However, we differ from those which only found immunoreactivity in macrophages near the deposits, correlating overexpression with residual Hg deposits. HLA-DR was overexpressed in intraepithelial dendritic cells, as well as at the perivascular level and in stromal elements. HLA-DR was expressed in antigen-presenting cells and its overexpression could be interpreted as a phenomenon induced by the metallic components in the context of pigment degradation processes.

Conflict of Interests

The authors have no conflict of interests to declare.

References

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