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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.

PALABRAS CLAVE
Amalgama;
Inmunohistoquímica;
HLA-DR;
Metalotionina

Estudio clínicopatológico e inmunohistoquímico de la pigmentación oral por amalgama

Resumen El tatuaje por amalgama, la pigmentación exógena oral más frecuente, puede en ocasiones simular lesiones melánicas y ser motivo de estudio biópsico. 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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a una infiltración por mastocitos (CD117+), así como a una sobreexpresión de metalotionina y HLA-DR a diferentes niveles tisulares.

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%[6] 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 pigmentation[8,9] 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 only 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 the 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.

Denomination	Type of antibody	Clone	Source	Dilution
CD-68	Mouse monoclonal Ab	KP1	Dakoppats®	RtU
CD-117 (c-kit)	Rabbit polyclonal Ab	Ab Poly	Dakoppats®	1/400
HLA-DR α-chain	Mouse monoclonal Ab	TAL.1B5	Dakoppats®	1/30
Metallothionein	Mouse monoclonal Ab	E-9	Dakoppats®	1/100

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