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# Expression of Mmp-20 in Dental Germs of Human Fetus

## Expresion de Mmp-20 en Gérmenes Dentales de Fetos Humanos

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**SUMMARY:** During experiments in animal studies, it has been observed that enamelysin (MMP-20) is expressed during tooth development in the late secretory stage of amelogenesis but not in the mature enamel. The aim of this research was to determine the location of MMP-20 in human tooth germs in the different structures of the enamel organ. The detection of MMP-20 was performed by immunohistochemistry in 20 specimens obtained from human fetuses. Immunostaining of MMP-20 was observed from the presecretor stadium in stellate reticulum and intermediate stratum and in the basal portion of ameloblasts in the secretory stage in stellate reticulum, stratum intermedium, secretory ameloblasts, odontoblasts and dental papilla. The results of this research show the location of MMP-20 in tooth germ development in humans and provides the foundation for future research about the process of dental organ formation.

**KEY WORDS: Enamelysin; Immunohistochemistry; Ameloblasts; Odontoblasts.**

## INTRODUCTION

The process of embryogenesis of the face and associated intraoral structures consists of a complex series of events highly integrated that include extensive cell migration, tissue interactions, cell growth and differentiation (Aguirre *et al*., 1997).

The oral ectodermal epithelium at the beginning is built up of two layers: a superficial of flattened cells and a basal of high cells, which are connected to the embryonic mesenchymal or connective tissue through the basal membrane (BM). Research about epihtelium-mesenchyma induction in cell cultures postulate that BM is an important factor in dental cell differentiation and organogenesis (Graveson *et al*., 1997; Lumsdem *et al*., 1987).

During tooth development, take place morphogenetic interactions between the epithelium covering the facial process (ectoderm) and the underlying mesenchyme. Morphogenesis involves differentiation of various types of dental cells, ectodermal epithelium originates the enamel, and the ectomesenchyme (which will originates dental papilla) forms the dentin-pulp complex, cement, periodontal ligament and alveolar bone (Orban *et al*., 1957).

The tooth buds follow in their evolution a series of stages that, according to their morphology, are termed: solid bud stage, cap stage, bell stage, stage of dental follicle and terminal or mature.

Dental enamel is chemically constituted by an organic matrix (1-2%), an inorganic matrix (95%) and water (3–5%) (Gomez de Ferraris *et al*., 2002). The dental enamel is initially formed by an organic matrix containing about 30% of protein. Ameloblasts produce two kinds of matrix proteins: amelogenin, a relatively homogeneous product, that constitutes approximately 90% of the secreted matrix of the enamel, and a heterogeneous group of non-amelogenin proteins such as tuftelin, ameloblastin, enamelin, metal proteinase, and serine proteinases, which makes the remaining 10%. Information about the composition, mechanism of action during mineralization and maturation changes in the enamel proteic matrix have been difficult to achieve since many of them are found only in small amounts and most undergo proteolytic processing following secretion.

Matrix metalloproteinases (MMPs) hydrolyze extracellular matrix components (Brinckerhoff & Matrisian, 2002; Chaussain-Miller *et al*., 2006). Enamelysin (MMP-20) is the enzyme mostly found in tooth formation, being only secreted by odontoblasts and ameloblasts, is expressed in the newly formed dental enamel, having as function to degrade amelogenin, the most abundant enamel matrix

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protein (Sulkala *et al*., 2002; Nagase *et al*., 2006; Ryu *et al*. 1999). In studies with experimental animals has been reported that MMP-20 is expressed during tooth development primarily in the late secretory stage of amelogenesis but not in the mature enamel (Bourd-Boittin *et al*., 2004; Papagerakis *et al*., 2008; Margolis *et al*., 2006; Kim *et al*. 2005).

In the present study, the localization of MMP-20 in different structures of the enamel organ in human tooth germs was determined.

## MATERIAL AND METHOD

**Obtaining of specimens**. Five fetuses between 14-22 weeks of age were analyzed; a macroscopic examination was performed on each specimen to check for any apparent malformation present. The specimen dissection was performed, obtaining five upper and five lower maxillas, which were divided into right and left for 20 test samples, which were fixed, in 10% neutral formalin.

### Preparation of specimens

**Immunohistochemistry.** The specimens were processed for paraffin embedding technique. Serial sections of five μm in thickness of the jaws were made. The samples were placed in a decalcifying solution of 10% ethylenediaminetetraacetic acid (EDTA) for 15 days, periapical radiographs were taken to check the decalcification progress, once obtained, the specimens were washed in 1% PBS for 30 min and embedded in paraffin.

Cross sections of 5 μm thickness were prepared and antigen retrieval was performed with 0.01 M sodium citrate with pH of 6.0 for 2.5 min in a pressure cooker. Blocking endogenous peroxidase was performed applying an enamelysin polyclonal antibody (Chemicon International, Billerica, MA, United States) diluted 1:50, using the developing system Dako LSAB HRP (Dako, Glostrup, Denmark).

Denmark and counterstaining with hematoxylin (Allen *et al*., 1994). Images were obtained with a microscope Axioscop 40 (Carl Zeiss, Jena, GmBH) equipped with an image analyzer (Image Pro Plus Cybernetics).

**Ethical considerations.** The Education, Research and Ethics Committee of the Zacatecas General Hospital, the institution that made the donation, provided permits for the use of human fetuses in this investigation.

The sample size calculation was conducted according to the Cummins and Hulley formula (Hulley & Cummins, 1988). Obtaining 20 samples to analyze.

Table I. General characteristics of the specimens.

| Specimens | Sex | Age (Weeks) | Weight (Grams) | Skull length – rachis (cm) |
|---|---|---|---|---|
| Fetus 1 | Female | 22 | 347 | 17 |
| Fetus 2 | Male | 20 | 208 | 14 |
| Fetus 3 | Male | 20 | 100 | 12 |
| Fetus 4 | Male | 14 | 125 | 12 |
| Fetus 5 | Male | 16 | 225 | 13 |

## RESULTS

In studies with experimental animals as rats and pigs has been observed that MMP-20 is expressed during tooth development primarily in the late secretion stage of amelogenesis but not in the mature enamel (Bourd-Boittin *et al*.). In the present study, 20 specimens from human fetuses were analyzed and general characteristics including sex, age and weight were observed (Table I), as well as the detection of MMP-20 at the different stages of tooth formation.

**Detection of MMP-20.** At the presecretor stadium a morphogenic and a differentiation stages were observed. In the morphogenic stage a slight immunostaining on stellate reticulum and on intermediate stratum of the enamel organ as well as in the basal portion of the ameloblasts was observed, no presence of MMP-20 was observed in the dental papilla (Fig. 1B). Furthermore, in the differentiation stage is observed a defined immunostaining in stellate reticulum, intermediate stratum, ameloblast, and dental papilla (Fig. 1D).

In the secretory stage a large presence of MMP-20 in stellate reticulum, stratum intermedium, secretory ameloblasts, odontoblasts and dental papilla is identified; MMP-20 was not observed in calcified dentine (Figure 2B).

In the maturation stage, a strong pattern of MMP-20 expression in secretory ameloblasts around enamel prisms, dentin enamel junction and odontoblasts was observed, along with a slight expression in predentine with an absence of MMP-20 in calcified dentine (Fig. 2D).

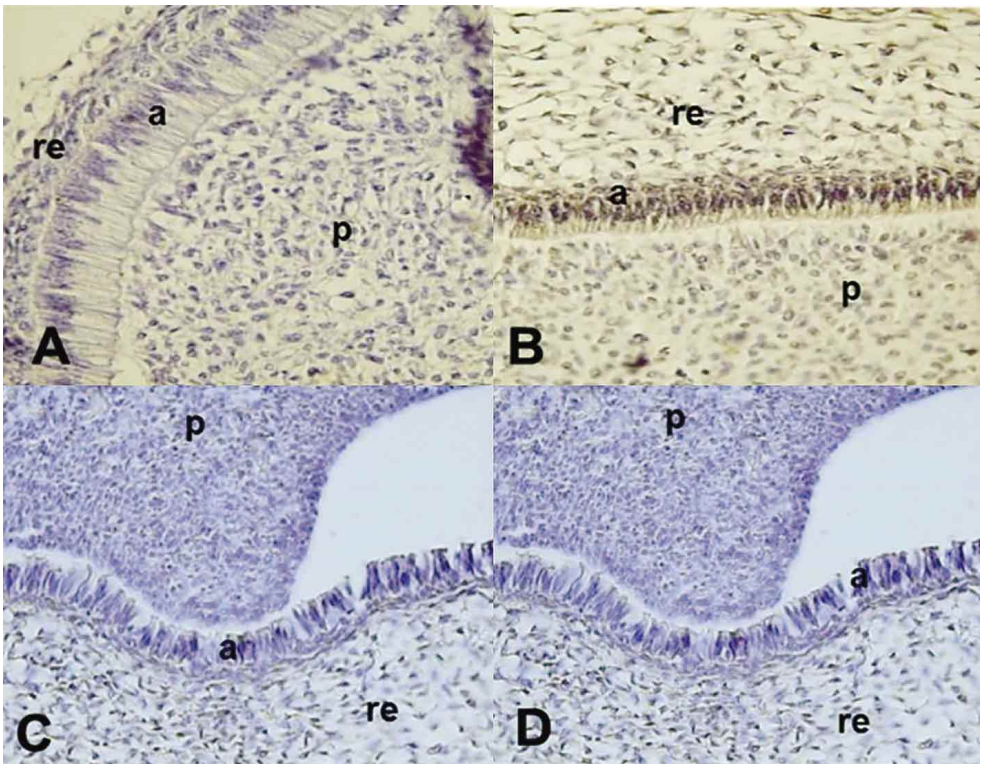


Fig. 1. Enamelysin immunohistochemistry labeling in human dental germs. Early bell stage, cells are in a presecretor stage. 600X. A and C, Staining with H–E. B and D. inmmunohistochemistry using. Anti-enamelysin (Chemicon International. Papilla (p); Stellate reticulum (re); Ameloblast (a). The inset shows the early bell stage, where organic matrix is not present.

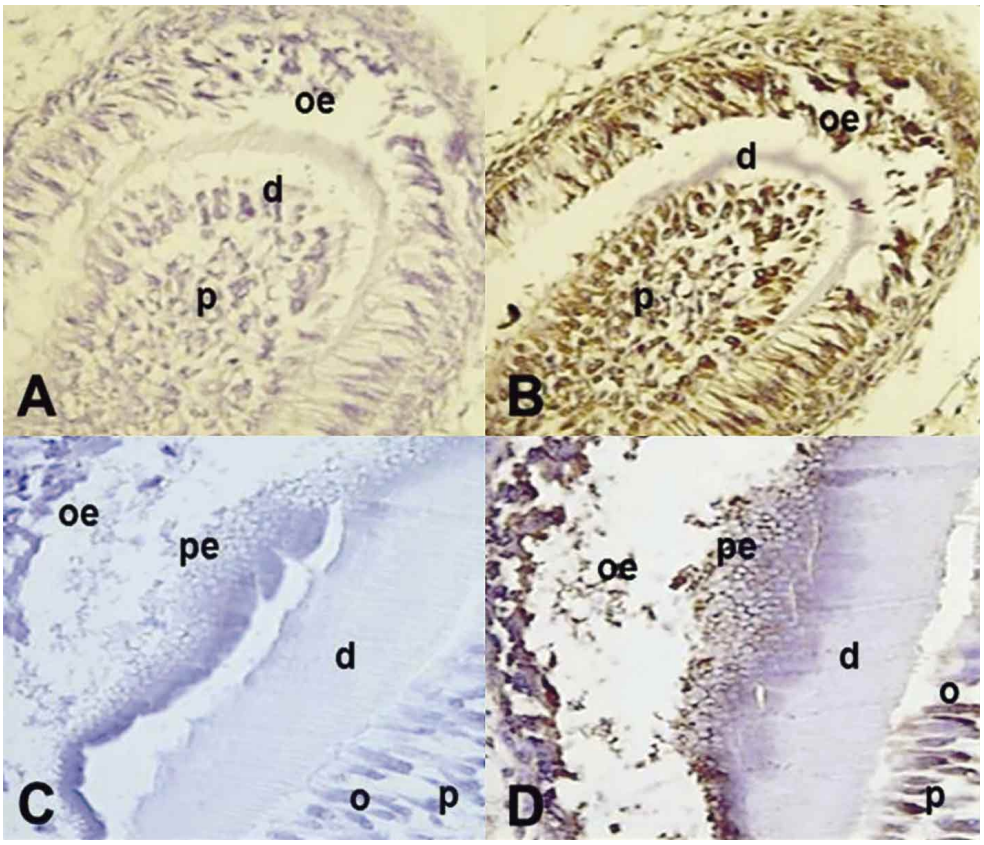


Fig. 2. Enamelysin immunohistochemistry labeling in human dental germs. Late bell stage. Confocal microscopy, X 600: A and C, Staining with H–E. B and D. inmmunohistochemistry using. Anti-enamelysin (Chemicon International. Papilla (p); enamel prisms (ep); enamel organ (oe). extracellular matrix of newly formed dentinl.

## DISCUSSION

Previous studies indicate the detection of MMP-20 in experimental animals during the secretory stage (Bourd-Boittin *et al*.; Caterina *et al*., 2002). In this study upper and lower jaws of human fetuses were used to identify patterns of localization of MMP-20 during the formation and mineralization of enamel.

MMP-20 has unique structural and enzymatic features enabling the degradation of amelogenin, which is the largest component of the organic matrix of the enamel and therefore is believed to play an important role during enamel development (Bourd-Boittin *et al*.). Our results agree with this approach since it was observed the presence of MMP-20 around the crystals or enamel prisms (Fig. 2D). The role of MMP-20 expressed by odontoblasts remain unknown (Bourd-Boittin *et al*.). In this study the expression of MMP-20 in the presecretor, secretory and ameloblast maturation stadiums, as well as into odontoblasts were observed. For this, one should consider that these cells have a different origin; epithelial to ameloblasts and cells derived from the neural crest to the odontoblasts and that each cell type produces different matrices, respectively enamel and dentin. In studies, using scanning electron microscopy and *in situ* hybridization was observed that enamelysin is secreted by ameloblasts and odontoblasts within enamel and dentin matrices (Bourd-Boittin *et al*.; Furkae *et al*., 1996).

In this work, it was noted that the papilla odontoblasts also express high levels of enamelysin, by the above; it is believed that the MMP-20 may be involved in the dentine formation process.

MMP-20 is expressed in functional odontoblasts during the initiation of predentine secretion, continuing after the mineralization of dentine in mature odontoblasts of mouse (Bourd-Boittin *et al*.). In this study slight expression of MMP-20 was observed from presecretor stadium during the differentiation stage (Fig. 1D) increasing its expression in secretory and maturation stage (Figs. 2B and 2D).

The presence of MMP-20 in odontoblasts and ameloblasts might indicate that pre-odontoblasts and pre-ameloblasts share the same basal membrane and degrade products trapped in the formation of dentin (Bourd-Boittin *et al*.). Our results showed slight expression of MMP-20 in the basal portion of the ameloblasts in the presecretor stadium during morphogenic stage (Fig. 1C), indicating the start of its discharge into the cell before the enamel organic matrix production, with subsequent presence of MMP-20 in odontoblast and immature dentin matrix, the above suggests its involvement in the formation of immature dentinal tissue (Fig. 2B).

## CONCLUSION

The results of this research show the localization of MMP-20 in development of human tooth germ and provides the basis for future research on the process of formation of dental organs. In summary, we suggest that MMP-20 can play an important role in the early development of the dental organ by creating protein cleavage products that are necessary for the formation of enamel and dentin.

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**RESUMEN:** En estudios realizados en animales de experimentación se ha observado que la enamelisina (MMP-20) se expresa durante el desarrollo dental durante el estadio de secreción tardío de la amelogénesis pero no en el esmalte maduro. El objetivo de la presente investigación fue determinar la localización de MMP-20 en gérmenes dentarios humanos en las diferentes estructuras del órgano del esmalte. Se analizaron 20 especímenes obtenidos de fetos humanos, efectuando la detección de MMP-20 por Inmunohistoquímica. Se observó inmunolocalización de MMP-20 desde el estadio presecretor en retículo estrellado y estrato intermedio, así como en porción basal de ameloblastos; en el estadio secretor en retículo estrellado, estrato intermedio, ameloblastos secretores, odontoblastos y papila dental. Los resultados de la presente investigación muestran la localización de la MMP-20 en el desarrollo del germen dentario en humanos y aporta las bases para futuras investigaciones acerca del proceso de formación de los órganos dentales.

**PALABRAS CLAVE: Enamelisina; Inmunohistoquímica; Ameloblasto; Odontoblasto.**

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