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Original Article

Immunohistochemical evaluation of inducible nitric oxide synthase (iNOS) expression in the dental follicle, follicular cyst, and calcifying cystic odontogenic tumor

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ABSTRACT

Objective: The calcifying cystic odontogenic tumor (CCOT) is a benign cystic neoplasm with a variety of histopathological and clinical features in comparison with the follicular cyst. iNOS is an enzyme that produces free radicals and is a mediator regulator of the inflammatory response. It has been implicated in tumorigenesis as well. The aim of this study was to compare iNOS expression in the dental follicle and two odontogenic lesions with different aggressive behaviors.

Methods: In this cross-sectional study, 44 paraffin blocks were selected from the dental follicle, follicular cyst, and CCOT, and immunohistochemical staining was done by iNOS. The percentage and intensity of staining (total score) were calculated in the cytoplasm of the epithelial cells.

Results: The highest total score of iNOS staining was found in the cytoplasm of the epithelial cells of CCOT when compared to the follicular cyst and dental follicle ($p = 0.000$). There was a significant difference in the final score between the follicular cyst and dental follicle ($p = 0.001$).

Discussion: The overexpression of iNOS in the CCOTs plays a role in its pathogenesis and iNOS, because of producing free radicals and damaging the oral tissue, may contribute to more aggressive behaviors of CCOT.

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1. Introduction

The calcifying cystic odontogenic tumor (CCOT) is a relatively rare lesion. It was previously known as the calcifying odontogenic cyst (the Gorlin cyst). Due to diverse clinicopathological appearances and the various neoplastic potentials, there is still disagreement on its terminology and also whether to classify CCOT as a cyst, neoplasm, or a combined lesion. Treatment varies on the basis of histopathological type from surgical enucleation to more aggressive treatments because of its risk of recurrence.[1,2]

A dentigerous cyst (follicular) is the most common developmental odontogenic cyst that covers the crown of the impacted tooth and is asymptomatic in most cases if it is not infected or inflamed. Treatment includes enucleation of the cyst and extraction of the involved tooth. It is reported that the dental follicle may transform to the dentigerous cyst.[3,4]

In the literature, little is known about the pathogenesis and biological growth process of CCOT. It has an aggressive behavior when compared with the follicular cyst and dental follicle.[5]

Nitric oxide synthase is a member of the family of enzymes that catalyzes the production of nitric oxide from L-arginine. Nitric oxide has an important role in physiologic and pathologic events and is active as a short-effect toxic gas. It induces free radicals formation and might enhance angiogenesis, which can lead to an accelerated growth of the primary tumor and metastasis.[6] Overexpression of unregulated NO has been implicated in pathologic conditions. Three isoforms of nitric oxide synthase include nNOS (neural), iNOS (induced), and eNOS (endothelial). iNOS has an important role in inflammatory and neoplastic events and its production is increased by inflammatory mediators of inflammatory cells.[6,7] The iNOS expression has been studied in some cysts.[8,9] In one study, the expression of iNOS in the dental follicle and follicular and radicular cyst was reviewed, which showed no significant difference. iNOS induces bone resorption and expansion of the cysts, which may be due to overexpression of matrix metalloproteinase.[8] There is considerable controversy over understanding its role in tumorigenesis.[10]

It is believed that nitric oxide in low concentrations in the initial stages of cancer has a role in cell proliferation, and its high density is cytotoxic for tumoral and normal cells.[11] The iNOS expression has been reported in benign and various malignant tumors and some researchers suggest NO may play a main role in tumor growth, progression, and metastasis.[12] There are no articles in the literature comparing the expression of iNOS in the dental follicle, follicular cyst, and CCOT. Therefore, the aim of this study was to compare and evaluate iNOS expression in the dental follicle and two odontogenic lesions with different aggressive features by the immunohistochemical method and to see whether there is any relationship between the aggressive behavior of odontogenic lesions and the expression of iNOS.

2. Methods

This cross-sectional study was approved by the Ethical Committee of Babol University of Medical Sciences. The study samples were 44 cases, CCOT (15 cases), follicular cyst (15 cases), and dental follicle (14 cases), which were obtained from paraffin-embedded section archives of the Pathology Department of Dental Universities of Babol and Mashhad. Sections (4 μm) were cut and counterstained with hematoxylin to make a definite diagnosis by two independent pathologists who were unaware of the clinical findings, and then, different histopathologic types of CCOT were classified according to Saghafi et al.[13]:

1. Simple cyst: only has a cystic epithelium similar to ameloblastoma with ghost cells.
2. Cystic neoplasm: consists of Gorlin cystic epithelium with ameloblastic nests in the connective tissue.
3. Solid neoplasm: consists of cystic epithelium of the Gorlin cyst with ameloblastic proliferation, and dentinoid and ghost cells in the connective tissue.
4. Combined lesion: Gorlin cyst accompanied by odontogenic tumors, such as ameloblastoma and odontoma.

Samples whose tissues were not adequate for evaluation or had inappropriate quality or fixation were excluded. Then, other 4-μm slides of paraffin-embedded blocks were obtained and stained using the immunohistochemical method with iNOS antibody. All moderate and severe inflamed CCOT and dental follicle samples were deleted. The follicular cyst was classified as inflamed (8 cases) and noninflamed (7 cases).

The specimens of paraffin-embedded blocks were first deparaffinized with xylene and then rehydrated in decreasing concentrations of ethanol and covered with sodium citrate buffer (pH 6) for 5 min. They were then transferred to microwaves (Bhutan) for antigen retrieval. The endogenous peroxidase activity was quenched by incubation in 3% peroxidase-blocking solution so that it covered all the tissues. The tissues were then incubated with the initial iNOS antibody (Rabbit Anti-Human iNOS, Abcam, UK. Cat Na: ab3523, 1/50) for 16 h at 4 °C and then with a secondary antibody for 30 min (Novolink polymer, Leica Microsystem Corporation. REF: RE7112, LOT: 6017103); Diaminobenzidine tetrahydrocloride (DAB) (DAB/Chromogen/DAB/Substrate buffer (kit), DAKO, USA) sections were counterstained with Mayer's hematoxylin for 30 s. Oral squamous cell carcinoma was the positive control and deletion of the primary antibody and its replacement with PBS and phosphate buffer was the negative control. The stained slides were reviewed and scaled independently by two pathologists using a light microscope (Olympus Bx41, Olympus Corporation, Tokyo, Japan). If there was any disagreement between the two pathologists, a third pathologist evaluated the slides and reported the final results.

In the study, the percentage and intensity of staining was considered and buffy-colored cytoplasmic staining was considered positive for the iNOS marker.[14]

3. Assessment of immunohistochemical staining

Microscopic iNOS-stained slides were evaluated at (100×) magnification, and in the fields with the highest staining, evaluation of 100 cells by (400×) magnification was done in