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Coincidence of Expression of Syndecan 4 with VEGF in Pulp Tissue of Mesiodens

Mukhaled L Ali[1*], Abdul Karim A Al-Mahammadawy[2], Noor Natik Raheem[2], Athraa Y. Al-Hijazi[2]

[1]College of Dentistry, Babylon University, Babel, Iraq
[2]College of Dentistry, Al-Mustansiriya University, Baghdad, Iraq

ABSTRACT

Background: Supernumerary teeth can be found in almost any region of the dental arch Mesiodens, is the most frequent supernumerary tooth present in the region of maxillary central incisor and most of the times they are asymptomatic. Syndecan-4 is a proteoglycan receptor, act as central mediator of cell adhesion, migration, proliferation and mechanic-transduction.
Aim of the study: This study explores the coincidence of the expression of Syndecan-4 and Vascular Endothelial Growth Factor (VEGF) in pulp tissue of mesiodens in comparison to primary central incisors.
Materials and Methods: Twenty human mesiodens samples and ten healthy primary central incisors teeth extracted (at shedding time) for children aged (6-15 years) are examined for Immunohistochemical localization of Syndecan-4 and VEGF.
Results: Results show highly significant difference for positive pulp cells that expressed Syndecan-4 and VEGF by mesiodens in comparison to primary teeth. Present findings also illustrate positive role and coincidence of intense expression of VEGF with strong expression of Syndecan-4.
Conclusion: Vascular endothelial growth factor (VEGF) has possible involvement in Syndecan-4 expression.

Key words: Mesiodens, Vascular endothelial, Syndecan-4, Dental pulp

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Corresponding author: Mukhaled L Ali
e-mail✉: isamhtk@hotmail.com
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INTRODUCTION

Mesiodens is supernumerary tooth located at midline, commonly seen in the maxillary arch. It is considered as the most significant dental anomaly that affects permanent teeth mainly as well as primary teeth. It may be associated with a syndrome or may occur as an isolated dental anomalous condition [1]. Many theories have been suggested to explain its etiology. One theory suggests of dichotomy of the tooth bud. Another theory, suggests that it is formed as a result of local, independent, conditioned hyperactivity of the dental lamina. Morphologically, mesiodens may be of three types: the most commonly seen is conical, more than tuberculate type [2,3].

Supernumerary tooth provides an excellent model to use for determining the factors/processes regulating signaling and cell differentiation [4]. Some adhesion molecules characterized to have an active role in controlling cell differentiation, like Syndecan that bind to fibroblast growth factors (FGFs) and vascular endothelial growth factors (VEGFs), bring them to the receptor on the same cell [5,6]. Any change in certain proteoglycan could cause developmental defects, like disordered distribution or inactivation of signaling molecules; in contrast over-expression of Syndecan-4 may enhance signaling.

Syndecan-4 has a molecular weight of ~20 kDa and considered the best-plasma membrane proteoglycan. Their intracellular domain of membrane protein interacts with actin (cytoskeleton) and signaling molecules in the cell membrane [7]. They interact with fibronectin on the cell surface, and signaling proteins inside the cell to modulate the function of integer in for cell-adhesion. Syndecan are normally found on many cell surfaces like, fibroblasts and epithelial cells [8].

The present study is designed to explore the expression of Syndecan-4 in pulp tissue of mesiodens and primary teeth and to identify the potential role of VEGF focusing in particular on its possible involvement in Syndecan expression.

MATERIALS AND METHODS

The study population consisted of thirty (30) male children who visited the Private clinic. Ranged in age

from (6-15) years. Supernumeraries are detected by clinical examination and radiographs using IntraOs 70 X-ray Equipment, (21 CFR 872-1800), Bluex, Italy.

Twenty human mesiodens samples and ten healthy primary central incisors teeth extracted (at shedding time) are included in this study.

Teeth were fixed in 4% Para-Formaldehyde (PFA) in Phosphate-buffered saline (PBS) for 48 h at 4°C. After fixation teeth were dematerialized in acetic acid 0.1 N in 0.5% PFA in PBS for 7 days and then washed with PBS for 4 h.

Teeth were then dehydrated and embedded in paraffin wax. Ten serial sections (4 µm thickness) were prepared, five sections for hematoxylin and eosin stain other five mounted on positively charged slides and stored in air-tight boxes at 4°C until immunohistochemistry [9].

Immunohistochemistry on tissue sections

The Anti-Syndecan-4 antibody (ab74139), anti-VEGFA antibody (ab46154) from ABCAM, company (USA) are used. Preparation and characterization of these monoclonal antibody has been described and verified according to the manufacture data sheets.

Positive peroxidase staining produces brown color on light microscopy. The percentages of positively stained cells were counted at 5 representative fields (40X).

RESULTS

Pulp tissue for primary central incisor shows displacement of odontoblast with pulp cell degeneration (Figure 1), while pulp of mesiodens shows well organized odontoblast with numerous blood vessels (Figure 2).

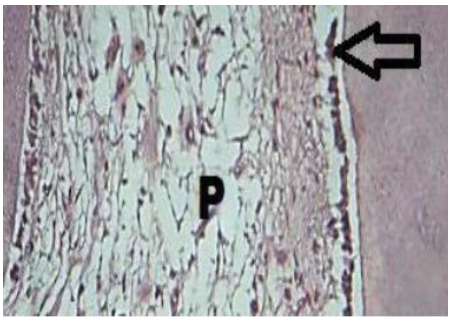

Figure 1: Pulp tissue of primary central incisor, arrow=odontoblast; P=pulp; H&E; 20X

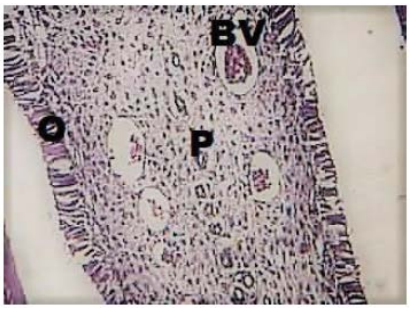

Figure 2: Pulp tissue of mesiodens P=pulp; BV=blood vessel; O=Odontoblast; H&E; 10X

Immunohistochemical investigation for localization of VEGF and Syndecan-4 by Pulp tissue of primary central incisor show faint, weak immune-reaction as illustrated in Figures 3 and 4.

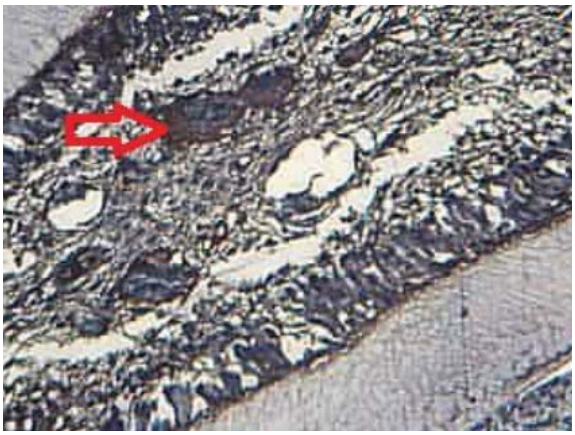

Figure 3: Imunohistochemical view VEGF in primary tooth arrow=endothelial cell; DAB; 20X

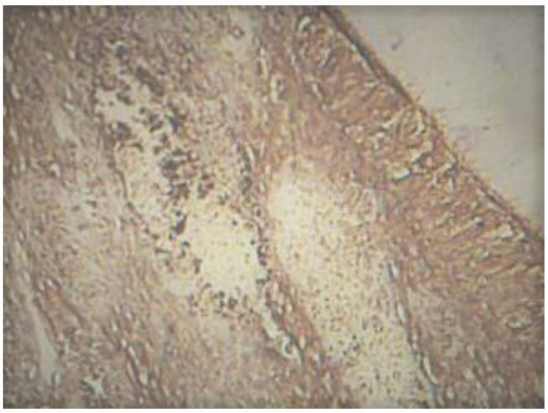

Figure 4: Immunohistochemical Syndecan-4 in primary tooth; DAB; 20X

Immunohistochemical investigation for localization of VEGF by endothelial cell and Syndecan-4 by fibroblast, odontoblast, mesenchymal cell and defense cells of Pulp tissue of mesiodens show intense, strong positive immuno reaction as illustrated in Figures 5 and 6.

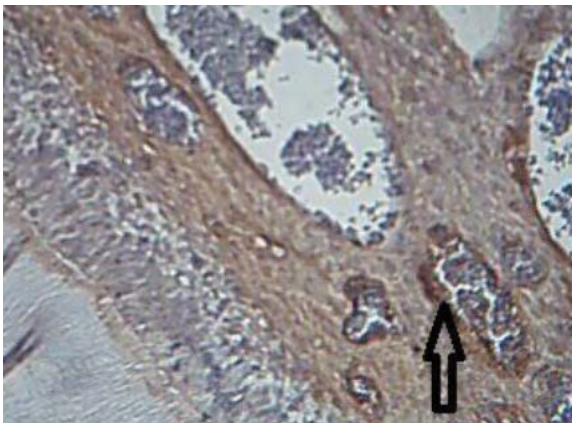

Figure 5: Immuno-localization view of VEGF by mesiodens arrow=endothelial cell; DAB; 40X

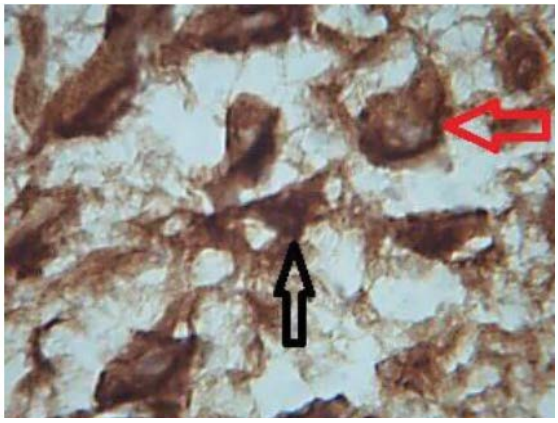

Figure 6: Immuno-localization view of Syndecan-4 by mesiodens; red arrow=mesenchymal cell; black arrow=fibroblast; DAB; 40X

Analysis of variance (ANOVA) is an analysis tool used in statistics that splits the aggregate variability found inside a data set into two parts: systematic factors and random factors. According to ANOVA test of groups differences, high significant values for differences in the mean of the number of pulp cells that expressed positive immuno-reaction for Vascular Endothelial Growth Factor (VEGF) and Syndecan-4 between primary (control) and mesiodens teeth, are illustrated in Tables 1 and 2.

Table 1: Analysis of variance (ANOVA) test for positive pulp cell expressed VEGF in primary and mesiodens teeth

Groups	Sample No.	Positive cell % expressed VEGF				
		Mean	S.D.	S.E.	F-test	P-value
Primary	10	5.5	0.35	0.13	27.70	0.000*
Mesiodens	20	10.5	1.5	0.52		
*Highly significant						

Table 2: Analysis of variance (ANOVA) test for positive pulp cell expressed Syndecan-4 in primary and mesiodens teeth

Groups	Sample No.	Positive cell % expressed Syndecan-4				
		Mean	S.D.	S.E.	F-test	P-value
Primary	10	5.25	0.79	0.30	24.50	0.000*
Mesiodens	20	13.5	1.95	0.73		
*Highly significant						

DISCUSSION

It was stated that secretion of growth factors and presences of extracellular matrix molecules provide molecular signals for the proliferation of dental pulp tissue that controls tooth development during its formation, deposition of dental structure and it's maturation [10].

Present study shows that Syndecan-4 is distributed in distinct areas of mesenchyme pulp tissue for both primary and mesiodens tooth indicated that Syndecan-4 is involved in the signaling of odotogenesis process that correlates with proliferation of mesenchymal cells which is necessary for normal progression of tooth development. These results coincide with previous studies [11].

Once more, strong expression of Syndecan-4 in mesiodens revealed that pulp fibroblast cell is associated with intensive proliferative activity. In addition, increased expression by undifferentiated mesenchymal cell which act as precursors for other specialized cell indicated that Syndecan-4 may play an additional role in differentiation events [12]. Moreover, intense expression in these specialized dental cells suggests its implication in self-renewing, spread and stimulate stem cells to differentiate to be functional differentiated cells during tooth homeostasis and repair.

In contrast, decrease of Syndecan-4 expression in dental pulp cells of primary teeth may correlates with the progression of mesenchymal cell differentiation, and cell activity, especially teeth were in shedding time.

And as Syndecan-4 has a biological function includes cell growth regulation, differentiation, and adhesion which isn't being more needed during shedding mechanism.

Vascular endothelial growth factor (VEGF) is the most important essential mediator for angiogenesis that is secreted by many cell types and illustrates to be involved in hematopoietic development and chemo-taxis for other cells. VEGF also act as signaling molecules which is important to regulate stem cell fate and function [13].

Present study reported strong expression of VEGF by endothelial cell and mesenchymal cell of pulp tissue of mesiodens with simultaneous presence of Syndecan-4, which may involve in its activity and could modulate its function [14].

Moreover, although Syndecan-4 is constitutively expressed by several cells but its expression is also induced during many biological phenomena as tissue regeneration and during organ development and growth factors have been the prime candidates to induce Syndecan-4 expression in these situations [15]. In our results Syndecan-4 expression varies in mesiodens when compared with primary teeth, similarly, expression of VEGF coincide with Syndecan depending on different biological sequence [16-18].

In conclusion, the present results highlight the important and dynamic nature of Syndecan-4 during the biologic tooth life. These results suggest that there is a close relationship between VEGF and Syndecan-4 and concerning the potential role of VEGF focusing in particular on its possible involvement in Syndecan expression.

CONFLICT OF INTEREST

The authors' declares that they have no conflict of interest.

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