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ALPHA- SMOOTH MUSCLE ACTIN EXPRESSION IN THE HUMAN DENTAL PULP TISSUE OF MOBILE TEETH

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ABSTRACT

Tooth mobility is a degree of tooth looseness in its bony socket. A slight degree is accepted as a normal physiologic mobility of all types of the teeth in the oral cavity. During the tooth mobility due to different causes several biological processes take place, during these biological processes the dental pulp tissue is subjected to structural and protein expression modifications in order to maintain their integrity and functional morphology. The aim of this study is to evaluate Alpha- smooth muscle actin expression in the human dental pulp tissue of mobile teeth. Method: Acrosssectional study of 66 extracted teeth with their pulp tissue (49 with mobility and 17 as non-mobile control group); the samples were processed, and the immunohistochemical reaction of the Alpha- smooth muscle actin (α-SMA) marker for each sample were analyzed. Results: Among the mobile teeth group (29) were with mild to moderate mobility, (20) with sever mobility. The Alpha- smooth muscle actin (α-SMA) positive results were obtained in (7) samples of the teeth with sever mobility and (21) samples of teeth with mild to moderate mobility were compared to (9) positive cases of control group. Conclusion: The data demonstrate that the dental pulp tissue does not expresses any irreversible histological alterations with mild to moderate teeth mobility compared to the irreversible changes that obtained in the pulp tissue of the sever mobile group. In conclusion the type of treatment required for the mobile teeth will depend on the responses of the pulp and the supporting periapical tissues to determine the outcome of the involved teeth sequel.

KEYWORDS: Dental pulp tissue, Tooth movement, Immunohistochemistry, Alpha-Smooth muscle actin.

INTRODUCTION

Tooth mobility is a degree of tooth looseness in its bony socket. A slight degree of teeth mobility is accepted as a normal physiologic mobility of all types of the teeth in the oral cavity [(15)]. Dental pulp is composed of mucous connective tissue which is rich in blood vessels and nerves entering the pulp chamber from the periodontal ligament (PDL) through the apical foramen [(9)]. The pulp tissue has its main neurovascular supply through the apical foramen. any kind of trauma from apical area may cause severance of the apical blood supply, when the tooth has been chronically moved in the alveolar socket from its normal resting position will leading to inflammatory response that assessing the pulp tissue healing. The pulp tissue cells involved in the activation and repair process of both pulp and periodontal tissues [(2)]. The periodontal tissues composed of connective tissue rich with blood vessels and bundles of collagen fibers, these periapical tissue shows histological changes during tooth mobility, and should not consider each tissue response including pulp and periodontal tissue as a final isolated response to the effects of mobility forces. A better understanding of the cellular events and pulp tissue responses to the mobility of the teeth is mandatory to facilitate the tissue regeneration that may changes the priority of the treatment plane steps for patients with mobile teeth in the future [(16)]. Actins are proteins involved in the intracellular transport, integrity, and, cell motility [(8)]. The α-SMA is commonly used as a marker of myofibroblasts formation typically in wound healing. After tissue injury, fibroblasts differentiate into contractile and secretory myofibroblasts that contribute to tissue repair during wound healing [(1)].

AIMS OF THE STUDY

1- This study will localize the α-SMA expression in the pulp tissue of mobile teeth by immunohistochemical (IHC) techniques.

2- To compare the expression of the α-SMA in the pulp tissue of the mobile teeth groups to the non-mobile teeth group.

3- To evaluates the expression of the α-SMA among the mobile teeth groups.

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MATERIALS AND METHODS

The teeth were extracted (with their pulp tissues) for prosthodontic and pain relive purposes and collected from different private dental clinics and the dental health center of Duhok/ Iraq. Sampling in this study was approved by the Ethical Committee of Duhok Director of General Health Center.

Teeth were diagnosed clinically and radiographically to determine the periodontal diseases and the degree of mobility before teeth extraction. Pulp tissue sample from 66 extracted teeth were obtained.

The dental pulp tissues were fixed in 10% formalin and histopathological processed into formalin fixed paraffin embedded (FFPE), sectioned with 4 microns' thickness and stained by hematoxylin and eosin (H and E) stain. The IHC staining protocol was performed for α-SMA (Monoclonal Mouse Anti-Human Actin (HHF35 0.08 mol/EDTA) according to the avidin-biotin complex (ABC) detection system. Primary and secondary antibody kits were used, provided by the DAKO Company detected with the EnVision+ system that employs peroxidase-labeled polymer conjugated to anti-mouse immunoglobulin antibodies. Immune complexes were identified by using the peroxidase reaction with DAB+ as chromogen (EnVision+ detection system, K4006, Dako Corp, Carpinteria, CA) [5].

Statistical Analysis Data were analyzed using the statistical software for Windows version 17.0 (SPSS), in which the crosstab was applied to analyze the statistical significance of the data when applicable. The critical level of significance was set at $p< 0.05$.

RESULTS

From 66 teeth 29 was considered as moderate mobile teeth group whereas 20 teeth considered as severely mobile teeth group, the remaining 17 were teeth from the control group.

The results of the relation between the α-SMA results, the mobility of the teeth group and the IHC analysis for the α-SMA marker showed greater expression in moderately mobile teeth as seen in Table 1 out of the 21 are positive (about 77.1% which represents 69.3 of all mobilt teeth) and low in both severely mobile teeth and control group. The α-SMA expression as shown in figure 1 was high around the blood vessel cells. These result are statistically significant.

Table (1): - The frequency and the relation between the α-SMA results and the mobility of the teeth group is seen in

	Tooth mo bility		α-SMA	
			Positive (+ve)	Negative (-ve)
No mobility	17	40.9	9	8
moderate mobility	29	43.9	21	8
sever mobility	20	15.2	7	13
Total	66	100.0	37	29

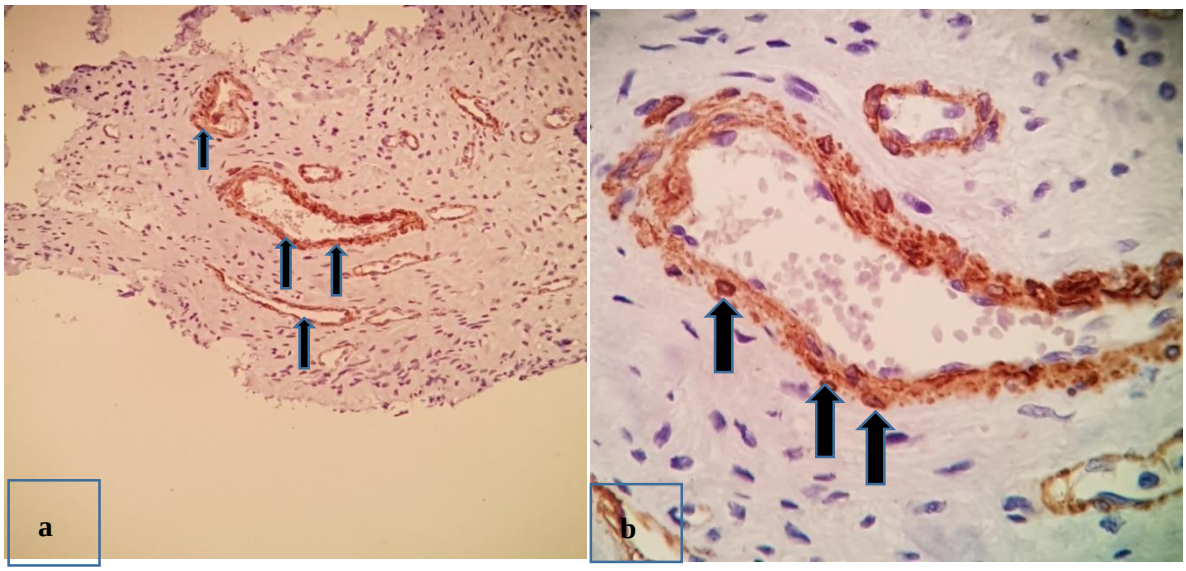

Fig. (1): - The IHC markers expression in tooth pulp tissue: brawn stain- black arrows; a: perivascular cells with α-SMA expression in pulp tissue samples of moderately mobile teeth (10X); b: perivascular cells with α-SMA expression (40X).

DISCUSSION

The highly mineralized enamel and dentine layers of human teeth constitute a protective barrier prevent the pulp soft tissue from different external stimuli and microbial invasion, this protection may be affected by progressive demineralization of the enamel by the acids that are released from certain bacteria when placed in a sugar-rich environment or the pulp affected by bacterial invasion from the periapical tissues as in case of chronic periodontal diseases which subsequently triggers inflammatory responses in the dental pulp [10]. For these reasons additional studies, which target the molecular interactions within the periapical tissue are required as these may identify novel clinical therapies for dental tissue repair. In this study the patients presented with mobile teeth and pain, this may reflect the delay in attending dental clinics for chronic periodontal diseases. The

IHC analysis for α-SMA found statistically higher positive results in mobile teeth group than the control group [13]. However, the significant values was seen in moderately mobile teeth results but not in sever mobile teeth group, the presence of dental caries may be the cause of high expression of the α-SMA around the perivascular cells as a result of the repair and regenerative response of the pulp tissue to the inflammation and bacterial invasion [12]. The current study concentrated on the relation of positive results in α-SMA, with the severity of teeth movement,the IHC positive results for α-SMA were lower in sever mobility than the moderate mobility. This can be explained by the fibrosis of the pulp tissue in the severely mobile teeth as are of old age with increase the fibers content of the pulp and decrease of the cellularity this agree with the [2]. The α-SMA positive expression in this study was significantly higher in mobile teeth than the control group. The statistically high positive results for α-SMA obtained in the teeth with moderate degree of mobility that reflect the importance of this medical problem. The unexpected low positive results for severely mobile teeth may be due to that the healing process is becoming more chronic and the myofibroblasts continue to regulate the connective tissue remodeling [7,11]. Since the α-SMA has more relation with the healing and remodeling which continue simultaneously with the injury and inflammation [14], therefore the expression of α-SMA is observed in both the pericytes and smooth muscle cells of blood vessels as indicated by [3]. In the current study, the α-SMA positive expression was frequently seen in the arterioles and capillaries of the dental pulp. More studies dealing with the treatment of periodontal disease that elicit more destructive inflammation, to get the best options for pulp tissues preservation [6]. This study need further analysis for other inflammatory molecules that

might be beneficial to incorporate more effective and useful therapeutic regenerative potential of the periapical and dental pulp tissue.

CONCLUSION

The inflammatory process occurs in the soft tissue supporting the teeth and the dental pulp, yet the degree of the histological changes in relation to teeth mobility is seldom studied in researches. A better therapeutic protocol may be obtained from the research activity in the area combined with the clinical translational approaches this study which reflect the host defense and repair events that occur within and surround the teeth roots.

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