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The Role of Epithelial Mesenchymal Transition Process in Inflammatory Gingival Hyperplasia

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ABSTRACT

Background: Gingival enlargement, the currently accepted terminology or an increase in the size of the gingiva. Local and systemic factors influence the gingival conditions of the patient. These factors result in a spectrum of diseases that can be developmental, reactive and inflammatory to neoplastic. Inflammatory gingival enlargement is the most common one; it is an inflammatory restraint to local irritant correlating with the gingiva.

Material and method: The study involved 15 tissue blocks of inflammatory gingival hyperplasia taken from the archives of oral pathology, laboratory of oral diagnosis department, collage of dentistry/university of Baghdad. Immunohistochemical expression of Vimentin, E-Chdaherin and α-SMA was assessed.

Results: Vimentin and E-Chadherin immunoreactivity of the connective tissue fibroblasts showed 100% positivity, while Alpha-smooth muscle actin staining was mostly seen in the endothelial lined blood vessels with a few myofibroblast with in the connective tissue being stained positive. In this article we sought to investigate if the Epithelial mesenchymal transition theory participitated in the advancement of this benign lesion.

Key words: Gingival hyperplasia, Traditional treatment, Cytoplasmic expression, Myofibroblast

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INTRODUCTION

Inflammatory gingival hyperplasia is an inflammatory restraint to local irritant correlating with the gingiva; the irritant could be microbial like plaque and calculus. Clinically present as deep red or bluish, considerably friable and fine with smooth glossy surface and commonly bleed easily [1]. Histologically, inflammatory gingival enlargement characterized by thicking of the epithelium with increased volume of the connective tissue with different degree of inflammation and fibrosis [2]. Gingival overgrowth usually treated with traditional periodontal treatment such as scaling and root planning, but if it include significant fibrotic component that don't respond to the traditional treatment so it will be treated by surgical removal of the excess tissue [3].

EMT process is one of the diverse theories attempted to explain the mechanism of advancement of this benign lesion. EMT is a process in which epithelial cells migrate in to the connective tissue and trans-differentiate into fibroblast-like cells, this occurs as the epithelial cell-cell and cell- extracellular matrix interactions are destabilized [4]. In this regard, Vimentin is the most abundant and highly conserved type III intermediate filaments protein. It is mainly expressed in cells of mesenchymal origin and it is often used as a marker for epithelial mesenchymal transition. Vimentin has an important role in adhesion and cell-cell interaction through their association with hemidesmosome and desmosome [5]. E-Cadherin is considered as a prototypical epithelial marker of EMT. The reduction in epithelial expression of E-Cadherin also called the Cadherin switch has been known to promote EMT by facilitating weakening of the intercellular junctions and promoting movement of epithelial cells towards the connective tissue [6]. Alpha smooth muscle actin positive myofibroblast have been demonstrated in type 2 EMT [7].

MATERIALS AND METHODS

Study samples

The study involved 15 tissue blocks of inflammatory gingival hyperplasia were obtained from the archives of oral pathology, laboratory of oral diagnosis department, collage of dentistry/university of Baghdad from 1971 to 2018 after approval of the ethical committee. The specimen was taken surgically from different age range patient (from 13–60) years old, suffering from gingival hyperplasia caused by plaque accumulation as a result to poor oral hygiene.

Four micron serial sections for Immunohistochemistry were performed for each tissue blocks and staining was done according to the manufactures instructions using three primary antibodies (Table 1); Manufactured by Abcam (Cambridge, UK) and modified from manufactures datasheets in an assay dependent dilution.

Detection was carried out using Rabbit specific HRP/DAP Detection kit (Abcam, ab236469; 30 ml) that used for the detection of all primary antibodies. Negative controls were carried out on consecutive sections with omitting of the primary antibody resulting in no detectable staining.

Evaluation

Slides were observed and registrated with a light microscope (Olympus CH). All slides were scanned at low power (10x) to select 5 representative fields, visualized and scored with a 400x objective; a mean positive percentage was recorded and an experienced pathologists evaluated the slides independently with statistical correlation to assure acceptable agreement, the percentage of positive cells was scored semi quantitatively as shown in Table 2.

The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation and ranges. Categorical data presented by percentages. Independent t-test (two tailed) was used to compare the continuous variables between study groups accordingly. Chi square test was used to assess the association between markers score and study groups.

RESULTS

Immunohistochemical analysis

Table 3 shows the pattern of distribution of the study markers in samples analyzed. Regarding Vimentin; all samples of inflammatory gingival hyperplasia expressed vimentin, 6 out of 15 was score (3) and the other 9 samples score was 4. Figure 1 shows positive cytoplasmic expression

Table 1: Characteristics of the antibody used for the Immunohistochemical study.

Antibody	Source	Isotype	Specific reactivity	Dilution
Polyclonal anti-vimentin antibody	ab45939	IgG	Mouse, Rat, Rabbit, Human	1:700
Polyclonal anti-E-Chadherin antibody	ab15148	IgG	Human, Pig	1:50
Polyclonal anti-Alpha-smooth muscle actin antibody	ab5694	IgG	Mouse, Rat, Human	1:100

Table 2: Scoring system of the study markers.

Markers	Score (1)	Score (2)	Score (3)	Score (4)
Vimentin	Scattered spotty Staining	up to 25% of Cells positive	25% to 50% cells positive	more than 50% of cells positive
α-SMA	Scattered spotty Staining	up to 25% of cells positive	25% to 50% cells positive	more than 50% of cells positive
E Cadherin	<10% positive cells	10 to 20% positive cells	>20 to 50% positive cells	>50% positive cells

Table 3: Distribution of markers in study samples.

Markers	Inflammatory gingival hyperplasia	Mean ± SD
Vimentin		
Score (3)	6 (42.9)	52.26% ± 10.66%
Score (4)	9 (52.9)	
E Cadherin		
Score (3)	9 (52.9)	46.3% ± 12.4%
Score (4)	6 (46.2)	
Alpha-SMA		
Score (1)	9 (39.1)	23.4% ± 8.2%
Score (2)	6(75.0)	

of vimentin in the fibroblast cells of connective tissue (CT).

Regarding the the E-Cadherin; all samples of inflammatory gingival hyperplasia expressed E-Cadherin, 9 out of 15 was score (3) and 6 out of 15 score (4). Figure 2 shows positive cytoplasmic and membranous expression of E-Cadherin in fibroblast cells of CT and in the epithelial surface which use as internal control for E-Chadherin.

Regarding the α-SMA; all samples of inflammatory gingival hyperplasia weakly expressed α-SMA, 9 out of 15 was score (1) and 6 out of 15 score (2). Figure 3 shows positive cytoplasmic expression of α-SMA in myofibroblast cell of the CT.

DISCUSSION

The present study was carried out in an effort to determine if EMT operates in the pathogenesis of inflammatory gingival hyperplasia to serve as a source of fibroblasts. To confirm this mechanism, we investigate the Immunohistochemical expression of three specific markers assessing EMT mechanism namely α-SMA, Vimentin and E-Cadherin. Alpha-SMA is a putative myofibroblast marker. Since myofibroblasts are implicated in EMT induced fibrosis we sought to analyse α – SMA expression in the samples.

As mentioned previously, the mean number of myofibroblast positive alpha-sma was; 23.4%

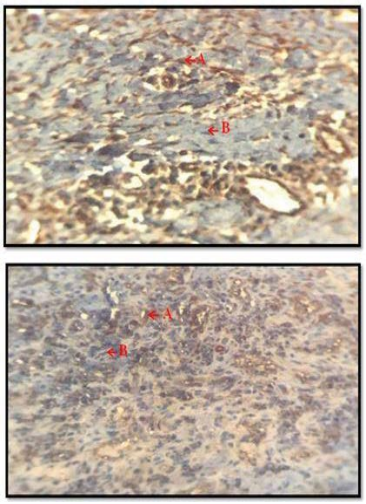

Figure 1: Section of inflammatory ginigival hyperplasia showing vimentin expression in fbroblast cells of connective tissue (40x) (A) Positive cytoplasmic expression of spindle cell fibroblast. (B) Negatively stained.

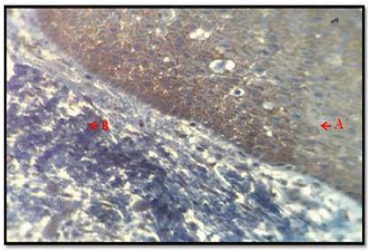

Figure 2: Section of inflammatory ginigival hyperplasia showing Echadherin expression in fibroblast cells of connective (40x). (A) surface epithelia (internal control for E-chadherin) positive membranous and negative cytoplasmic and nuclear staining. (B) positive membranous expression of spindle cell fibroblast.

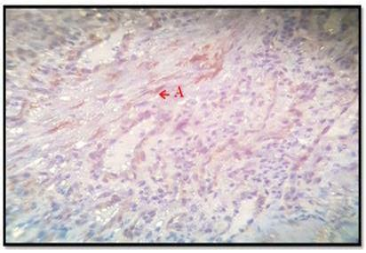

Figure 3: Section of inflammatory ginigival hyperplasia showing alpha smooth muscle actin expression in fibroblast cells of connective tissue (40x). (A) positive cytoplasmic expression of spindle cell fibroblast.

in samples analyzed, as the Alpha-sma is one of the cytoskeletal biomarkers for the epithelial mesenchymal transition process [8] so first of all this study suggested that EMT process may be participated at least partly in pathogenesis of inflammatory gingival hyperplasia and this result was in accordance with [9,10].

Regarding the weak expression of Alpha-sma in the study group which is the weakest expression compared with other two markers of this study (Vimentin and E Chadherin) this can be explained based on the concept that in the granulation tissue, fibroblast cells progressively become the majority population and takes myofibroblast phenotype, including expression of Alpha-actin [11] then these myofibroblast can long remain silent or disappear by apoptosis after wound healing. This probably the situation that occurred in case of gingival inflammation when the number of Alpha-sma positive cells was very low if not reduced to cells from the blood vessel wall [9].

E-Cadherin is required for the maintenance of normal intercellular adhesion and barrier integrity in oral tissues [12]. Vimentin is one of the most familiar members of intermediate filaments (IFs), as it is the major IF protein in mesenchymal cells and it is frequently used as a developmental marker of cells and tissues [13].

As mentioned previously; the mean number of vimentin& E-Chadherin positive fibroblast were 51.43% & 48.56%, respectively, so as these two markers are biomarkers for EMT, it is suggested that EMT process may be involved at least partly in the pathogenesis of inflammatory gingival hyperplasia. Similar result reported by [8,14,15] but it disagree with [12] who claimed that vimentin should not be considered as a marker of type 2 EMT in the setting of fibrosis.

Tumor growth factor beta one (TGF-B1) has emerged as a potent inducer for EMT, not only through SMAD pathway, but also through pathway not mediated by SMAD, so increased expression and activation of TGF-B1 in inflammatory gingival hyperplasia (14) promote an epithelial cell plasticity that may progress to EMT [15].

Regarding Vimentin; Previous study on renal fibrosis provide an evidence that fibroblast can form locally by EMT during the pathological stress of tissue fibrosis, in renal fibrogenesis about 36% of new fibroblasts come from local EMT, about 14-15% from bone marrow and the rest from local proliferation. This finding reinforce the notion that EMT play arole in the genesis of fibroblast during organ fibrosis in adult tissues [10,16]. And as vimentin is expressed in mesenchymal cells, so connective tissue fibroblast can be used as internal control for vimentin [17]. Therefore as a result to the implication of EMT process in the pathogenesis of inflammatory gingival hyperplasia, this will lead to the overproduction of connective tissue fibroblast which is vimentin positive.

Regarding E-Chadherin; Arora and other researchers try to investigate if the epithelial mesenchymal transition process operates in Cyclosporin A induced gingival overgrowth. They demonstrated that when primary human gingival epithelial cells were treated with TGF-B1 it induced reduction in barrier function as evidenced by reduced electrical resistance and paracellular permeability and simulantanously decreasing the expression of cell surface E-Chadherin [15]. Jeopardized E-Chadherin expression could alter the cell phenotype from epithelial to fibroblast with spindle shape morphology [17]. Okada H and coworkers have shown that the epithelial cells migrate from the epithelial layer, travel through the basement membrane and accumulate in the interstititium of the tissue; here they eventually get rid of their epithelial markers and gain a fully fibroblastic phenotype [18,19]. Mousa and co-workers have shown that TGF-B1 was higher in inflammatory gingival hyperplasia compared to normal gingiva [14].

CONCLUSION

Epithelial mesenchymal transition process in inflammatory gingival hyperplaia may be driven by elevated level of TGF-B1.

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