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Correlation Between the Immunohistochemical Expression Of Basic Fibroblast Growth Factor In Gingival Tissue With The Clinical Periodontal Parameters (Plaque Index, Gingival Index, Bleeding On Probing) And Angiogenesis.

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ABSTRACT

The objectives of this study were to investigate the Correlation between clinical periodontal parameters (PLI, GI, and BOP) with immunohistochemical findings (matrix FGF2 and angiogenesis) as well as Correlation between the expressions of matrix basic FGF and angiogenesis. A total of 44 subjects(15-30) years old with plaque- induced gingival overgrowth indicated for gingivec to my attending the clinics of the Department of Periodontics at the teaching hospital at College of dentistry - University of Baghdad / Iraq. At time of periodontal surgery, collection of data including: plaque index (PLI), gingival index (GI), and bleeding on probing(BOP) were performed. Gingival tissue were taken from patients undergoing conventional gingivectomy, washed by normal saline, and fixed with 10% formalin for immune his to chemical analysis of bFGF. There were negative correlations between immune his to chemical findings (basic FGF2 and angiogenesis) and clinical periodontal parameters (PLI, GI, and BOP).There was a significant positive correlation between basic FGF and angiogenesis. Basic fibroblast growth factor (bFGF) is important soluble mediator participate in tissue growth during the formation of granulation tissue that takes an essential role in angiogenesis.This adverse correlation may indicate that after scaling and polishing and with the removing of periodontal pathogenic bacteria, inflammatory processes were reduced and healing processes had been started.

Keywords: basic fibroblast growth factor, angiogenesis, plaque index, gingival index, bleeding on probing.

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INTRODUCTION

In normal tissue, basic fibroblast growth factor (bFGF)(which also known as FGF2) is found as membrane-bound in basement membranes and in the sub-endothelial extracellular matrix of blood vessels as long as it is not activated. It has been theorized that, in the course of both wound healing of normal tissues and tumor development, bFGF is activated by the action of heparan sulfate-degrading enzymes, thus facilitating the formation of new blood vessels by process known as angiogenesis. [1]

FGF-2 acts on various cell types as it promotes proliferation of fibroblasts and osteoblasts, and enhances angiogenesis. These activities are directly associated with periodontal tissue regeneration. However, periodontal ligament cells are the key players during periodontal tissue regeneration. To reveal the molecular and cellular mechanisms by which FGF-2 induces periodontal tissue regeneration, various IN VITRO experiments were conducted in which the effects of FGF-2 on periodontal ligament cells were examined. Experiments showed that periodontal ligament cells express FGFR1 and FGFR2 mRNA. [2] In contrast, gingival epithelial cells express mRNA of FGFR 1, 2, 3, and 4. [2]the responsiveness to FGF-2 is higher in undifferentiated periodontal ligament cells than in mature periodontal ligament cells.

In a study performed by Shirakata*et al* (2010) [3] to investigate the regenerative effect of basic fibroblast growth factor on periodontal healing in two-wall intrabony defects in dogs in 8 week follow up, using EMD and PDGF/*β*-TCP serving as benchmark controls, they found that there is a significant increase in bone formation in bFGF group as compared to EMD where as there is no significant difference between bFGF group and PDGF/*β*-TCP group.

In another study conducted in 74 patients displaying a 2- or 3-walled vertical bone defect as measured ≥3 mm apical to the bone crest, using different concentration of recombinant human FGF-2 with 3% hydroxy propyl cellulose (HPC) as vehicle and conducted a randomized double-blinded controlled trial involving 13 facilities. Subjects comprised 74 patients displaying a 2- or 3-walled vertical bone defect as measured ≥3 mm apical to the bone crest. Results show a significant increase in alveolar bone height in group receiving bFGF as compared to control group. These suggest that some efficacy could be expected from FGF-2 in stimulating regeneration of periodontal tissue in patients with periodontitis. [4]

The objective of this study were to investigate the Correlation between clinical periodontal parameters (PLI, GI, BOP) with immunohistochemical findings (matrix FGF2 and angiogenesis) as well as Correlation between the expressions of matrix FGF2 and angiogenesis.

MATERIAL AND METHOD

A total of 44 patients with plaque-induced gingival overgrowth / age range (15-30) years attending the clinics in the Department of Periodontics - College of dentistry - University of Baghdad / Iraq.

All the subjects were examined for checking their appropriateness for the study and underwent the 1st phase of periodontal treatment including oral hygiene instructions, scaling and polishing. At the time of periodontal surgery; the clinical examination and collection of clinical periodontal parameters: plaque index (PLI), gingival index (GI), and bleeding on probing (BOP) were performed.

Gingival tissue collection:

Gingival tissue were collected from patients undergoing conventional gingivectomy procedure in which excess gingival tissue to be removed was excised in total by an incision made at an angle of 45 degree to the long axis of the tooth, after which the surgical area was covered by periodontal dressing.

Tissue preparation and sectioning:

All the gingival tissue samples were washed in saline solution immediately after excision and fixed in 10% formalin for at least 24–48 h before processing and then processed routinely into paraffin embedded tissue blocks.

Sections: from each of these paraffin embedded tissue block; serialized sections were cut as follows:

- One 4µm thick sections for each case were cut and mounted on positively charged slides (Fisher scientific and Eschosuperfrost plus, USA) for immunohistochemical staining with bFGF polyclonal antibodies (figures 4 and 5)).
- For each immunohistochemistry run, one slide of positive controls, which obtained according to antibody manufacturer, was included. For fibroblast growth factor 2 polyclonal antibody, two tissue blocks of hepatocellular carcinoma were used(figures 1 and 2)).
- For each immunohistochemistry run, a negative tissue controls were obtained by adding washing buffer instead of the primary antibodies to any one of test group slides (figure 3)).

Staining procedure

1. Slide baking: The slides were placed in a vertical position in the hot air oven at 60˚ C. overnight.
2. Slides were deparaffinized and hydrated by dipping through three changes of xylene and alcohol and distilled water 5 minutes for each one.
3. The tissue sections were subjected to heat-induced epitope retrieval (HIER) at 95-99c, and then, the slides allowed to be cooled for a minimum of 20 minutes.
4. Endogenous peroxidase were added for 10 minutes, and then rinsed with washing buffer.
5. Primary antibody or washing buffer for negative control were added and incubated for 30 minutes (at 37c), and then rinsed with washing buffer.
6. Enhancer where added and incubate for 20 minutes, after that rinsed with washing buffer.
7. Then the prepared DAB and were added and incubated for 10 minutes and then rinsed with washing buffer.
8. Slides were counterstained with hematoxylin for 10 seconds, followed by rinsing with tap water.
9. Slides were rehydrated by dipping in a serious of alcohol, and then dipped two changes of xylene.
10. The final step consisted of mounting with DPX (one to two drops were applied to slide section wetted with xylene) and covered with cover slips lightly to allow excess and air bubbles to be removed then left to dry.

FGF-2 expression:

Immunohistochemical signal specificity was demonstrated by the absence of immunostaining in the negative control slides and its presence in recommending positive controls. For FGF-2 cells with clear brown cytoplasmic staining pattern were considered positive.

Cytoplasmic expression was the parameter scored for FGF-2 expression. The extent of staining was scored using the following scale: Score 0 = 0-10% of cells (negative), Score I = staining 10-25% of cells (weak positive), Score II =staining 25-50% of cells (moderate positive), Score III = staining up to 50% of cells (strong positive). [5] Also the number of newly formed blood vessels (angiogenesis) were calculated and compared between the groups. [6]

We certify that this study involving human subjects is in accordance with hilsinki declaration of 1975 as revised in 2000 and that it has been approved by relevant institutional ethical committee. [7]

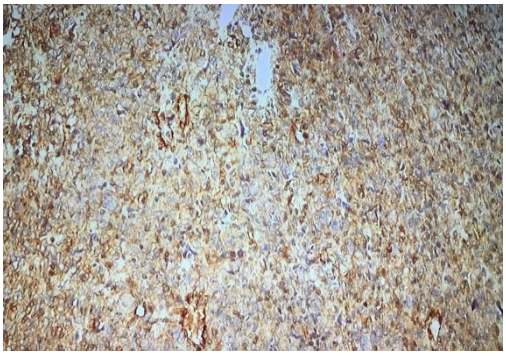

Figure 1: IHC staining of hepatocellular carcinoma(positive control)(20X).

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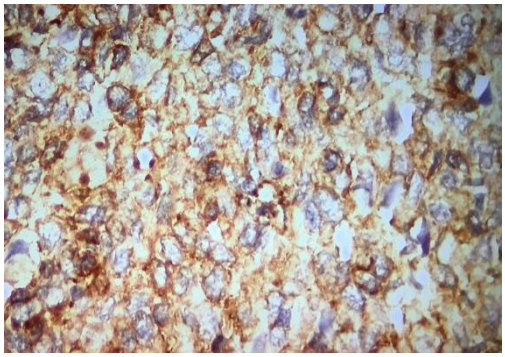

Figure 2: IHC staining of hepatocellular carcinoma(positive control)(40X).

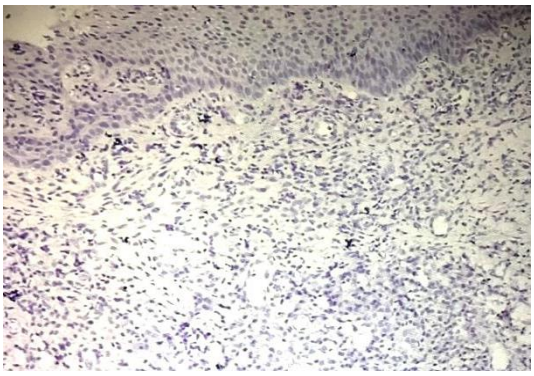

Figure 3: IHC staining for negative control (20X)

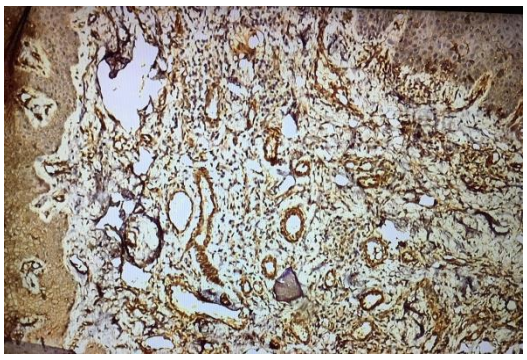

Figure 4: IHC staining for study sample (20X)

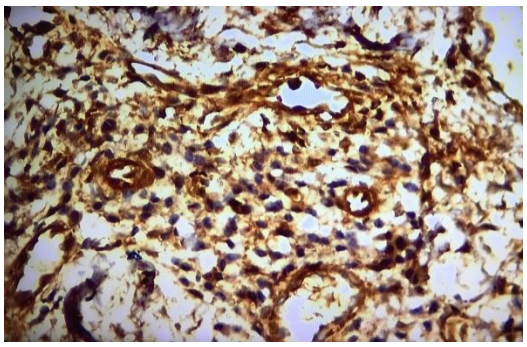

Figure 5: IHC staining for study sample (40X)

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RESULTS

Correlation between the clinical periodontal parameters (PLI, GI, BOP) with matrix FGF2 and angiogenesis:

Table (1) shows correlations between clinical periodontal parameters (PI, GI, and BOP) with matrix bFGF and angiogenesis.

Plaque index, gingival index, and bleeding on probing showed negative non-significant correlation with matrix FGF2 as r-value and p-value were (-0.085, 0.584), (-0.195, -0.204), and (-0.242, 0.114) respectively while showed a negative high significant correlation with angiogenesis in plaque index as r-value and p-value were (-0.417, 0.005)(figure 5)) and a negative significant correlation in(GI, BOP) as r-value and p-value were (-0.317, 0.036) and (-0.371, 0.013) respectively(figures 6 and 7)).

Matrix FGF2 with angiogenesis:

Table (2) figure (3) correlation between basic FGF and angiogenesis in all cases, there was a positive significant correlation as –value and p-value were (0.298, 0.038) respectively (figure8)).

Table (1): correlations between clinical periodontal parameters (PI, GI, BOP) in all cases at 2nd visits with basic FGF and angiogenesis.

Groups		Matrix	Blood vessels
PLI2	r	-.085	**-.417**
	P	.584	**.005****
GI2	r	-.195	**-.317**
	P	.204	**.036***
BOP2	r	-.242-	**-.371**
	P	.114	**.013***

* = Significant at P<0.05. ** = highly significant at p<0.01.

Table (2): correlation between basic FGF and angiogenesis.

Groups	Statistics	
Correlation	R	0.298
	P	**0.038***

***** Significant at P<0.05.

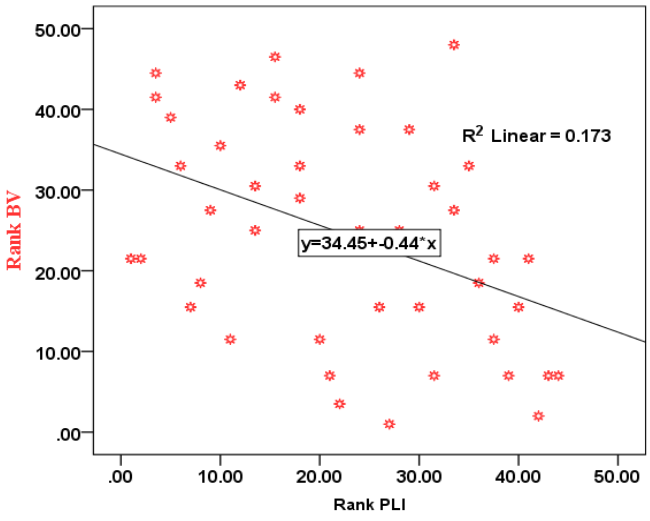

Figure 5: Correlation between plaque index and angiogenesis in all cases .

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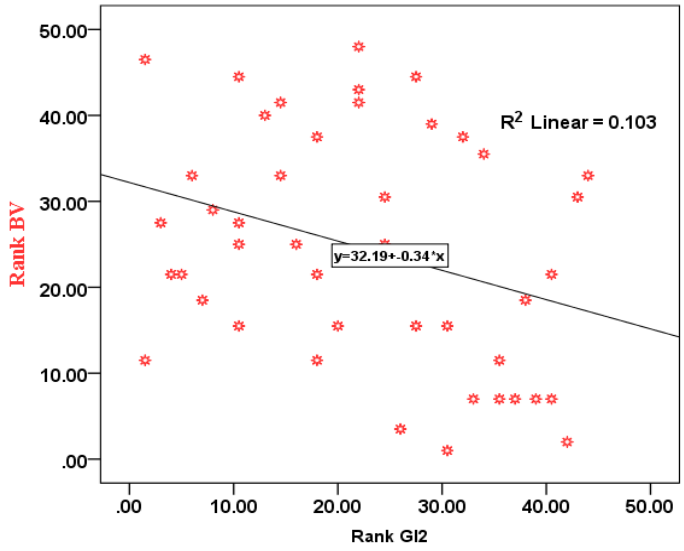

Figure6: Correlation between rank gingival index and angiogenesis in all cases.

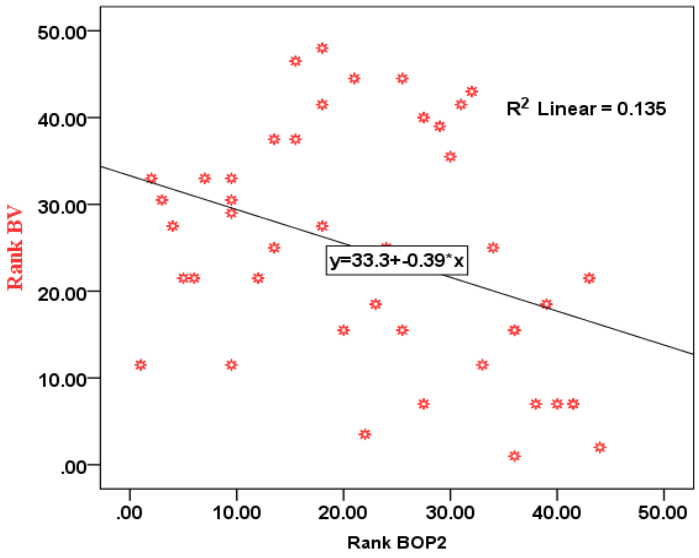

Figure7: Correlation between bleeding on probing and angiogenesis in all cases.

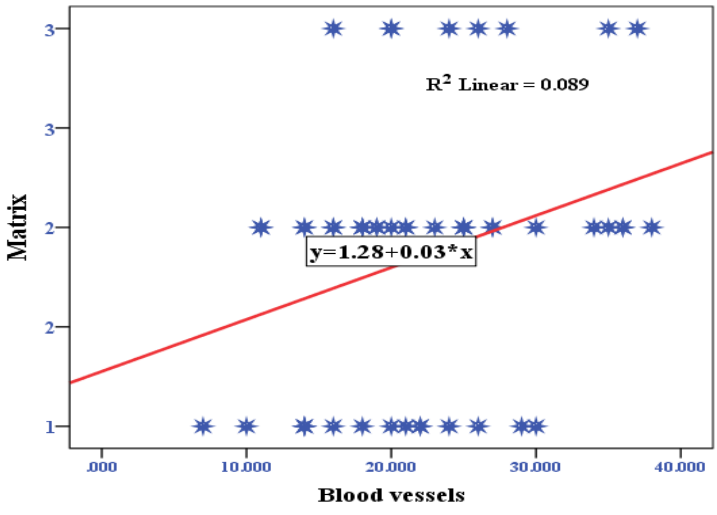

Figure8: showes correlation between basic FGF and angiogenesis in all cases.

DISCUSSION

There were negative correlations between clinical periodontal parameters with matrix FGF2 and angiogenesis; they were significant between PLI and BOP with angiogenesis while no significant in the others. This adverse correlation may indicate that with scaling and polishing and with removing of period on to pathic bacteria, inflammatory processes were reduced and healing processes had been started. Healing process required precise coordination of connective tissue repair, re-epithelialization, and angiogenesis, and can be divided in to three phases: inflammation, proliferation, and remodelling [8, 9].In proliferative phase, which started 2-10 days after injury, fibroblasts in gingiva take an essential role to generate new tissue, they don't proliferate to increase in number but also they produce large number of growth factor as well as extracellular matrix proteins in an aim to reconstruct the injured tissue among these growth factors: FGF2, VEGF, Keratinocyte growth factor-1(KGF-1).[8]

The correlation between bFGF and angiogenesis showed a significant positive. This may indicates that these are two markers for granulation tissue formation which is an important step in healing process. During tissue formation phase, newly formed blood vessels and fibroblast associated capillaries and macrophage replace temporary fibrin matrix. [10]

Basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are important soluble mediators participate in tissue growth during the formation of granulation tissue that takes an essential role in angiogenesis. [11]

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