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Expression of TGFB3 in Pyogenic Granuloma

Asmaa Ali Hussein

College of Medicine, University of Fallujah, Anbar, Iraq

Abstract

Objective: The aim of the study was to determine the expression of TGFB3 in pyogenic granuloma. **Material and Methods**: Pathologist used a double-headed light microscope to calculate cutoff values for all the antibodies employed in the investigation. Stained slides were thoroughly washed for 4 minutes with Gill's Hematoxylin Solution, dehydrated, and mounted using DPX mounting solution. **Results**: Transforming growth factor beta-3 protein expression was measured using immunohistochemistry. In pyogenic granuloma tissues expression, negative scores (0) were found in 4.5 of 16 samples (18.1%), whereas positive values (+) were found in 10 of 16 samples (33.06%). **Conclusion**: TGFB3 is expressed in most pyogenic granulomas.

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Email: mmss2006@uofallujah.edu.iq

Introduction

Pyogenic granuloma is a benign tumor that grows in the mouth or skin tissues. Because of the vascular effects of female hormones, it mostly affects young girls in their second decade of life. Proliferation of granulation tissue with inflammatory infiltration and high angiogenic potential is the most prevalent form of hyperplasia in the mouth. This uncommon disease necessitates accurate diagnosis and treatment, particularly during pregnancy [1,2]. Pyogenic granuloma is a lesion that responds to a variety of low-level stimuli, including recurrent trauma, aggressions, hormonal variables, and some medications. High levels of estrogen and progesterone during pregnancy are linked to a higher occurrence of this lesion. Lesions are more prevalent in the upper jaw, anterior regions, and gingiva's vestibular zone [3,4].

The gums are the most common site (75% of cases), although it can also occur on the lips, tongue, oral mucosa, and palate. In diverse cells and tissues throughout the body, the TGF-beta superfamily of proteins has a variety of often conflicting actions. TGF-3 (transforming growth factor beta-3) is a protein produced by the TGFB3 gene. This protein may be found across the body and is necessary for development both before and after birth. To fulfill its responsibilities, it connects (binds) to cell surface receptor proteins. This binding initiates signal transmission inside the cell, controlling a variety of cellular functions [5,6].

TGF-beta-3 is a protein that controls cell differentiation and embryogenesis. Different stages are involved in maturation into mature form: Latency-associated peptide (LAP) and Transforming growth factor beta-3 (TGF -beta) chains remain non-covalently connected after cleavage of the proprotein in the Golgi apparatus [7,8]. The effects of TGF- on bone resorption vary depending on the target cell and the settings of the experiment. TGF stimulates the replication of osteoblastic cells and bone production in mice, but it does not promote osteoblastosis [9,10]. The primary objective of this research was to investigate the expression levels of transforming growth factor (TGF) protein in pyogenic granuloma, aiming to better understand its potential role in the lesion's pathogenesis.

Material and Methods

After a first wash with washing buffer, all the slides were coated with Peroxidase-Blocking Reagent and incubated for 10 minutes. After allowing the slides to cool for 20 minutes at room temperature, the margins around the samples were marked with a liquid blocker pap pen to prevent the contents from leaking out during the IHC run.

The ideal primary antibody dilution (the dilution of each Ab and the volume were calculated and adjusted earlier) was applied to the slides, incubated for 60 minutes, and then thoroughly washed with washing buffers. Negative controls included incubations without the particular antibodies [11,12]. The stained slides were thoroughly washed for 4 minutes with Gill's Hematoxylin Solution, dehydrated, and mounted using DPX mounting solution. The stained slides were kept in the fume hood for at least one hour to dry at room temperature [13,14]. A pathologist utilized a double-headed light microscope to calculate cutoff values for all the antibodies employed in the investigation.

Overexpression of TGFB3 was defined as positive nuclei staining in at least 10% of the cells, whereas normal expression was defined as fewer than 10% positive cells. Staining was carried out according to the instructions of the Dako detection system, with the following software and protocol:
"pouring the solutions (on the slide for each location 100 l maximum 300 l per slide)". The slides were then de paraffin zed by running them through xylene (twice) and graded dilutions of alcohol (100 percent twice, 95 percent, 70 percent, and 50 percent) before incubating them in distilled water for 10 minutes. The slides were incubated for demasking (Antigen Retrieval). Solution for retrieval, Citrate Buffer; pH9 for (TGFB3) in a water bath for 60 minutes at 97°C. Following many experiments utilizing various incubation durations (20, 30, 45, 60, and 90 minutes) for Ag recovery of samples, the 60-minute optimum incubation time was established.
The slides were allowed to cool for 20 minutes at room temperature before the liquid blocker pap pen was used to mark the edges around the samples to prevent the contents from leaking out during the IHC run. After a first wash with washing buffer, all slides were coated with Peroxidase-Blocking Reagent and incubated for 10 minutes. Because some cells or tissues have endogenous peroxidase, it includes H2O2, which is a peroxidase substrate. The washing buffer was used to clean the slides for 30 minutes, blocking reagent (Ab diluent) was administered to block the excess site of another nonspecific protein and reduce background generation. Washing buffer was used on the slides. The optimal dilution of primary antibody was applied to the slides (the dilution of each Ab and the volume were previously calculated and adjusted), then forcefully rinsed with washing buffer after incubation for 60 minutes.
Negative controls were incubations that did not include antibodies previously adjusted to minimize the creation of a strong background in the dyed slides that would interfere with the results) and rinsed two times thoroughly. The slides were rinsed thoroughly with water after being counterstained with Gill's Hematoxylin Solution for 4 minutes.
The slides were dehydrated before being mounted using DPX mounting media. For at least one hour in the fume hood, the stained slides were permitted to cure at room temperature [15,16]. With the help of a trained pathologist and a double-headed light microscope, the stained slides were scored together. With the aid of a pathologist, cut off values for all the antibodies employed in the study were determined. Cut-off values for the DAKO procedure are as follows:
According to Sophia et al., TGFB3 scoring was utilized. TGFB3 oveexpression was defined as positive nuclei staining in at least 10% of the cell nuclei overexpression, Normal expression was defined as those with fewer than 10% positive cell nuclei. (Negative, score 0; weak or mild staining (5–10% score 1); moderate staining (10–25% score 2); severe staining (25–50% score 3) and extremely strong staining (25–50% score 4) (over 50 percent score 4).

Statistical Analysis

As a cutoff criterion, we utilized the median IRS=4 score. Patients with higher IRS scores (>4) were categorized as having strong TGFB3 expression. p=0.05 was used as the statistical significance cutoff. To examine the pT and Clark level variables, Cochran–Armitage statistics were used.

Results

In this work, immunohistochemistry methods were used to investigate the protein Transforming growth factor beta-3, which is generated by the human TGFB3 gene, in patients from Iraq who developed pyogenic granulomas. Negative results (-0) were seen in 4.5 of 16 samples (18.1%) when TGFB3 expression in pyogenic granuloma tissues was confirmed by immunohistochemistry. Ten of the 16 samples, or 33.06 percent, received positive ratings (+), one out of 16, or 2 percent, had positive ratings (++), and one out of 16, or 4.5 percent, had positive ratings (+++). The control had lesions (1) and a Negative score (0), as shown in Table. The expression of TGFB3 in tissues from pyogenic granulomas was markedly different from control samples (P = 0.001) in comparison. Figure 1 displays the expression of TGFB3 with the nucleus stained with Brown to demonstrate positive TGFB3 expression and without nucleus staining to show negative TGFB3 expression. Figure 2 displays the immunohistochemical staining of TGFB3 in pyogenic granuloma slices with counterstained blue hematoxyline and brown peroxidase/DAB (400X) (400X).

Discussion

A driving force behind growth Among other ways, beta-3 can stop development, cause cell death, alter the cell matrix on the outside, and lessen the body's reaction to sickness [17]. TGFB3 has also been demonstrated to enhance the production of endothelial growth factors (TGF-I), as well as having a variety of additional anti-swelling properties. TGFB3, a novel host factor that can't be weakened by infection, is another essential cytokine, organize tissue development, proliferation, and growth (using different expressions).
When individuals with pyogenic granuloma are followed with obvious health values, TGFB3 is commonly accessible [18]. Patients with erosive form had substantially greater TGFB3 tissue expression levels than those with retinal form. Regardless, there is no recurrent disparity in numbers, suggesting that TGFB3 levels differ significantly between patients with pyogenic granuloma and healthy controls. Another study found a strong link between TGFB3 levels and various kinds of pyogenic granuloma, as well as an increase in saliva levels for patients with TGFB3 in pyogenic granuloma. In comparison to the retinal form, the corrosive and destructive forms included more TGFB3 [19].
In addition, 8 of 11 individuals with pyogenic granuloma had an unaffected response to TGFB3. Despite this, pyogenic granuloma was shown to be negative in recent tissue samples from healthy people; also, the levels of TGFB3 and different types of pyogenic granuloma were shown to have a significant relationship. The reticular type had lower TGFB3 levels than the atrophic and erosive forms. In addition, in 8 out of 11 individuals with pyogenic granuloma, an unable to harm response was shown against TGFB3 [20].

Conclusions

According to the results of the study, TGFB3 expression is high in pyogenic granuloma.

Conflict of Interested Statement

None to declare.

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Table 1. TGFB3 expression in pyogenic granuloma was investigated using immunohistochemistry.

Score group	Negative scored	+	++	+++
Pyogenic granuloma	4.5 (18.1%) A	10(33.06%) B	1 (2%) B	1 (4.5%) C
Control	Negative scored (0)			

*** P <0.005

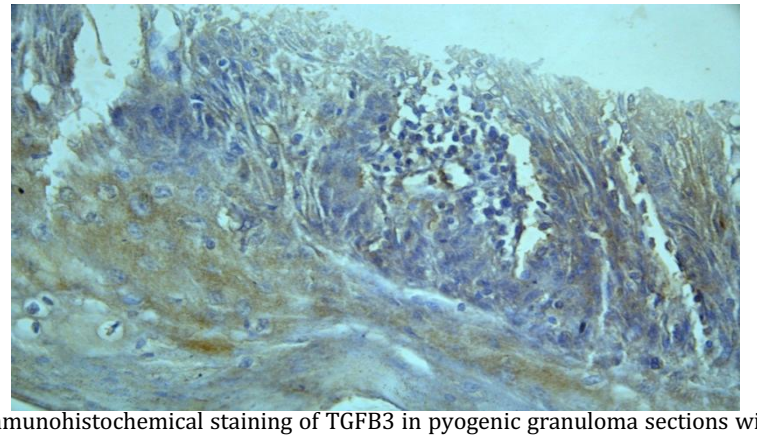

Figure 1. Immunohistochemical staining of TGFB3 in pyogenic granuloma sections with peroxidase/DAB (brown) counterstained with blue hematoxyline (400X).

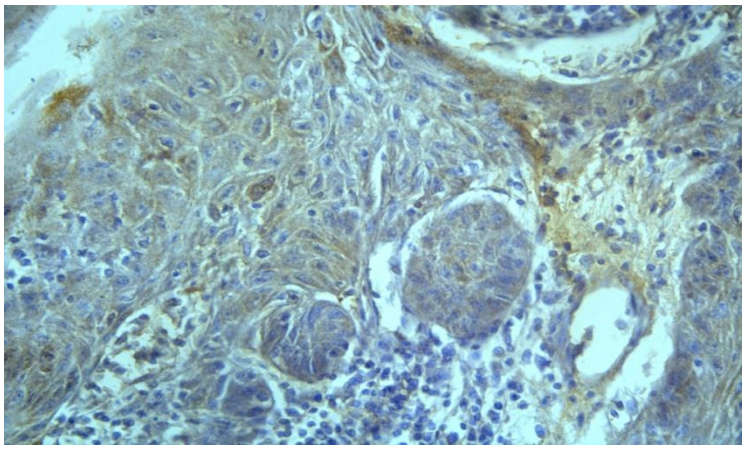

Figure 2. Immunohistochemical staining of TGFB3 in pyogenic granuloma slices with peroxidase/DAB (brown) and blue hematoxylin counterstained (400X).