Human Apical Periodontitis Tissue And CD44 Expression.

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Introduction

Apical periodontitis is an inflammatory disorder of periradicular tissues caused by aetiological agents of endodontic origin. The subject has been reviewed by Nair. [1][2]
They are believed to result from inflammatory processes in the periapical tissues associated with bacterial infection and necrosis of the dental pulp in carious teeth. As a consequence of these processes and the inability of host defense mechanisms to eradicate antigens, periapical lesions such as apical abscesses, granulomas, and periapical cysts may be formed, with the aim of restricting microbial invasion. [3]

Periapical granulomas are chronic inflammatory lesions caused by complex polymicrobial infections. They are typified by damage to supporting tissue, including alveolar bone resorption around the apical areas, and by granulomatous tissue with a large number of inflammatory cells, such as macrophages, lymphocytes, plasma cells and polymorphonuclear leukocytes (PMNs) [4]. Migration of inflammatory cells, which predominantly express various cytokines [5], [6], and growth factors that augment immune responses, seem to play an important role in the progression of periapical lesions. In particular, infiltrating activated macrophages and lymphocytes are believed to initiate periapical inflammation, eventually leading to progressive bone loss. [7]

The localization of circulating leukocytes within inflamed tissues occurs as the result of interactions with and migration across vascular endothelium, and is governed, in part, by the expression of adhesion molecules on both cell types. Recently, a novel primary adhesion interaction between the structurally activated form of the adhesion molecule CD44 on lymphocytes and its major ligand hyaluronan on endothelial cells under physiologic laminar flow conditions has been described, and it has been proposed that this interaction functions in an extravasation pathway for lymphocytes in vascular beds at sites of inflammation. CD44 on lymphocytes binding to its carbohydrate ligand hyaluronan can mediate primary adhesion (rolling interactions) of lymphocytes on vascular endothelial cells. This adhesion pathway is utilized in the extravasation of activated T cells from the blood into sites of inflammation and therefore influences patterns of lymphocyte homing and inflammation. Hyaluronan (HA) is a glycosaminoglycan found in the extracellular matrix and is involved in a number of biological processes. In addition, HA exerts its functions as a signaling molecule through the cell surface receptor, CD44. Although the mechanism of HA-CD44 signaling events has not been clearly understood, HA modulates cell adhesion, motility, proliferation and differentiation, which are implicated in wound healing, tumor formation and metastasis, inflammation and tissue remodeling. [8]

Previous study has demonstrated CD44 level in healthy and inflamed pulp tissue. Symptomatic and asymptomatic pulpitis when compared with healthy pulp tissue. When both groups were compared with each other, a slight difference between both pathologic groups was observed. [9]

This study aimed to determine CD44 levels in inflamed periodontal tissue in comparison with healthy and inflamed pulp tissue.

Materials and Methods

Work was carried out on periapical tissue from patients at the Surgery Department of the Dentistry School in Rosario. These patients had previously signed informed consents. Teeth were extracted for surgery reasons. The patients' ages ranged from 18–45 years. Gender was not determined. The selected teeth had the apex completely developed. The patients excluded were those whose medical history presented a systemic condition that could affect the biologic response to repair. The samples of inflamed periodontal tissue were removed with tooth and sectioned with surgical knife #16. All the samples were preserved until they were processed in physiologic saline in Eppendorf tubes and frozen at –32°C and were used to investigate the levels of the soluble hyaluronate receptor (CD44). The immune hematologic technique applied was agglutination inhibition of CD44-hyaluronate system.

In the agglutination inhibition reactions, specificity and antibody titers were estimated according to the competition between particulate and soluble antigens for the combination sites. This can be equaled to the hyaluronate receptor (CD44) – hyaluronate reaction.
The reagents used were Bromeline (Sigma.B22.52; Sigma, St Louis, MO), phosphate-buffered saline (PBS), hyaluronate (Sigma-Aldrich H 1876; hyaluronic acid, sodium salt from human umbilical cord), and anti-D. The biologic material was periapical tissue lysate.

In the first stage, equal volumes of hyaluronate were confronted in sub-agglutinating levels, with periapical tissue lysate in geometric dilutions (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, and 1:512) and were incubated for 12 hours at 4 °C. Later, the non-neutralized hyaluronate activity was measured through its adhesive (agglutinating) action over the developer system.

Indicator Cells.

To prevent interference in the readings, erythrocytes from the same blood group (ABO-compatible) and the same Rh as the patient's were used and were bromelinized. A volume and a half of bromeline (5 mg/mL) was added to a volume of centrifuged red blood cells at the end of the test tube, and it was incubated for 15 minutes at 37°C. Later the red blood cells were washed 3 times in PBS and suspended at 2% in the same environment.

Then the bromelinized red blood cells were added and incubated for 24 hours in a refrigerator.

After 24 hours, readings were taken (Table 1). The results were expressed in a score, and later the sensitivity parameter was applied to them.
The results were expressed in a score, as follows: (10)

Score 10	+++	1 to 3 big agglutinated red cells.
Score 8	++	3 to 10 agglutinated red cells.
Score 5	+	Over 10 agglutinated red cells.
Score 2	±	Tracing.
Score 0	-	No agglutinated red cells.

The statistical analysis methods applied were the Kruskall-Wallis test and the multiple contrasts test.

Discussion

Bacterial infection of the tooth root canal system leads to the development of periapical lesions that represent local defense reactions against intracanal pathogens. Recent studies have indicated that macrophages may have a central role in the lesion development. Macrophage-associated antigen-expressing cells comprise the most prevalent immune cells [11] [12] [13] [14].

Hyaluronon is a major glycosaminoglycan in the periodontal ligament, comprising repeated disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine. It plays important roles in cell adhesion, migration and differentiation mediated by various hyaluronon-binding proteins and cell-surface receptors such as CD44. [15] [16]

Several functions of low molecular-weight hyaluronan associated with inflammation have been demonstrated. [17] Furthermore, low molecular-weight hyaluronan induced nitric oxide synthesis in murine macrophages, which is associated with connective tissue destruction. [18]

28 biological samples of granulation tissue from teeth extracted for pulp death and periapical infection diagnosis reasons were analyzed, and CD44 levels were established.

Once the samples were subjected to statistical tests, it was concluded that there are significant differences between the periapical process groups and the pulp inflammatory process groups. The results agree with Tasman's [19] work, which showed high levels of CD44 expression, both on blood vessel endothelial walls and on the connective tissue, participating especially in the organization of mononuclear elements and lymphocytes. Although a marked CD44 sensitivity was observed, and its participation in inflammatory processes and cell migration was proved, further research should be done.

Results

The periodontal tissue samples were analyzed to determine the presence of CD44 by using a specific marker. The results are expressed as sensitivity parameter (a) [11]. Rouleaux are assessed according to score (E), which is a measurement of the degree of red cell adhesion that, combined with serum dilution (D), determines the sensitivity parameter (a), an adimensional parameter and defined by the mathematical expression:

$$\alpha = \sum \frac{E\,i}{Di} \times 10^{-3}$$

This value must be independently calculated for each incubation period (constant parameter) on the basis of the two other variables: titer and score.

Using the sensitivity parameter, however, gives a more accurate quantification of the sensitivity increase of a technique, in contrast with another technique that is standard or not so improved. The sensitivity parameter values for each group are displayed in Table 1.

After the Kruskall-Wallis test was applied, the conclusions indicated the existence of significant differences in the sensitivity parameter medians in the groups considered (p=0.001).

Identification			Geometric dilution									Sensitivity parameter
N°	Processes	Blood Group	P	2	4	8	16	32	64	128	256	αs
115	Apical	O+	-	±	±	+	+	-	±	±	±	0.0024921
112	Apical	O+	++	+	±	±	+	+	+	±	±	0.0118203
116	Apical	O+	+++	++	+	+	++	+	±	±	±	0.0165859
111	Apical	A+	++	++	±	+	±	±	-	-	-	0.0133125
120	Apical	O+	+++	+++	±	±	+	±	±	±	-	0.0161718
B	Apical	A+	-	++	±	-	±	-	-	-	-	0.04625
C	Apical	O+	-	++	+	+	+	±	±	±	±	0.0063046
A	Apical	O+	++	+	±	±	±	-	-	-	-	0.011375
121	Apical	O+	+++	++	±	±	±	-	±	±	-	0.0149218
151	Apical	O+	-	+++	+++	+++	+++	++	+	+	±	0.00975
152	Apical	O+	+++	++	++	+	+	±	±	±	±	0.0170546
M	Apical	A+	+++	+++	+++	++	+++	+++	++	++	++	0.0196562
160	Apical	A+	+++	++	++	+	+	+	±	±	±	0.0171484
161	Apical	O+	+++	+++	++	++	+++	++	++	++	++	0.0190937
162	Apical	A-	+++	+++	+++	+++	++	+	+	±	±	0.0195078
163	Apical	O+	+++	+++	++	+	++	+	±	±	±	0.0183359
F	Apical	A+	++	++	+	+	+	+	±	±	±	0.0144609
D	Apical	A+	+++	++	+++	++	+	+	+	±	±	0.0180703
156	Apical	O+	++	+++	++	+	-	±	-	++	+	0.015832
157	Apical	O+	+	++	±	±	+	+	-	-	-	0.0102187
154	Apical	O+	+	++	±	±	±	-	±	+	-	0.0099453

Table 1:
Samples identification, geometric dilution and sensitivity parameter.

The group of periapical processes (n=21) was compared to healthy pulps (n=10); to open asymptomatic pulp inflammatory processes (n=36); and to closed asymptomatic pulp inflammatory processes (n=28) previously analyzed. Sensitivity parameter values for each group are illustrated in Table Nº 2, where the data main descriptive measures are observed.

Processes	Apical	Healthy	Closed Symptomatic	Open asymptomatic
Mean	0.0136516	0.0037093	0.0125013	0.0196367
Median	0.0149218	0.00275	0.008	0.00935935
Standard deviation	0.00498304	0.00197565	0.0212762	0.0346873
Interquartile range	0.0069297	0.004	0.0067852	0.0078515

Table 2: Sensitivity parameter values for each group are illustrated.

As the variances' standard and equality assumptions were not met, the comparison was performed through the Kruskal-Wallis technique. It can be concluded that there are significant differences between the values of the sensitivity parameters in the groups analyzed ($p<0.0001$). After the Kruskal-Wallis multiple comparison technique was applied, the following conclusion can be drawn:

Processes	N =	Average Range	Homogeneous group
Apical	21	66.90	*
Healthy	10	15.10	*
Closed Symptomatic	28	43.68	*
Open asymptomatic	36	49.47	* *

Tabla 3: Multiple comparison technique was applied and the following conclusion can be drawn.

The sensitivity parameter median for the periapical process group is significantly higher than the one for the healthy process group and for the closed symptomatic process group. This, in turn, is significantly higher than the healthy group. The open asymptomatic process group has an ambiguous position between the closed group and the periapical group ($p=0.005$).

The sensitivity parameter values for each group are illustrated in the following chart:

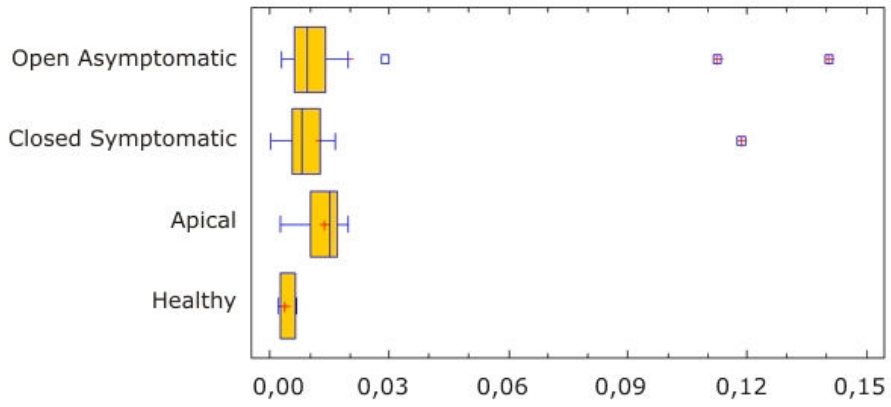

Figure 1:
The sensitivity parameter values for each group are illustrated in the following chart.

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