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A COMPARISON OF IL-2 IN NORMAL AND SYMPTOMATIC PULPS

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ABSTRACT

Normal healthy pulpal tissues were obtained from 19 impacted molars and symptomatic samples were obtained from 18 carious molars and premolars clinically diagnosed in all vital pulpal tissues. Student's t-test revealed significant differences in IL-2 concentrations, comparing symptomatic pulpal tissues with normal healthy samples (657, $p<0.01$). These results suggest that IL -2 may serve as a marker of changes in pulp tissue.

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INTRODUCTION

Toothache of pulpal origin is a sign of inflammation and release of chemical transmitters in pulp tissue. The presence of immune competent cells and their roles in inflammation and production of different chemical transmitters is well known. Injury to the soft tissues causes the release of biological molecules such as cytokines from the injured cell wall membranes.[1] Cytokines are low molecular weight glycoproteins with specific and non-specific functions.[2] Nearly 30 lymphokines have been described.[3] Their fundamental role is to control the inflammatory immune responses. In addition, they affect various cells and boost immune responses.[4] Certain cytokines can regulate the behaviour of the cells as well as cells which make them; these cytokines are called regulator cytokines. Some cytokines cause proliferation of cells such as T and B-lymphocytes.[5]

Interleukins (ILS) are cytokines which are produced by leukocytes. IL-2 is a potent stimulant, and is released by T-helper cells.[6] IL-2 plays an important role in pathogenesis or progression of disease.[1] It is a major T cell growth factor.[5]

Cytokines such as IL-2 and interferons can enter the systemic circulation and cause systemic effects such as fever, ACTH production and release of neutrophils from bone marrow.[7]

The normal biologic reaction of pulp to injuries is an inflammatory response, which is probably mediated through T-lymphocytes. An increase in the number of T-lymphocytes and concentration of IL-2 has been reported in inflamed symptomatic pulps.[8,9]

The purpose of this study was to investigate the presence of IL-2 and its relation to clinical symptoms (pain) in symptomatic pulps compared to normal pulps.

MATERIAL AND METHODS

Based on their clinical symptoms our samples were divided into two groups of twenty samples each. The first group were normal pulps. The teeth in this group had no decay or symptoms and were extracted for orthodontic reasons. Clinical tests and radiographs were used to diagnose normal teeth.[10]

The samples in the second group were twenty symptomatic (painful) pulps that had clinical sings of irreversible (painful) pulpitis. These samples came from teeth which were not restorable and were scheduled for extraction. Diagnosis of irreversible pulpitis in the teeth was made by periapical radiographs, dental history and clinical examinations.[10]

Following administration of local anesthesia, the teeth

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were extracted with elevator and forceps. After placing buccal and lingual vertical grooves on each tooth and using fissure burs and water spray the teeth were split into two halves and the pulps were removed and placed in Indorf tubes.

The samples were coded and kept at -76°C. After collection of all the samples, they were brought to room temperature for 30 min. They were then weighed using a Sartoizus balance (N: 195626-Sartoizus-Germany) with an accuracy of 0.0001 grams (Table I).

To prevent the enzymatic changes in the samples and cell destruction, one hundred microliters of phosphate buffered saline (PBS), pH=7 was added to each sample. The samples were then pressed between two glass rods for three minutes until their interstitial fluids were released. The Indorf tubes containing pulps and their fluid were kept at 4°C for thirty minutes. They were vibrated for 30s using a Heidolph vibrator.

Table I. IL-2 concentrations in different samples in the study.

Sample	Tooth	Weight	Clinical state	IL-2 (pg/mg)
1	17	0.009	normal	
2	31	0.019	normal	10084
3	16	0.44	normal	2613
4	21	0.010	normal	9750
5	18	0.005	normal	1700
6	17	0.005	normal	13823
7	16	0.0150	normal	9333
8	16	0.0118	normal	8898
9	32	0.0223	normal	6502
10	11	0.0124	normal	12298
11	15	0.0025	normal	10576
12	11	0.345	normal	3623
13	32	0.0072	normal	14583
14	21	0.008	normal	17500
15	11	0.0098	normal	8163
16	17	0.010	normal	13875
17	32	0.0255	normal	3431
18	12	0.0164	normal	7164
19	16	0.0250	normal	4000
20	13	-	normal	-
21	36	0.0035	symptomatic	47857
22	14	0.009	symptomatic	17777
23	26	0.007	symptomatic	16428
24	31	0.005	symptomatic	21000
25	16	0.0055	symptomatic	23636
26	32	0.0127	symptomatic	13000
27	11	0.0032	symptomatic	67968
28	31	0.0043	symptomatic	43023
29	15	0.006	symptomatic	34583
30	14	0.0055	symptomatic	30000
31	36	0.0085	symptomatic	18970
32	14	0.0075	symptomatic	18666
33	36	0.005	symptomatic	36000
34	15	0.0127	symptomatic	13779
35	3	0.0016	symptomatic	87500
36	16	0.015	symptomatic	11666
37	21	0.0061	symptomatic	22950
38	17	0.0033	symptomatic	39393
39	32	-	symptomatic	-
40	18	-	symptomatic	-

Table II. The means and standard deviations of IL-2 in normal and symptomatic pulps.

	Mean	SD	Normal
Normal	9701	3867.87	19
Symptomatic	31344.22	20280.646	18

T= -4.568, $p<0.01$

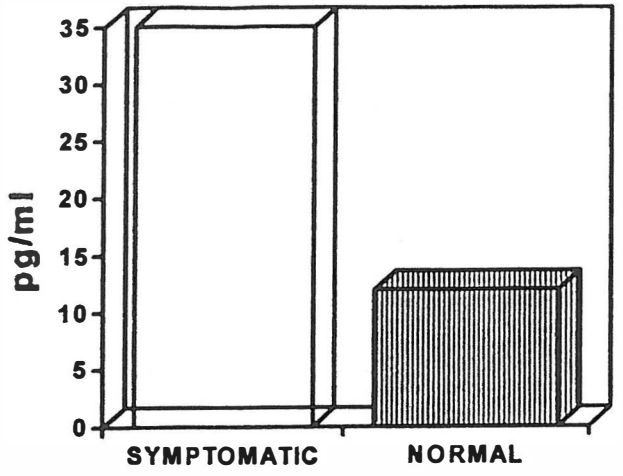

Fig. 1. Comparison of IL 2 concentrations in normal and symptomatic pulps.

Finally the samples were centrifuged for two minutes at 9800 r.p.m. Fifty microliters of the top fluids of each centrifuged sample was collected and the amount of IL-2 protein was measured using ELISA kits.

The standard curve was first drawn using the optical density (OD) values of the standard samples (with known IL-2 value) and then the average value of OD was determined for all standards and test samples.

The IL-2 level was calculated in picogram per milligram (pg/mg)for each pulp using the following formulas:

a) pulp weight (mg) 0-100 (mL)/ pulp concentration (mg/mL)= comparison of IL-2

b) IL-2 concentration (pg/mL)/ pulp concentration (mg/mL)= IL-2 pulp (pg/mL). The Student's t-test was used to determine statistical difference between the two groups.

RESULTS

Three samples (one normal pulp and two symptomatic pulps) were eliminated because the samples were too small to include in our evaluation.

Table I provides the information regarding tooth number, weight of each pulp sample, clinical diagnosis and concentration of IL-2 in each sample.

Table II shows the mean and standard deviation value of IL-2 in normal pulps (9701 pg/mL) and in symptomatic pulps (31344 pg/mL).

Figure 1 shows that the average concentration of IL-2 in symptomatic pulps is three times higher than those found in normal pulps.

Statistical analysis of the results show a significant difference between the value of IL-2 in normal pulps compared to symptomatic pulps ($p<0.01$).

DISCUSSION

Our data shows that IL-2 exists in normal pulp as well as in inflamed pulps. However, the concentration of IL -2 in inflamed and symptomatic pulps is significantly higher than that found in normal pulps.

The results of this study corroborate the findings of Reuschenberger and associates who showed increased levels of IL-2 in inflamed pulps[1] and the findings of McFarlane and co-workers[9] who showed that the levels of IL-2 in gingivae of laboratory mice with periodontitis was much higher than that of normal gingivae in these animals.

A comparison of the results of previous studies with our findings shows that IL-2 does exist in normal gingiva and pulps and increases dramatically during inflammation and in painful pulps.

The results of our study regarding an increase in IL-2 are in agreement with those reported by Abbas and Lichtman[11] as well as Bailey and Rauchenberger. These results show that existence of a significant concentration of IL-2 in the pulp is indicative of pulp disease.

The biological reaction of pulp to injuries and mechanisms, which control this process, are not completely understood. Currently certain clinical symptoms and various laboratory tests are used for evaluation of the pulp after injuries. However none of these clinical methods have been able to determine pulp disease accurately.[2]

Determinations of pulpal status obtained with present methods are very subjective. A biological assay such as determination of the level of a mediator of inflammation like IL -2 could be more accurate than the use of present methods.

The use of mediators of inflammation such as IL- 2 for pulpal conditioning may increase the validity of endodontic diagnostic tests.

If IL- 2 and other cytokines act as inflammatory mediators[9,10] and chemotactic factors attract inflammatory cells to the site on injury, theoretically, we might be able to reduce acute inflammation by using antagonists for those cytokines.

Based on the results of this study in appears that IL-

2 is present in high concentrations in symptomatic pulpitis and plays a role in the pathogenesis of pulpal diseases and could be used as a marker for determination of pulpal conditions.

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