Medical Journal of the
Islamic Republic of Iran

Volume 17
Number 2
Summer 1382
August 2003

# 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.

*MJIRI, Vol. 17, No. 2, 137-140, 2003.*

**Keywords:** pulp tissue, inflammation, chemical transmitters, cytokines, interleukins, T lymphocytes, IL-2.

## 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 ás 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, $p<0.01$

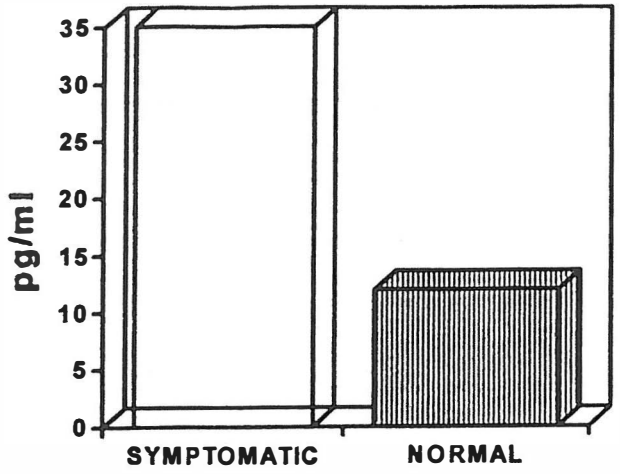


**Fig. 1.** Comparison of IL 2 concentrations in normal and symptomatic pulps.

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.

## ACKNOWLEDGEMENTS

This research was performed in Professor Torabinejad Research Center and the Immunology Laboratory of Isfahan Medical University. The assistance given by the staff of these centers particularly Mr. F. Oreizi is acknowledged. The authors also thank the research vice-chancellor of the university for his financial support.

## REFERENCES

1. Rauschenberger CR, Bailey JC, Cootauco CJ: Detection of human IL-2 in normal and inflamed dental pulps. Am J of Endo 23: 366-370, 1997.
2. Trowbridge HO, Emling R: Inflammation: a review of the process. Chicago: Quintessence, Fifth Edition, 97: 109, 1997.
3. Torabinejad M, William CA, Naidor F, Laving J: Inflammatory and immunological aspects of the pathogenesis of human periapical lesions American JOE 11(11): 479-488, 1985.
4. Jawetz E, Melnick JL, Adelberg EA: Medical Microbiology, 20 th ed., East Norwalk: Appleton and Lange Co., p. 141, 1995.
5. Roitt I, Brostoff J, Male D: Immunology. London: Mosby, Fourth Edition, pp. 1-18, 69-81, 1996.
6. Hosseini F: Immunology, Mashad, 3rd Edition, p. 297, 1994 (in Persian).
7. Benjamin E, Leskowitz S: Immunology, a short course. Third edition, Nashre Tayeb Tehran, Supervised by Dr. Khansari, 1996, p. 251.
8. Rauschenberger BJ: Detection of LI-2 in healthy and inflamed dental pulps (abstracts). American JOE 19(4): 191-2, 1993.
9. Mcfarlane CG, Meikle MC: Interleukin-2 receptor, and interleukin-4 levels are evaluated in the sera of patients with periodontal disease. J Peridont Rest 26: 402-408, 1991.
10. Walton R, Torabinejad M: Principles and Practice of Endodontics. Philadelphia: Saunders Company, Second edition, p. 58, 1996.
11. Abbas AK, Lichtman AH, Pober J: Cellular and molecular Immunology. Philadelphia: Saunders Co., Second Edition, pp. 313-325, 1994.