Measurement and Comparison of Endogen 17β-Estradiol Levels in Healthy and Symptomatic Pulp Tissues with Irreversible Pulpitis

Aim: The aim of this study was to measure the endogen 17β-estradiol (E₂) level in human pulp tissue and to investigate whether it was different in symptomatic irreversible pulpitis (SIP).

Materials and Methods: The tissue samples were obtained from 23 impacted third molar teeth (8 male and 15 female) that were surgically extracted and 33 teeth (8 male and 25 female) that underwent endodontic treatment. The endogen E₂ level in the tissue samples was measured through immunoassay method. All the results were statistically evaluated using Student’s unpaired t-test and Mann-Whitney U test.

Results: The endogen E₂ level in the samples obtained from the females was higher than that of the samples obtained from the males (p<0.001). In the analysis conducted according to tissue type, endogen E₂ level was higher in the tissues with SIP (p=0.024). In the evaluation based on gender and tissue type comparisons, the rate of increase in the endogen E₂ level of the SIP tissue of the female patients was statistically significant (p=0.007).

Conclusion: As a result of this study for the first time ever estrogen levels in the human pulp tissue were recorded and it was shown that in the presence of SIP there was an increase in endogen E₂ level.

Keywords
17β-estradiol, estrogen, pulp, irreversible pulpitis, tissue

Amaç: Bu çalışmanın amacı insan pulpa dokusunda endojen 17β-estradiol (E₂) düzeyini ölçüp semptomatik irreversible pulpitis (SIP) varlığında miktarında farklılık olup olmadığını araştırmaktır.

Gereçler ve Yöntemler: Doku örnekleri cerrahi olarak çekilen 23 gömülü üçüncü molar dişten (8 erkek ve 15 kadın) ve 33 endodontik tedavi uygulanan dişten (8 erkek ve 25 kadın) elde edilmiştir. Doku örneklerindeki endojen 17β-estradiol (E₂) düzeyi immunoassay metodü ile ölçülmiştir. Sonuçlar Student’s unpaired t-test ve Mann-Whitney U test ile istatistiksel olarak değerlendirilmiştir.

Bulgular: Kadın hastalardan elde edilen örneklerdeki endojen E₂ düzeyi erkek hastalardan elde edilen örneklerde göre daha yüksek tespit edilmiştir (p<0.001). Örneklerin elde ettiği doku tipine göre yapılan analizde endojen E₂ düzeyi SIP’li dokular arasında daha yüksek bulunmuştur (p=0.024). Cinsiyet ve doku tipine göre yapılan değerlendirilmede ise SIP dokusundaki endojen E₂ düzeyindeki artış kadın hastalarda istatistiksel olarak anlamlı çıkmıştır (p<0.007).

Sonuç: Bu çalışmanın sonucunda insan pulpa dokusunda ilk defa östrojen düzeyleri tespit edilmiştir ve SIP varlığında endojen E₂ seviyesinde artış olduğu gösterilmiştir.

Anahtar Kelimeler
17β-östradiol, östrojen, pulpa, irreversible pulpitis, doku
INTRODUCTION

Estrogen hormones are prominent steroid hormones that regulate growth, differentiation, and the functions of reproductive as well as non-reproductive tissues. The most important estrogens are estrone (E1), 17β-estradiol (E2), estriol (E3), and of these, E2 is the most functional estrogen during reproductive age. E2 is synthesized intraglandularly in the ovaries of females and in the testicles of males. The extraglandular synthesis of this hormone is realized in the tissues that contain aromatase enzyme (fat tissue, muscle, skin, liver, brain...etc) through peripheral aromatization. The cellular effect of E2 is an outcome of the binding of receptor-hormone complex, which forms in the presence of estrogen receptors (ER), to deoxyribonucleic acid (DNA). Numerous studies that show the effect of E2 in various organs and tissues of the dentomaxillofacial region (oral mucosa, salivary glands, periodontal tissues, temporomandibular joint...etc) were performed after detection of ER in the targeted organs.

The presence of ERs was first shown in the pulp tissue (the predentinal-odontoblast region and pulpal blood vessels) by Hietala et al in 1998, and in the odontoblasts, endothelial cells, and Schwann cells by Jukié et al in 2003. Odontoblasts have a role in type I collagen synthesis and secretion during formation of dentin matrix, which suggests that these cells may be sensitive to the estrogen hormone that affects the collagen fibril content and characteristics of the organism (an increase in type III collagen content and a decrease in the type I/III collagen ratio).

Estrogen hormone changes the expression of cytokines and neuropeptides directly or indirectly during various tissue infections. Moreover, estrogen receptors were detected in Schwann cells, which are a constituent of the pulp tissue, as well as in the endothelial cells, which are a component of the vascular structure. Both of these are suggestive of a potential role of estrogen hormone in the pathophysiology of pulpal inflammation.

Recent studies have failed to accurately reflect the hormone concentrations in the tissues based on measured plasma estrogen levels. Thus, the determination of hormone levels at a cellular level may clearly define the biological effects of hormones. In this study the endogenous 17β-estradiol (E2) level in human pulp tissue composed of cells, essential matter, fibers, nerves, and blood vessels was measured and whether it was different in symptomatic irreversible pulpitis (SIP) was investigated.

MATERIALS AND METHODS

Patient selection

The study was approved by the University Scientific Ethics Committee, and all the subjects (n: 56) gave their written informed consent. The healthy pulp group consisted of 23 subjects (median age: 28 ± 6.3 years, age range: 16-40 years) who required extraction of healthy impacted third molar teeth. The SIP group comprised 33 subjects (median age: 24.88 ± 8.7 years, age range: 13-40 years) complaining of toothache, who attended a dental hospital for relief of dental pain.

The following information was recorded for each patient: Age, gender, tooth, history of previous local treatment, and radiographic findings. The day of the menstrual cycle was also recorded for the female patients.

The inclusion criteria for the SIP group were diagnosis of SIP, presence of deep interproximal or occlusal carious lesions or secondary caries under a restoration, no exposure of the dental pulp in clinical and/or radiographical evaluations. None of the teeth showed radiographic evidence of periapical inflammation. The teeth were classified into the healthy pulp group based on the following criteria: No radiographic evidence of peripheral pathosis and no symptoms.

Exclusion criteria were conditions requiring good general health judged according to medical history, blood pressure, pulse rate; and no his-
coated paramagnetic microparticles are combined with 17-beta-estradiol molecule. Following the washing step, the acridinium labeled conjugate initiates the chemiluminescent reaction in the presence of sodium hydroxide and hydrogen peroxide (CMIA, Abbott Architect IL, USA). The limit of detection was 6 pg/ml. Within run imprecision of the assay for low, medium, and high controls were 4.8–6.8%, 2.9–3.6% and 2.1–2.9%.

**Protein determination**

Protein was determined by the method of Lowry et al (3) using bovine serum albumin as the standard 18.

**Statistical analyses**

All the results were analyzed statistically by using the Statistical Package for Social Sciences, SPSS 11.5 (SPSS® Inc., Chicago, IL, USA). Student’s unpaired t-test was used for continuous variables with normal distribution. Mann-Whitney U test was used for variables without normal distributions in the studied population. Mean, standard deviation, median, minimum-maximum values were given as descriptive statistics. Significance level alpha was taken as 0.05.

**RESULTS**

In this study, two groups composed of healthy pulpal tissue samples and SIP tissue obtained from the extracted teeth of 56 patients were evaluated, and E₂ was found in all the human pulp tissue samples. E₂ levels of all the samples were measured and the results were compared according to gender, menstrual cycles of the female patients, tissue type, location and restoration of the tooth from which the tissue was extirpated (Table I).

In the male patients, the median tissue level of E₂ was 2659, (167-5959) (median, (min-max)) fmol/mg pr, and in the female patients, the median tissue level of E₂ was 7456.5, (703-23050) fmol/mg pr. The statistical analysis showed significantly higher E₂ levels in the female patients than in the male patients (p< 0.001).
The female patients were divided into two groups according to their menstrual cycles as follicular phase (1-14 days) and luteal phase (14-28 days). In the follicular phase, the $E_2$ level in the pulp tissue was $7822 \pm 1583-23050$ fmol/mg pr, and in the luteal phase, $7091 \pm 703-18385$ fmol/mg pr. However, the difference between the two values was not statistically significant ($p=0.520$).

Comparisons of the menstrual phases according to pulp tissue type showed no statistically significant results for both the healthy and SIP groups ($p>0.05$) (Table II).

The comparisons of the groups according to the tissue type showed that the $E_2$ level of the SIP tissues was $7091 \pm 270-23050$ fmol/mg pr, and the $E_2$ level of the healthy pulp tissues was $3900 \pm 167-11167$ fmol/mg pr. The difference between the two values was statistically significant ($p=0.024$). According to the comparisons based on gender and tissue types together, there was a significant difference ($p<0.007$) between the female group of samples (SIP and healthy pulps), whereas there was no difference between the $E_2$ levels of the male group ($p=0.161$) (Table III).

In our study, $n=20$ samples were obtained from the maxilla, and $n=36$ samples were obtained from the mandible. The $E_2$ level of the samples from the maxilla was $5113.5 \pm 167-21277$ fmol/mg pr, and the $E_2$ level of the samples from the mandible was $6027.5 \pm 703-23050$ fmol/mg pr. The distribution of $E_2$ levels according to the location was not statistically significant ($p=0.442$). Similarly, the distribution of $E_2$ levels according to gender and location was not statistically significant (Table IV).

No statistical comparison of the groups for $E_2$ levels according to restoration was made because in the male group, there were only two samples with resin restoration. In the female group, no statistically significant differences were found between the $E_2$ levels according to the presence of cavity and composite resin restoration ($p=0.246$) (Table V).

DISCUSSION

The most important factor determining the response of the cells to hormones is the level of the particular hormone and the number of
### TABLE II

*Distribution of E₂ levels (fmol/mg pr) according to tissue type and menstrual phase*

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Menstrual Phase</th>
<th>n</th>
<th>Median</th>
<th>Min-Max</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP</td>
<td>Follicular</td>
<td>7</td>
<td>6028</td>
<td>1583- 10673</td>
<td>0.397</td>
</tr>
<tr>
<td></td>
<td>Luteal</td>
<td>8</td>
<td>4077.5</td>
<td>703- 11167</td>
<td></td>
</tr>
<tr>
<td>SIP</td>
<td>Follicular</td>
<td>14</td>
<td>8737.5</td>
<td>4567- 23050</td>
<td>0.936</td>
</tr>
<tr>
<td></td>
<td>Luteal</td>
<td>11</td>
<td>4077.5</td>
<td>867- 18385</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE III

*Distribution of E₂ levels (fmol/mg pr) according to sex and tissue type*

<table>
<thead>
<tr>
<th>Sex</th>
<th>Tissue Type</th>
<th>n</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Median</th>
<th>Min-Max</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>HP</td>
<td>8</td>
<td>3177,25</td>
<td>1385,31</td>
<td>3624,5</td>
<td>167- 4388</td>
<td>0.161</td>
</tr>
<tr>
<td></td>
<td>SIP</td>
<td>8</td>
<td>2436,5</td>
<td>1712,23</td>
<td>2072,5</td>
<td>270- 5959</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>HP</td>
<td>15</td>
<td>5407,0</td>
<td>3743,62</td>
<td>4255,0</td>
<td>703- 11167</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>SIP</td>
<td>25</td>
<td>10412,3</td>
<td>6060,2</td>
<td>9912,0</td>
<td>867- 23050</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE IV

*Distribution of E₂ levels (fmol/mg pr) according to sex and location*

<table>
<thead>
<tr>
<th>Sex</th>
<th>Location</th>
<th>n</th>
<th>Median</th>
<th>Min-Max</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Maxilla</td>
<td>5</td>
<td>1799,0</td>
<td>167- 5959</td>
<td>0.583</td>
</tr>
<tr>
<td></td>
<td>Mandibula</td>
<td>11</td>
<td>2710,0</td>
<td>1405- 4388</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Maxilla</td>
<td>15</td>
<td>5673,0</td>
<td>1568- 21277</td>
<td>0.233</td>
</tr>
<tr>
<td></td>
<td>Mandibula</td>
<td>25</td>
<td>9653,0</td>
<td>703- 23050</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE V

*Distribution of E₂ levels (fmol/mg pr) according to sex and the presence of cavity and composite restoration*

<table>
<thead>
<tr>
<th>Sex</th>
<th>Issue</th>
<th>n</th>
<th>Median</th>
<th>Min-Max</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male+Female</td>
<td>Cavity with no Restoration</td>
<td>25</td>
<td>6038,2</td>
<td>867- 21277</td>
<td>0.606</td>
</tr>
<tr>
<td></td>
<td>Composite Restoration</td>
<td>8</td>
<td>9761,5</td>
<td>270- 23050</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Cavity with no Restoration</td>
<td>19</td>
<td>9653,0</td>
<td>867- 21277</td>
<td>0.246</td>
</tr>
<tr>
<td></td>
<td>Composite Restoration</td>
<td>6</td>
<td>12021,0</td>
<td>4567- 23050</td>
<td></td>
</tr>
</tbody>
</table>
receptors that are sensitive to this hormone\textsuperscript{19}. Although ER-\(\alpha\) was determined in the odontoblasts, endothelial cells, and Schwann cells of the pulp tissue, we could not come across with any study on the endogen E2 level in the pulp tissue.

The results of our study indicated that endogen E\(_2\) levels of the samples obtained from the female patients were higher than those of the male patients and this difference was statistically significant. This finding is parallel to the literature information indicating a difference between the serum E\(_2\) levels of male and female genders\textsuperscript{20}. The E\(_2\) levels of the samples obtained from the maxilla and mandibula were compared, and no statistically significant differences were determined.

In this study, the E\(_2\) levels of the healthy pulp tissues and SIP tissues were compared for the first time. The total E\(_2\) level of SIP group was statistically significantly higher than that of the healthy pulp tissue. Statistical studies have shown differences between the healthy pulp tissues and SIP tissues of female patients. In an adult female, the serum E\(_2\) level varies depending on the menstrual phase. According to the history of the female patients participating in the study, the menstrual cycles were determined as follicular or luteal phase\textsuperscript{21}. The E\(_2\) levels of the groups (follicular and luteal) were not statistically significant. In the male patients, the E\(_2\) level of the SIP tissues was higher than that of the healthy pulp tissues was. However, the difference was not statistically significant. This may have been due to lower number of samples obtained from the male patients.

Peripheral aromatization is an important source of estrogen\textsuperscript{1}. In one study, cytokines and matrix metalloproteinase during infections of the cornea were shown to affect the expression of the enzymes that contribute to aromatization and thus, E\(_2\) is synthesized in the tissue by peripheral aromatization\textsuperscript{22}. In case of an inflammation of the pulp, the expression of cytokines such as IL-6, IL-8\textsuperscript{23} and TNF-\(\alpha\)\textsuperscript{24} and of MMP\textsuperscript{25} increases. In our study, the endogen E\(_2\) level was increased in the inflamed pulp tissues. This is suggestive of peripheral aromatization of estrogen in this tissue. Aromatase enzyme has a local role in the expression of estrogen hormone by peripheral aromatization\textsuperscript{26}. Nevertheless, literature review revealed no studies on the presence of aromatase enzyme in the pulp tissue. In an earlier study, the increase in the estrogen level upon local aromatization determined after a pathological change was reported to cause no significant association between the serum and tissue E\(_2\) concentration\textsuperscript{27}. In our study, because serum E\(_2\) levels could not be measured, it was not possible to determine whether the elevated level of endogen was a local increase.

Pulp inflammation is a complex condition involving variations of vascular and neural system reactions. In the healthy pulp tissue, various neuropeptides such as substance P, calcitonin gene-related peptide, and neurokinin, which play an active role in homeostatic regulation, have been shown to increase in pulpal inflammation\textsuperscript{28}. Several studies have also shown inflammation modulating capacity of estrogen by interacting with various mediators that have a role in pain and inflammation\textsuperscript{29,30}. Bjorling et al\textsuperscript{31}, in their study on the bladder tissue, have reported that despite suspected pain and inflammation stimulating effect of fluctuations in estrogen concentration alone, estrogen may have a role in determination of the response to neurogenic inflammation. Determination of ERs in the Schwann cells of the pulp tissue\textsuperscript{9} suggests that neural structures in the area may be affected by E\(_2\) as well. As our study has found an increase in E2 levels in SIP tissues, relationship between estrogen with the pain experienced in pulpitis might be a subject for further research.

Bisphenol A (BPA) is a chemical with estrogenic effects and it exists in the structure of some dental materials used in dentistry, such as fissure sealants\textsuperscript{32}, composite filling substances\textsuperscript{33}, and polycarbonate based temporary crowns\textsuperscript{34}. Despite studies on the leak of bisphenol A from
dental materials to the saliva, its effect on the pulp is not clearly known. The presence of estrogen receptors in the pulp tissue suggests that if BPA and similar endocrine substances reach the pulp through dentin tubules, they may cause various chemical reactions. In our study, the endogenous E2 levels of SIP tissues with or without composite resin restoration were compared, and no statistically significant differences were found.

Lack of information on the serum E2 levels of the patients from whom the samples were obtained and relatively lower number of the samples obtained from the male patients comprises the limitations of our study.

CONCLUSION

Since the pulp tissue is frequently exposed to inflammatory agents through dentin tubules and apical foramen and is surrounded by dentin, it is attractive for studies on inflammation. To the best of our knowledge, our study is the first to compare the endogenous estrogen levels of healthy and inflamed pulp tissues. Higher level of endogenous E2 level in the pulp tissues of the female patients with SIP may pave the way for new hormonal approaches to the etiology and treatment of pulpal diseases.

REFERENCES


