Oral Hygiene in Children with Epilepsy: Effect of Interleukin-1β and VEGF Levels in Gingival Crevicular Fluid

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Abstract

Aim: Biochemical parameters in gingival crevicular fluid (GCF) are affected by some systemic diseases and poor oral hygiene. The aim of this study was to evaluate the total amounts of Interleukin-1β (IL-1β) and vascular endothelial growth factor (VEGF) in GCF in children with epilepsy. Material and methods: 80 children with epilepsy in free seizure period (Test group) and 80 healthy children (Control group) were evaluated. Gingival index (GI), plaque index (PI), probing depth (PD), clinical attachment level (CAL) was measured. GCF was collected and its volume was measured. The total amounts of IL-1β and VEGF in GCF were analyzed in Biochemistry Laboratory. Results: The biochemical (IL-1β and VEGF) and clinic parameters (GI, PI, PD and GCF volume) were significantly higher in the children with epilepsy compared with healthy children (p<0.001). When the epilepsy patients were divided into groups according to the drug used in this study, there were no significant differences in GCF levels of IL-1β and VEGF among the drug groups. Conclusion: According to these results it may be suggested that patients need an optimal oral care in epilepsy.

Key Words: Cytokine, Children, Epilepsy, GCF, Oral health

Introduction

The World Health Organization estimates that the 0.8% of world's population is affected from epilepsy, which is a brain disorder [1]. In 35% of the cases, epilepsy is due to an identifiable central nervous system (CNS) injury, whereas in the rest of the cases, it is as a result of either genetic or unknown causes [2].

For preventing seizure, different drugs are used in the treatment of epilepsy. But there are some side effects of these medications. For example, in 30 to 50% of the cases, phenytoin develops a significant gingival overgrowth. Although the reason of the gingival overgrowth is not fully understood, some studies have shown that inflammatory cytokines and growth factors may play an important role in the pathogenesis of gingival changes [3,4]. IL-1β, 17-kDa glukoprotein is a potent proinflammatory cytokine. It is produced predominantly not only by monocytes/macrophages, but also by fibroblasts and bone cells [5]. Its production may be induced by microorganisms, microbial products, inflammatory agents and antigens [6]. Some studies related to the role of cytokines have focused on IL-1β in epilepsy [7,8]. It has been found that IL-1β plays a role in the development of a neuronal cell death after traumatic, ischemic, excitotoxic and seizure-induced brain injuries [9].

Several clinical studies addressed the change of IL-1β levels in blood and cerebrospinal fluid (CSF) of patients with focal epilepsy. There were no significant differences in the IL-1β concentration in blood and CSF within 24 hours after tonic–clonic seizures when compared to the control subjects [10]. More recent studies in patients with focal epilepsy have similarly showed that postictal plasma concentrations of IL-1β after complex partial or secondary generalized tonic–clonic seizures have not significantly differed from baseline levels [11].

Gingival crevicular fluid (GCF) is an exudate that is originated from gingival crevices or periodontal pockets around teeth with inflamed gingiva. GCF contains a variety of materials, including leukocytes (mainly neutrophils), antibodies, complement proteins, various enzymes, and cytokines [12-16]. Therefore, GCF component or constituents measurements are useful to show the alterations in gingival tissues. Additionally, in oral cavity, poor oral hygiene, gingival and periodontal diseases without a gingival enlargement also can modify the contents of GCF. In some studies, poor oral hygiene, gingivitis, periodontitis or a gingival injury increases IL-1β and VEGF values in GCF [17-19]. IL-1β has an important role in regulating and increasing the inflammatory response in periodontal diseases. IL-1β levels in periodontal tissues and GCF have been closely associated with periodontal disease severity and it has been found that they significantly increased in diseased sites, compared to healthy sites [19].

A vascular endothelial growth factor (VEGF), 45-kDa homodimeric glycoprotein, is an angiogenesis and vascular permeability factor, that increases the microvascular permeability, stimulates the endothelial cell (EC) proliferation, and induces the proteolytic enzyme expression and the migration of ECs, monocytes, and osteoblast, all of which are essential for the angiogenesis [20]. An increase in VEGF in GCF affects the periodontal vasculature profoundly during the periodontal disease progression [17,18]. The increased concentration of VEGF in periodontitis may be one of the reasons of the increased vascularization and permeability, and it may be considered as a sign of severe periodontitis [17,21,22]. VEGF is detectable in periodontal tissues within endothelial cells, plasma cells, and macrophages, in junctional, sulcular, and gingival epithelium, and in GCF [22]. Moreover, VEGF expression may be induced by IL-6, IL-1, PGE2. VEGF also appears to be regulated by the oxygen concentration that cells are exposed to, with hypoxia inducing its expression [23].

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In this study, we aimed to investigate the total amounts of IL-1β and VEGF in GCF in epileptic patients in seizure-free period and focused on the relationship between oral hygiene and these biochemical parameters.

**Material and Methods**

**Patient selection**

This study was performed in the Department of Pediatric Neurology, Faculty of Medicine and Department of Pedodontics, Faculty of Dentistry Ataturk University at 2012. Samples were analyzed at the Biochemistry Department. A total of 160 subjects, 80 pediatric patients with epilepsy- test group (36 girls, 44 boys) and 80 systemically healthy children- control group (46 girls, 34 boys), aged 4-11 participated in this study. The study was approved by the local ethics committee of Medical School and performed in accordance with good clinical practice guidelines. Informed consent was obtained from the parents of patients. The children were excluded from the study if they had any systemic diseases, periodontal diseases, dental caries or filling in the maxillary anterior teeth, and had taken antibiotics, anti-inflammatory drugs. The children in test group had other systemic diseases except epilepsy and they had had no seizures in the last 1 month.

**Clinical measurements**

Periodontal examinations were carried out and recorded by the same investigator. None of the study patients has a gingival overgrowth. Four points (mesio-buccal, mesio-oral, disto-buccal, disto-oral) of per maxillary anterior primary teeth were selected for clinical measurements. If there were no maxillary anterior primary teeth, permanent maxillary anterior teeth were selected. The selected teeth were caries-free and unfilled. The gingival conditions of the patients were evaluated by Silness-Loe plaque index (PI) [24], Loe gingival index (GI) [25], clinical attachment loss (CAL) and probing pocket depth (PD) to the nearest millimeter with a Williams probe (Aesculap, Inc. USA). The obtained scores were calculated by dividing the total score by the total number of scored surfaces.

**GCF collection**

The selected teeth sites were mesiobuccal sites of maxillary central or lateral in primary or permanent dentition for collecting GCF. A total of 160 GCF samples were obtained, 80 from epilepsy patients and 80 from a healthy group. The upper anterior teeth were included in the study to improve the access and to reduce the risk of salivary contamination during these processes. To avoid the blood contamination and the possible stimulation of GCF flow during clinical measurements, samples were collected before any other clinical recordings except the PI. Prior to sampling, each selected site was carefully isolated using cotton rolls. Paper strip (Periopaper®, Ora Flow, NY, USA) was placed in the pocket until a mild resistance was felt and then left in the place for 30 s. In the case of the visible contamination with blood, the strips were removed. To eliminate the risk of vaporization, paper strips with GCF were immediately carried to a previously calibrated electronic gingival fluid measuring device (Periotron® 8000; Oralfow PO Box 219, Plainview, NY, USA) for a volume calculation. After the volume calculation by Periotron [26], periotron scores via a computer using ml convert program were converted to microliter (μl). The samples were put into an Eppendorf tubes in 300 μl phosphate buffered saline (PBS), containing 100 μl of 2% bovine serum albumin in PBS and immediately frozen at -80 °C until the day of the laboratory analysis.

**IL-1β and VEGF assay**

The level of IL-1β (eBioscience® Platinum ELISA, NY, USA) and VEGF (RayBio®, NY, USA) in GCF was measured by ELISA and the analysis was performed according to the manufacturer’s instructions using human recombinant standards in Biochemistry Laboratory. The results were reported in pg/ml per sample. The sensitivity was 0.3 pg/ml for IL-1β ELISA while it was 10 pg/ml for VEGF ELISA. The samples with IL-1β levels below the limits of the assay’s detectability were scored 0. The results of IL-1β were multiplied by the dilution factor (>2).

Results were converted to μl being divided by 1000. GCF volumes were added to the dilution factor and multiplied by IL-1β and VEGF levels.

**Statistical analysis**

Data analysis was performed using the statistical package SPSS 16 (2008, SPSS Inc., Chicago Illinois, USA). The results were expressed as means ± standard deviations. The data were firstly analyzed for the normal distribution with Kolmogorov-Smirnov test. The biochemical parameters between the groups were compared with Student’s t- test, while clinical parameters between the groups were compared with non-parametric Mann-Whitney U test. Non-parametric Kruskall Wallis test was used to evaluate the differences among the drug groups.

Pearson correlation test was used to verify the correlations between the biochemical parameters, while Spearman’s Rank correlation test was used between the clinical parameters. Spearman’s Rank correlation test was used to evaluate the relationship between the clinical and biochemical parameters.

The qualitative non-parametric variable of CAL was not analyzed because CAL of both healthy and unhealthy children with epilepsy was ≤2 mm.

**Results**

In the epilepsy group, the clinical and biochemical parameters were significantly higher than in the control group (p<0.0001 for all) (Table 1). Looking at the distribution of the drug in epilepsy patients, in the 46, 12, 10, and 12 of the 80 cases, they were taking valproic acid (VPA), carbamezapine, phenobarbital, and the combinations of these drugs, respectively. These patients have used their drugs for at least 6 months. When the patients were divided into groups according to the drug used in this study, there were not any significant
differences in the clinical and biochemical parameters among the drug groups (p>0.05).

**Table 1.** The mean ± SD of total amounts of IL-1β and VEGF and clinic parameters of both groups.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Healthy</th>
<th>Epilepsy</th>
<th>p values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IL-1β (pg)</td>
<td>4.02 ± 0.89</td>
<td>17.15 ± 6.90</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total VEGF (pg)</td>
<td>48.46 ± 10.92</td>
<td>124.06 ± 35.57</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

| Clinic Parameters | | | |
| GI              | 0.26 ± 0.13 | 0.81 ± 0.14 | 0.0001 |
| PI              | 0.35 ± 0.20 | 0.75 ± 0.14 | 0.0001 |
| PD              | 0.82 ± 0.24 | 1.78 ± 0.17 | 0.0001 |
| GCF volume (µl) | 0.06 ± 0.02 | 0.17 ± 0.08 | 0.0001 |

The p values show the comparison of the values in the same row

There was a statistically significant positive correlation between total IL-1β and VEGF in both groups (p=0.0001). The correlation between IL-1β-PI (r=0.359 p=0.001), VEGF-GI (r=0.419 p=0.0001), VEGF-PI (r=0.347 p=0.002), GI-PI (r=0.466 p=0.0001), and PD-GCF volume (r=0.662 p=0.0001) was found in healthy children. Regarding the children with epilepsy, it was found between IL-1β-GI (r=0.651 p=0.0001), IL-1β-PI (r=0.481 p=0.0001), VEGF-GI (r=0.635 p=0.0001), VEGF-PI (r=0.534 p=0.0001), GI-PI (r=0.676 p=0.0001), and PD-GCF (r=0.319 p=0.004) volume in them.

**Discussion**

The present study has showed that in epilepsy patients, total amounts of IL-1β and VEGF in GCF were significantly higher than in the healthy children. To our knowledge, this is the first report measuring GCF levels of IL-1β and VEGF in epilepsy patients.

In our study, all patients were taking antiepileptic drugs in the seizure-free period. Researchers found that IL-1β and VEGF production increased in blood and brain during the epileptic seizure [10,27,28], Vezzani et al. [8] reported that cytokine levels approximate to the normal values in brain tissue in the next three to seven days after the seizure. In this study, an increase in biochemical parameters in GCF couldn’t be attributed to the epileptic seizures because all the epilepsy cases were seizure-free.

Moreover, increased total amounts of IL-1β and VEGF in GCF may be due to other some causes in epilepsy patients. Gingival enlargement may be one of the factors that increase IL-1β and VEGF to damage the integrity of the gingiva. Some antiepileptic drugs (especially phenytoin) have side effects such as gingival enlargement. This situation was found to be rare in patients taking other antiepileptic drugs such as phenobarbital, phenytoin, valproic acid, carbamazepin, and levetiracetam. In our study, none of the patients with epilepsy was taking phenytoin. Besides, we couldn’t find a significant gingival enlargement in the patient population. The authors reported that valproic acid prevented an inflammation [29], and carbamazepine could cause an inflammation and hepatic injury as a result of this [30]. We couldn’t find any information about possible anti-inflammatory effects of phenobarbital. But, in our study, when patients were classified according to the use of the drug, it was not found any differences in IL-1β and VEGF levels of GCF among the drug groups.

We thought that the cause of the increased total amounts of IL-1β and VEGF in GCF may be from different factors rather than seizure, drugs or gingival enlargement. IL-1β is one of the most important inflammatory cytokines in an oral cavity that plays a major role in gingivitis and periodontitis [19], while VEGF is one of the primary mediators of the neovascularization in chronic inflammation, including a periodontal disease and wound repair [31] that plays a major role in periodontal diseases to increase the vascularization and permeability and help the passage of inflammatory cells and it may be considered as a sign of the severity of periodontitis [8,18,19]. There are various bacterial species embedded in a matrix of bacterial products and host-derived factors in dental biofilm or dental plaque. This biofilm can stimulate an inflammation in gingival tissue. The increase of the GCF volume is an indication of inflammation and subsequent clinical signs of gingivitis [32]. Studies show that a plaque increases crevicular IL-1β and VEGF [18,19]. Pathogenic bacteria in the subgingival plaque cause a subepithelial infiltration by inflammatory cells, which produce and release IL-1β, thus an IL-1β concentration in GCF increases in injury as a result of this [30]. We couldn’t find any differences in IL-1β and VEGF levels of GCF among the drug groups.

Many systemic diseases may adversely affect the oral health due to the changes in metabolism and the drug use. In some studies, which support our study, it was shown that epilepsy cases have poor oral hygiene and it has been found that GI and PI scores are higher in epileptics than in healthy children [33,34].

Gunerli et al. [21] investigated the VEGF concentration in GFC in the patients with periodontal diseases. Some of these patients (group I) had diabetic diseases, while the others...
(group II) were healthy without any systematic diseases. The differences of VEGF levels were found statistically insignificant between the periodontally healthy sites of these two groups and the negative control group. But they found statistically significant differences between periodontally healthy and unhealthy sites of these two groups, which is similar to Booth et al. [22] Guneri’s study indicated that oral situation is more important than systemic disease for GCF mediators, because VEGF levels in GCF were not different between periodontally healthy sites of diabetic patients and control group. This result indicates that oral hygiene may be one of the factors increasing VEGF that is unrelated to epilepsy.

Sandalli [35] recorded a positive correlation between PD and GCF volume in gingiva of 3-5 years old children. The correlation was more statistically significant in a gingivitis region than in healthy gingiva. Yucel et al. [36] reported higher GCF volumes in a deep pocket compared to a shallow pocket. In our study, in addition, we found a positive correlation between PD and GCF volume in both groups.

In this study, we found that the children with epilepsy were more likely to have a periodontal disease than healthy children. We could not give any exact reasons that this situation was an immune system problem caused by epilepsy or neglected oral hygiene. In our study, there was a limitation that blood specimens were not taken from the patients. Thus, we could not compare these biochemical parameters in serum and GCF. Further studies are needed to clarify the effect of epileptic drugs on the immune system.

If this situation is not a problem originated from the immune system, it should be focused on why these children care less about oral hygiene than healthy children. They spend most of their lives at hospital and constantly have to take medication. For all these reasons, oral care may be difficult and tedious for them.

**Conclusion**

Our study revealed that IL-1β, an inflammation indicator, and VEGF, an angiogenesis mediator, increase in GCF of epilepsy patients and we can say that epilepsy patients may be at risk of periodontal disease in their future lives.

Further longitudinal clinical studies are required in order to study the oral health of the children with epilepsy and the probable diagnostic value of combinations of inflammatory mediators.

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**Declaration of Conflicting Interests**

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**Ethical Approval**

This study had ethical approval. The acceptance number is B. 30.2.ATA.0.01.00/118.

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