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ORIGINAL ARTICLE

The impact of the IL-11:IL-17 ratio on the chronic periodontitis pathogenesis: a preliminary report

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OBJECTIVE: An imbalance in the pro- and anti-inflammatory cytokines may be responsible for periodontal breakdown through immune responses. This study aimed to determine the total amount, concentration and ratio of interleukin (IL)-11 and IL-17 in gingival crevicular fluid (GCF) of chronic periodontitis (CP) patients.

MATERIALS AND METHODS: Forty CP patients and 20 healthy controls (C) were included. The CP group was divided into two subgroups in line with the probing depth (PD) in GCF-sampling sites (CPa: PD ≥ 5 mm, CPb: PD ≤ 4 mm). For each patient, gingival index, plaque index, gingival bleeding time index, PD, and clinical attachment level values were recorded. IL-11 and IL-17 in GCF were evaluated by enzyme-linked immunosorbent assays.

RESULTS: The total amount and concentration of IL-11 and IL-17 were significantly lower in the CPa group ($P < 0.05$). The C group has the highest IL-11:IL-17 ratio, followed by CPb and CPa groups respectively. The ratio was significantly lower in CPa group than the CPb and C groups ($P < 0.01$).

CONCLUSION: Our data confirm that the decreased ratio of IL-11:IL-17 may be a factor, which has shown this imbalance between the cytokines' activities in deeper pockets in our study.

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Keywords: IL-11; IL-17; chronic periodontitis; periodontal pathogenesis

Introduction

The initiation and progression of periodontal disease depend on complex interactions between periodontopathic bacteria and the host immune system. Like the other chronic inflammatory diseases, cytokines are considered to play an important role in the initiation, progression and host modulation of periodontal inflammation. The relative importance of Th1 and Th2 subsets and nature of their cytokine synthesis in periodontal inflammation have also been investigated. Th1 cells induce the cellular immunity with the production of interleukin (IL)-1β, interferon-γ, and IL-6. Th2 cells provide the humoral immunity with the B-cell growth and differentiation with the synthesis and secretion of cytokines like IL-4, IL-5, IL-10 and IL-13. There is an attributed balance between the pro- and anti-inflammatory cytokines to avoid tissue destruction (Seymour and Gemmell, 2001; Kinane and Lappin, 2002; Silva *et al*, 2007). Thus, the investigations about this balance, especially the presence/absence; the decrease/increase of the total amount/concentrations of the cytokines are important in clarifying the process of the periodontal pathogenesis.

Interleukin 11 is a pleiotrophic cytokine, produced by a variety of stromal cells including fibroblasts, epithelial cells, and osteoblasts (Kishimoto *et al*, 1995). IL-11 has many biologic activities and roles; e.g. in hematopoiesis, immune responses, the nervous system and bone metabolism (Du and Williams, 1997). IL-11 was reported to have anti-inflammatory and Th2-polarizing effect and has a clinical potential in Th1-predominant inflammatory diseases and exerts its modulatory effect on naïve T cells (Curti *et al*, 2001) and on the expression of pro-inflammatory cytokines such as IL-1β, tumor necrosis factor (TNF)-α, IL-6, IL-12 and nitric oxide by macrophages (Redlich *et al*, 1996; Trepicchio *et al*, 1997; Martuscelli *et al*, 2000; Seymour and Gemmell, 2001). However, the effects of IL-11 on connective tissue cells are diverse. Similar to both IL-6 and TGF-β, IL-11 can potentially regulate inflammation by inducing TIMP-1 in synoviocytes and chondrocytes *in vitro* (Hermann *et al*, 1998).

Interleukin 17 is a pro-inflammatory cytokine, produced by Th17 cells (Kramer and Gaffen, 2007; Nakae *et al*, 2007; Lubberts, 2008). Polymorphonuclear cells

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were also reported to produce IL-17 (Witowski *et al*, 2000; Ferretti *et al*, 2003). IL-17 activates the fibroblasts and endothelial cells to increase their secretion of IL-6 in the presence of TNF-α (Katz *et al*, 2001; Nakchbandi *et al*, 2001). IL-17 induces exacerbation of periodontal disease by activating the gingival fibroblasts to produce inflammatory mediators and was found to be associated with cells producing receptor activator of nuclear factor kappa B (RANK), which may be related to bone resorption activity (Takahashi *et al*, 2005). However, Yu *et al* (2007) have reported an essential role for IL-17 in preventing pathogen-initiated bone destruction via neutrophils mobilization.

Although studies aimed to explain the possible relations of cytokines produced from Th1 and Th2 cells in periodontal diseases, the results are inconclusive. An imbalance of the cytokines derived from Th1 and/or Th2 cells is suggested as being responsible for periodontal breakdown through cellular and/or humoral hyperimmune responses. The balance between the Th1/Th2 cytokines has been investigated relatively extensively when compared with the Th17 cytokines, although the importance of the IL-17 cytokine family has been known for several years.

In this regard, it is obvious that a balance between Th1/Th2 and Th17 cells and cytokines in periodontal disease should not be disregarded and it would be necessary to clarify the role of this balance. Thus, the aim of this study was to evaluate the levels and the ratio of an anti-inflammatory (IL-11) and a pro-inflammatory (IL-17) cytokine and this local cytokine response in relation to clinical periodontal status in periodontal health and disease.

Materials and methods

Informed consent was obtained from the subjects and the protocol was approved by the Ethics Committee of Süleyman Demirel University Faculty of Medicine (01.06.2006, 04/09).

Selection of subjects

The volunteered individuals fulfilling the study criteria below were selected from patients referred to receive periodontal treatment at Süleyman Demirel University, Faculty of Dentistry, and Department of Periodontology between the period June 2006 and December 2006.

All of the subjects were non-smokers and had at least 20 teeth. The chronic periodontitis (CP) ($n = 40$) patients were selected according to the clinical and radiographic criteria (loss of clinical attachment >4 mm at two or three sites in more than two teeth in each quadrant, and radiographic evidence of bone loss). The CP group was then divided into two subgroups in line with the probing depth (PD) values of the GCF sampling sites as CPa: PD ≥ 5 mm and CPb: PD ≤ 4 mm. In addition to these two CP groups, periodontally healthy individuals had constituted the healthy control group (C, $n = 20$), who were admitted to the clinic for their annual controls and had no evidence of clinical attachment and radiographic bone loss in the dentition. All of the GCF samples were obtained from the sites having PD ≤ 3 mm in C group.

None of the subjects had any known systemic disorder or used prescribed antibiotics and/or anti-inflammatory medications in the last 3 months. Patient and control subjects with active infectious diseases such as hepatitis, HIV infection, and tuberculosis or those chronically treated with medications (phenytoin, cyclosporin-A, or calcium channel blockers), as well as females who were lactating or pregnant, were excluded.

Periodontal examination

For each patient, gingival index (GI, Löe and Silness, 1963), plaque index (PI, Silness and Löe, 1964), gingival bleeding time index (GBTI, Nowicki *et al*, 1981), PD, and clinical attachment level (CAL) values were recorded. The clinical examination was carried out by the same examiner (ZYA) and calibrated before the study. PD and CAL values were measured with a Williams probe (Hu-Friedy, Chicago, IL, USA). The probe was directed in line with the long axis of the tooth. The CAL measurements were made from the cemento-enamel junction to the bottom of the sulcus/pocket.

Gingival crevicular fluid sampling

Clinical measurements were recorded and gingival crevicular fluid (GCF) sampling sites were preselected 1 week before sampling. Maxillary teeth were selected for sampling, to reduce the possibility of contamination with saliva. GCF samples were collected from at least four non-adjacent maxillary anterior sites, which were fulfilling the criteria (CPa: PD ≥ 5 mm, CPb: PD ≤ 4 mm, C: PD ≤ 3). In CPa group, 122 samples from 98 teeth, in CPb group 131 samples from 102 teeth and in C group 120 samples from 120 teeth were obtained.

After isolating the tooth with a cotton roll, supragingival plaque was removed with curettes (Hu-Friedy), without touching the marginal gingiva. The crevicular site was then dried gently with an air syringe. A saliva ejector and cotton rolls were used to avoid salivary contamination. Periopaper Gingival Fluid Collection Strips (Periopaper, Amityville, NY, USA) were held within the gingival sulci for 30 s in the sampling site of each patient. In case of visible contamination with blood, the strips were discarded and other sites fulfilling the same criteria were selected. The strips were transferred, to the chair-side located Periotron 8000 (Oraflow Inc., Plainview, NY, USA), for volume determination. After the measurement, the strips of each person were transferred to the same Eppendorf tubes and isolated with parafilm to avoid evaporation. Each sample was stored at −80°C until assayed.

IL-11 and IL-17 analysis

After thawed, strips were eluted with 700 μl of Hank' s balanced salt solution containing 0.5% bovine serum albumin by centrifugation (3000 *g*, 4°C, 15 min). For quantitative detection of IL-11 and IL-17 enzyme-linked immunosorbent assays (ELISA) (IL-11: United States Biological, Swampscott, MA, USA; IL-17: BioSource

International, Inc, Camarillo, CA, USA) employed. IL-11 and IL-17 present in the sample were bound to anti-IL-11 and anti-IL-17 monoclonal coating antibody absorbed to the microwells. The second polyclonal antibodies were added, and following incubation colored products were formed in proportion to the amount of IL-11 and IL-17 present in the sample. The reactions were measured at 450 nm. The total amount of IL-11 and IL-17 were determined in picograms (pg) and calculations of the concentration in each sample were performed by dividing the total amounts of IL-11 and IL-17 by the volume of the sample (pg ml^{-1}).

Statistical analysis

Continuous variables (age, GI, PI, GBTI, PD, CAL, IL-11 total amount and concentration, IL-17 total amount and concentration) are presented as median, minimum and maximum, while categoric variables (gender) are presented as frequencies. Correlations between clinical periodontal parameters and GCF cytokine total amounts and concentrations were tested by the calculation of Spearman's *rho* (for skewed variables) correlation coefficients. The presence of the statistically significant differences among the groups was defined with Kruskal–Wallis test. Analyses of difference between continuous variables and the two groups were performed by the use of Mann–Whitney *U*-test. All reported *P*-values are based on two-sided tests and compared with a significance level of 5%. However, because of multiple significance comparisons, we used the Bonferroni correction in order to account for the increase in Type I error. The *P*-value after this correction and acceptance of the significance threshold, $P < 0.0125$. SPSS 9.0 soft-ware (SPSS Inc., Chicago, IL, USA) was used for all the statistical calculations.

Results

The characteristics and clinical periodontal parameter values

The characteristics and clinical periodontal parameter values of the groups in sampling were shown in Table 1. All of the clinical parameters for the whole mouth and sampling sites values were significantly lower in CPb group when compared with the CPa group ($P < 0.0125$).

Total amount and correlations of IL-11 and IL-17

The GCF volume, total amounts and correlations of IL-11 and IL-17 have been shown in Table 2. The GCF volume was found to be the highest in CPa group (significantly higher than CPb and C groups, $P < 0.0125$), followed by CPb and C groups, respectively.

The total amount and concentration of IL-11 was significantly higher in C and CPb groups than in CPa group ($P < 0.0125$), not significantly different from each other ($P > 0.0125$). No significant differences were found among the groups in the total amount of IL-17 ($P > 0.0125$). The CPa group had significantly lower IL-17 concentrations than CPb and C groups ($P < 0.0125$). The difference between the CPb and C groups was found to be statistically insignificant ($P > 0.0125$).

Correlations

The statistically significant correlations among the clinical periodontal parameters in sampling sites, and total amounts and concentrations of IL-11 and IL-17 have been shown in Table 3. The total amount of IL-11 expressed significant positive correlation with CAL values in CPa group ($P < 0.0125$), and GBTI values in C group ($P < 0.0125$, Table 3.). The GCF IL-11 concentrations had significant positive correlations with CAL values in CPa group and with GBTI values in C group ($P < 0.0125$). No correlations between the total amount and concentrations of IL-17 and the periodontal parameter values in any groups were observed (data not presented, $P > 0.0125$).

Cytokine ratios

The IL-11:IL-17 cytokine ratios (total amount and concentration) were significantly higher in C group than CPa group, and the CP groups found to be significantly

Table 1 The characteristics and periodontal parameter values of the groups in sampling sites [median (minimum–maximum)]

	CPa (n = 20)	*CPb (n = 20)*	*C (n = 20)*
Age	42.50 (28–65)*	43.50 (32–55)*	27 (25–48)
Female/male	10/10	8/12	8/12
GI	2.52 (1.16–2.75)*	1.10 (0.33–2.66)*	0.30 (0–1.23)
PI	2.82 (1.25–2.15)*	0.96 (0.15–2.92)*	0.30 (0.08–1.59)
GBTI	2 (0.48–3)*	1.16 (0.55–2.24)*	0.54 (0–0.73)
PD (mm)	3.75 (2.27–5.68)*	1.87 (1.14–3.53)	2.19 (1.09–3.12)
CAL (mm)	4.87 (2.52–8.46)*	2.35 (1.44–5.50)	2.30 (1.23–3.18)
GIs	2.5 (1–2.28)*	0.69 (0–3)*	0 (0–1)
PIs	2.88 (0.60–3)*	0.84 (0–3)*	0.07 (0–1.5)
GBTIs	2 (0.83–3)*	1 (0–2)*	0.11 (0–0.83)
PDs (mm)	6.25 (5–8.20)*	2.16 (1–3.33)*	1.5 (0.07–2.5)
CALs (mm)	6.83 (5–9.40)*	2.41 (0–4.33)	1.5 (0.07–2.5)

CPa, chronic periodontitis PD ≥ 5 mm; CPb, chronic periodontitis PD ≤ 4 mm; C, control; GI, gingival index; PI, plaque index; GBTI, gingival bleeding time index; PD, probing depth; CAL, clinical attachment level; GIs, gingival index in sampling site; PIs, plaque index in sampling site; GBTIs, gingival bleeding time index in sampling site; PDs, probing depth in sampling site; CALs, clinical attachment level in sampling site.
*Statistically significantly different than the C group (Mann–Whitney *U*-test, $P < 0.0125$).

Table 2 The gingival crevicular fluid (GCF) volume, total amount (pg) and concentrations (pg ml^{-1}) of the cytokines IL-11 and IL-17 [median (minimum–maximum), $n = 20$ for each group]

	Groups		
	CPa	*CPb*	*C*
GCF (ml)	0.004 (0.001–0.007)*	0.001 (0.005–0.004)	0.001 (0.001–0.002)
IL-11 (pg)	30.56 (2.50–165.63)*	166.76 (41.42–323.50)	166.76 (69.83–432.82)
IL-11 (pg ml^{-1})	0.01 (0–0.14)*	0.115 (0.02–0.58)	0.13 (0.06–0.39)
IL-17 (pg)	11.62 (6.76–18.93)	12.83 (0–26.25)	14.05 (6.76–21.37)
IL-17 (pg ml^{-1})	0.003 (0–0.02)*	0.01 (0–0.02)	0.01 (0.01–0.03)

CPa, chronic periodontitis PD ≥ 5 mm; CPb, chronic periodontitis PD ≤ 4 mm; C, control; GCF, gingival crevicular fluid.
*Statistically significantly different than the C group (Mann–Whitney *U*-test, $P < 0.0125$).

Table 3 The statistically significant correlations between the clinical periodontal parameters and the total amount and concentration of IL-11 and IL-17 in gingival crevicular fluid

	Parameters	*ρ*	P-*value*
CPa group	CALs – IL-11 (pg)	0.566	0.009**
	CALs – IL-11 (pg ml^{-1})	0.511	0.024*
C group	GBTIs – IL-11 (pg)	0.493	0.027*
	GBTIs – IL-11 (pg ml^{-1})	0.546	0.01*

CPa, chronic periodontitis PD ≥ 5 mm; C, control; GBTIs, gingival bleeding time index in sampling site; CALs, clinical attachment level in sampling site; IL-11, interleukin-11; Spearman Correlation test: *$P < 0.05$, **$P < 0.01$.

Table 4 Ratios of the IL-11:IL-17 in GCF in CPa, CPb and C subjects ($n = 20$ for each group)

	Groups			
Cytokine ratio	*CPa*	*CPb*	*C*	P-*value*
IL11:IL-17[a]	2.95:1*	8.72:1	11.23:1	< 0.01
IL-11:IL-17[b]	3.02:1*	8.42:1	11.22:1	< 0.01

CPa, chronic periodontitis PD ≥ 5 mm; CPb, chronic periodontitis PD ≤ 4 mm; C, control.
*Statistically significantly different than the C group (Mann–Whitney *U*-test, $P < 0.0125$).
[a]Ratio of the total amount of IL-11 to IL-17.
[b]Ratio of the concentration of IL-11 to IL-17.

different from each other ($P < 0.0125$). The C group presented any significant differences in total amount and concentration ratios of IL-11 and IL-17 when compared with the CPb group ($P > 0.0125$). The ratios found higher in C group, followed by CPb and CPa groups, respectively (Table 4).

Discussion

The intensity, prognosis and resolution of inflammation depend on shifting the balance between the activities of pro-inflammatory and anti-inflammatory cytokines during the periodontal inflammation stages (Kinane and Lappin, 2002; Honda *et al*, 2006). This study evaluated the total amount and concentration of IL-11 and IL-17 in GCF of CP patients. Our data confirm that the concentration and the ratio IL-11 and IL-17 were found to be significantly lower in CPa group when compared with the CPb and C groups.

As known, IL-11 is a pleiotrophic member of the IL-6-type cytokine family. IL-6 induces bone resorption by enhancing osteoclasts formation *in vitro* (Girasole *et al*, 1994). In contrast, IL-11 stimulates osteoblasts activity *in vitro* and bone formation *in vivo* (Takeuchi *et al*, 2002). It was predicted that IL-11 may have a regulatory role in modulating the host defense and is a candidate molecule for therapeutic modulation of the host response in the management of periodontal diseases with its anti-inflammatory properties (Girasole *et al*, 1994; Trepicchio *et al*, 1996, 1999; Martuscelli *et al*, 2000; Kinane and Attström, 2005; Salvi and Lang, 2005; Yucel *et al*, 2008). In a study, which evaluated the effect of the subcutaneous injection of recombinant human IL-11 (rhIL-11) in ligature-induced periodontal disease, it was reported that the subcutaneous injections of rhIL-11 were able to slow the progression of attachment and radiographic alveolar bone loss; and the inflammatory reaction was improved with IL-11 (Martuscelli *et al*, 2000). Taken together, these results allow us to suggest that IL-11 may be acting as a key mediator in preventing the progressive inflammation leading to periodontal tissue breakdown.

In this study, the total amount and concentration of IL-11 significantly decreased in the CPa group, which has the deepest PD and CAL in sampling sites, compared with the other groups (CPb and C). Our results have supported the results of Johnson *et al* (2004) who have reported that the decrease of IL-11 concentration in gingival tissues adjacent to the periodontal pockets deeper than 6 mm. They have suggested that this decrease may result in deficiency of the protective role of IL-11 in periodontal lesions. The authors have also referred to the 'altered microenvironment' suggesting an imbalance between pro- and anti-inflammatory influences on the inflammatory gingival tissues in their study. Supportive to their statements, the total amount and concentration of IL-11 were not found significantly different between of CPb and C patients who have the similar pocket depths in GCF sampling sites. Deeper periodontal pockets had decreased concentration and total amount of IL-11 in our study. The mentioned findings of our study may have pointed out the protective role, also anti-inflammatory feature of IL-11 in periodontal disease pathogenesis.

The role of Th cells in the pathogenesis of periodontitis is still a matter of conjecture. Although the Th1 and Th2 cells were investigated and reported with their relative ratio in stable and progressing lesions, the balance and the regulation of the cells and their productions, also cytokines and chemokines, remains to be clearly explained. A distinct subset of T cells was found. Park *et al* (2005) have provided compelling evidence that the pathway that leads to generation of IL-17-producing effectors, which has been termed the Th17 lineage, is distinct from that of the Th1 lineage, and therefore represents the third arm of the CD4 T-cell effector repertoire: Th1, Th2 and Th17. IL-17 acts *in vitro* and *in vivo* as a potent inflammatory cytokine by inducing the expression of other pro-inflammatory cytokines (Kolls and Linden, 2004). Harrington *et al* (2006) have suggested that IL-17 cannot directly potentiate its own differentiation by extinguishing Th1 or Th2 differentiation by way of IL-17, although it is possible that other factors that promote the development of, and/or are secreted by IL-17-positive CD4 T cells are capable of suppressing Th1 and Th2 development in favor of Th17 development. It seems that IL-17 plays an important role in the immunologic response and in the destruction bone. Besides, IL-17 was increased in many autoimmune diseases such as experimental autoimmune encephalomyelitis (Lees *et al*, 2008) and inflammatory conditions such as rheumatoid arthritis (Kotake *et al*, 1999; Shahrara *et al*, 2008).

In the literature, the studies investigating the role of IL-17 in periodontal diseases are few. The exact role of IL-17 in periodontal disease was not clearly described in the previous studies. It was stated by Gaffen (2004) that IL-17 is pro-inflammatory in nature, the determination of the IL-17 producing cells in experimental gingivitis and periodontitis models may be beneficial in clarifying the role of IL-17 in periodontal disease.

Takahashi *et al* (2005) have suggested that IL-17 is produced in periodontal lesions, which may be involved in Th1 modulation and enhanced inflammatory reactions via gingival fibroblast-derived mediators in periodontal disease and together with the other cytokines IL-17 has a potential role in the pathogenesis of periodontal disease. Fossiez *et al* (1998) have reported that IL-17 stimulates the mobilization and de novo generation of neutrophils by granulocyte-colony stimulating factor, thereby bridging innate and adaptive immunity. Yu *et al* (2007) have reported increased and allowed bone loss in IL-17-deficient mice. They have suggested that IL-17 has an important role in mediating host defense via neutrophils mobilization. In our study, the concentration of IL-17 was found significantly lower in the CPa group than the CPb and C groups.

Vernal *et al* (2005) have observed significantly lower GCF IL-17 concentration of CP patients than the healthy control group, similar to our study. The lower IL-17 concentration in chronic periodontitis patients than healthy controls may be depending on the higher GCF volume in diseased pockets.

The concentrations and the total amount of IL-17 were found quite lower in all of the groups in our study. The reasons of these differences may be listed as Takahashi *et al* (2005) have explained in several possible ways in their article: (i) IL-17 produced by activated T cells in periodontal lesions and bound to IL-17 receptor-positive cells; (ii) IL-17 may be degraded in the gingival crevice; and (iii) the amount of IL-17 in GCF is relatively low. The other reason may be the different methods used to determine IL-17. The researchers, Takahashi *et al* (2005) could not determine IL-17 in GCF; they have determined IL-17 in cell culture supernatants obtained from the periodontally diseased tissues with Western Blot and ELISA. The activities of the cytokines may be determined better in their complex immunologic environments, although the studies in cultures and animal models provide important insights for the cytokine biology (Yu *et al*, 2007).

As mentioned before, the ratio of the Th17 cytokines with the other Th1/Th2 cytokines and/or chemokines should be determined to clarify its exact role in periodontal pathogenesis. The ratio of the other cytokines and chemokines with IL-11 were investigated in various studies. Yucel *et al* (2008) found the IL-11:IL1-β ratio in gingivitis and control groups significantly higher than chronic periodontitis group. In addition, they reported progressively lower ratio in increasing pocket depths. It was also reported that IL-11:RANTES became progressively lower with increasing PD (Johnson *et al*, 2004). Similarly, the decreased ratio of IL-11:IL-17 in CPa patients (in deep pockets) or in other words higher cytokine ratio (total amount and concentration) in CPb patients and healthy sulcus (in shallow pockets) in C subjects in our study have shown that the anti-inflammatory properties of IL-11 may be impaired in deeper pockets.

The ratio of IL-11:IL-17 should be evaluated in larger populations, the limited sample size ($n = 20$) may be a limitation of this study. In addition, the role of the IL-17 and its ratio with the other cytokines in different phases of the periodontal disease activity should be evaluated, because different T-cell subsets predominate at different phases of the periodontal disease (Gemmell *et al*, 2002).

Further studies with larger populations evaluating the amount and ratio of the IL-17 with IL-11 and other key cytokines (IL-1β, TNF-α, IL-6, etc.) in the periodontally diseased tissues and GCF will be beneficial in clarifying the role in the pathogenesis of periodontitis.

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Author contributions

Z Yetkin Ay and E Berker designed the study and analysed the data. Z Yetkin Ay drafted the paper. E Berker reviewed

critically. R Sütçü, E Uskun and FY Bozkurt analysed the data.

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