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# Adjunctive Systemic Use of Beta-Glucan in the Nonsurgical Treatment of Chronic Periodontitis

Neslihan Nal Acar, Ülkü Noyan, Leyla Kuru, Tanju Kadir and Bahar Kuru  
*Department of Periodontology, Dental Faculty, Marmara University, İstanbul  
 Turkey*

## 1. Introduction

Periodontal lesions in chronic periodontitis are associated with a subgingival microbiota predominated by gram-negative anaerobic rods, spirochetes and other motile microorganisms (Tanner et al., 1979). Chronic periodontitis is a complex process itself involving periodontal microorganisms, immune system and host factors. One reason for the augmented colonization of these periodontopathogens is believed to be related with a weak specific T helper 1-mediated immunity (Bartova et al., 2000; Breivik & Thrane, 2001; Wassenar et al., 1998) and, the immune response in patients with periodontal lesions may be inclined towards a strong T helper 2-mediated immunity. Immune functions can be enhanced by activating macrophages and establishing Th1 dominance (Inoue et al., 2002; Lee et al., 2002). The use of certain immunomodulating agents may stimulate immune response and activate macrophages and neutrophils. Beta-glucan ( $\beta$ -glucan), a polysaccharide extracted from cell walls of *Saccharomyces cerevisiae*, has been found to have immunomodulatory effects in animals and humans (Babineau et al., 1994; Bleicher & Mackin, 1995; Chan et al., 2009; Engstad, 1994; Engstad et al., 2002). It increases resistance to bacterial infections and cancer cells while stimulating wound healing (Brown & Gordon, 2001; Chan et al., 2009; Yun et al., 2003). Numerous studies have shown that  $\beta$ -glucan is a stimulator activating phagocytosis, respiratory burst, and the production of cytokines and chemokines in macrophages (Kankkunen et al., 2010; Sherwood et al., 1987, Williams & Di Luzio, 1980). Recently, the possibility of subcutaneous injections of  $\beta$ -glucan being able to modulate allergic sensitisation has been demonstrated in children (Sarinho et al., 2009). The authors proposed a new therapeutic strategy in allergic diseases as  $\beta$ -glucan possesses a beneficial action in restoring T helper 2 function (Sarinho et al., 2009). Furthermore, besides its antibacterial effects, antiviral and antifungal properties of  $\beta$ -glucan have been put forward (Bedirli et al., 2003; Di Luzio et al., 1980; Jung et al., 2004; Kenyon, 1983; Kernodle et al., 1998; Leblanc et al., 2006; Nicoletti et al., 1992; Tzianabos, 2000). The protective effect of  $\beta$ -glucan has been established to *Staphylococcus aureus* (Di Luzio & Williams, 1978), *Escherichia coli*, *Listeria monocytogenes*, *Mycobacterium leprae*, *Candida albicans* (Williams et al., 1978), *Pneumocytis carinii*, *Leishmania donovani* and *Influenza virus* (Jung et al., 2004).

Transforming growth factor-beta1 (TGF- $\beta$ 1) plays a part in many different clinical processes, such as embryonal development, cellular proliferation and differentiation, wound healing, and angiogenesis via suppression of collagenase production by fibroblasts and macrophages

(Edwards et al., 1987; Page, 1991) and inhibition of the release of procollagenase. Moreover, it increases the synthesis of extracellular matrix molecules by stimulating numerous cell types including fibroblasts and osteoblasts (Chen et al., 1987; Hakkinen et al., 1996; Matsuda et al., 1992). Expression and production of this growth factor both at periodontally healthy and diseased sites suggest that it contributes to maintenance of tissue integrity (Buduneli et al., 2001; Kuru et al., 2004a; Kuru et al., 2004b; Steinsvoll et al., 1999; Wright et al., 2003). Thus, these properties of TGF- $\beta$ 1 show its important role in the pathogenesis of periodontal disease and wound healing.

Since  $\beta$ -glucan affects immune function with quickened macrophage activation and establishment of T helper 1 dominance, the tissue destruction seen in periodontal disease may be inhibited by the usage of this immunomodulating agent. Chaple et al. (1998) have reported that there was a failure of the recruitment and activation of macrophages in the gingival samples obtained from untreated advanced periodontitis patients, compared with those of patients with gingivitis. Thus, the ability of  $\beta$ -glucan to stimulate macrophages seems to be very crucial. Breivik et al. (2005) evaluated the effect of  $\beta$ -1,3/1,6 glucan on the progression of ligature-induced periodontal disease in animals. Their findings showed that orally administrated  $\beta$ -glucan significantly reduced periodontal bone loss as measured on digital x-rays. Stashenko et al. (1995) tested the effect of this biological response modifier on infection-stimulated alveolar bone resorption in an *in vivo* model. Their findings supported the concept that  $\beta$ -glucan can decrease hard and soft tissue destruction in animals. Although  $\beta$ -glucan has been suggested to enhance endogenous antibacterial mechanisms in neutrophils and to increase the healing potential of damaged tissues (Bedirli et al., 2003; Browder et al., 1988, Browder et al., 1990, Stashenko et al., 1995), so far, its effect on periodontal tissue healing as an adjunct to nonsurgical periodontal therapy (NPT) has never been investigated in humans.

The aim of this chapter is to present the results of a controlled study investigating the effects of NPT with an adjunctive use of systemic  $\beta$ -glucan on clinical, microbiological parameters and gingival crevicular fluid (GCF) TGF- $\beta$ 1 levels in chronic periodontitis patients over a 3-month period.

## 2. Materials and methods

### 2.1 Study population

Twenty subjects between 30-56 years of age were selected among chronic periodontitis patients who applied to the clinics of Department of Periodontology, Dental Faculty, Marmara University, Istanbul, Turkey. Medical and dental histories were obtained and intraoral examinations were carried out at pre-screening visit. Patients were diagnosed to have chronic periodontitis according to the clinical and radiographic findings (Armitage 1999). Inclusion criteria were as follows: to be systemically healthy, have at least two sites with a probing depth  $\geq 5$  mm in each quadrant and radiographic evidence of moderate to advanced chronic periodontitis. None of the patients had received antibiotics or periodontal treatment within the 6 months preceeding the study. Women who were pregnant, breast-feeding or using oral contraceptives were excluded. In addition, 10 systemically and periodontally healthy subjects were selected as the healthy control group. None of these subjects had bleeding on probing or a history of medication in the past 6 months. All

patients were non-smokers. The protocol of the study was approved by the Ethics Committee of Marmara University (Number: B.30.2.MAR.01.00.02/AEK-232). Patients who fulfilled the inclusion criteria provided written informed consent and participated in the study.

2.2 Study design

This study was a randomized, controlled, parallel group clinical trial of 3-month duration. With the purpose of evaluating the adjunctive effect of  $\beta$ -glucan, 20 chronic periodontitis patients were randomly divided into 2 groups: group 1 (n=10) received NPT only, group 2 received NPT and adjunctive  $\beta$ -glucan (n=10). A total of two sessions of NPT were applied to all patients and group 2 patients used systemic  $\beta$ -glucan (10 mg, 1x1) for 40 days (Fig. 1).

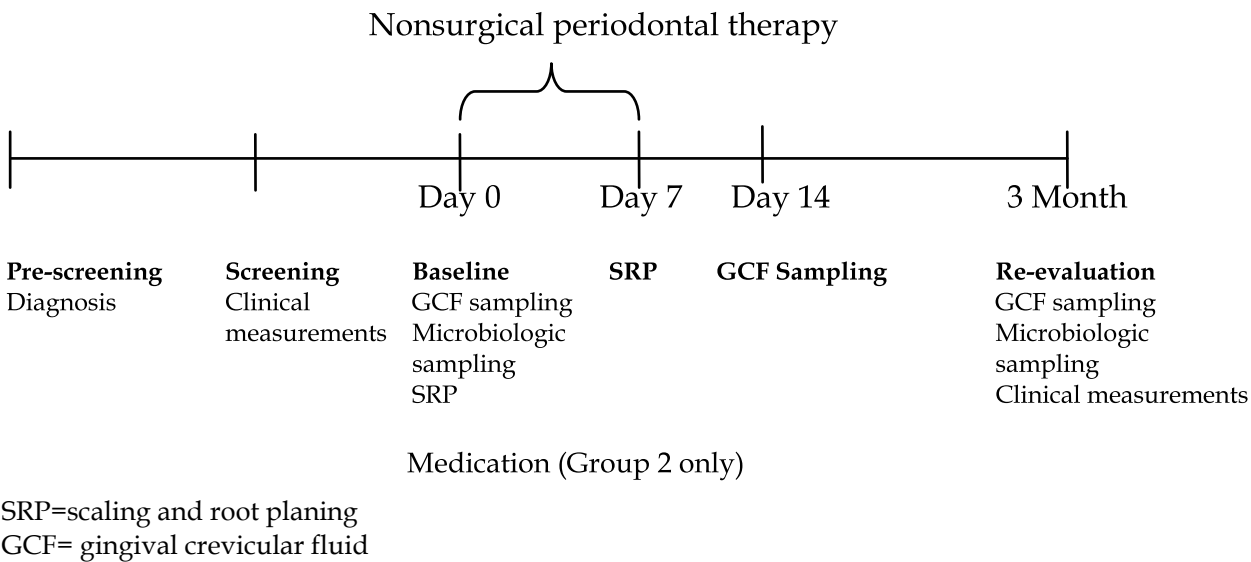


Fig. 1. Study design.

As shown in Fig. 1, this study was consisted of four main stages including pre-screening, screening, baseline (Day 0) and re-evaluation (3 month - day 91). Screening examination was conducted to assess the patient’s eligibility for participation. The periodontal status of each patient was assessed by a single blinded examiner (N.N.A.). The periodontal clinical measurements included gingival index (L e & Sillness, 1963), plaque index (Sillness & L e, 1964) and bleeding on probing. Additionally, probing depth and relative attachment level were measured to the nearest mm with a periodontal probe using an individual occlusal stent as a reference point for probe placement. Sampling was performed from sites with a probing depth  $\geq 5$  mm and subgingival microbiological and GCF samples were collected from different periodontal sites.

Patients who were eligible for the study, returned to our clinic at baseline visit for sampling and application of NPT. At baseline, oral hygiene instructions including brushing and flossing were given. Then, full mouth scaling and root planing (SRP) was performed in all patients using an ultrasonic scaler (Cavitron  EMZ, Switzerland) and Gracey curettes (Hu-Friedy Ins. Co, USA) until smooth root surfaces were achieved. The group 2 patients were further instructed to take one capsule of  $\beta$ -glucan (10 mg, 1x1) in the morning for 40 days.

At day 7, full mouth SRP was applied again in all patients. GCF samples were obtained at day 14. Clinical measurements and sampling procedures were repeated at 3-month re-evaluation stage.

### 2.3 Collection of GCF samples

GCF samples were obtained at days 0 and 14, and 3 months after therapy. Two GCF samples from each chronic periodontitis patient were collected from mesio- and disto-buccal aspects of single rooted teeth exhibiting probing depth  $\geq 5$  mm. In the healthy control group, two GCF samples were collected from mesio- or disto-buccal aspect of single rooted matching teeth exhibiting probing depth  $\leq 3$  mm without any clinical sign of gingival inflammation and alveolar bone loss.

Prior to GCF sampling, the selected sites were isolated with cotton rolls, saliva was removed using a high-power suction tip and supragingival plaque was removed using a periodontal probe to prevent saliva and/or plaque contamination (Griffiths et al., 1992). Paper strips (Periopaper; ProFlow, Inc., Amityville, NY, USA) were placed at the entrance of the crevice until mild resistance was felt and left in position for 30 seconds (Lamster et al., 1985). Strips contaminated with blood or saliva were discarded. The volumes of GCF collected were measured by weighing the papers, before and after sample collection, using a micro balance (AND 200-HM, Japan) (Kuru et al., 2004b). The weight of the fluid was converted to volume by assuming that the density of GCF was 1.0 (Cimasoni & Giannopoulou, 1988). The GCF samples were stored at  $-80^{\circ}\text{C}$  until the day of laboratory analysis.

### 2.4 TGF- $\beta$ 1 analysis in GCF

The paper strips were allowed to thaw at room temperature for 30 minutes. GCF samples were eluted from the strips by placing them in 100  $\mu\text{l}$  of phosphate-buffered saline (PBS) and stored at  $4^{\circ}\text{C}$  for up to 24 h prior to the laboratory procedures (Kuru et al., 2004a).

GCF TGF- $\beta$ 1 levels were analysed by enzyme linked immunosorbent assay, using a commercially available Colorimetric Sandwich enzyme linked immunosorbent assay Kit (Quantikine DB100, R&D Systems, Minneapolis, MN, USA). In order to measure biological active TGF- $\beta$ 1, GCF samples were acidified using 20  $\mu\text{l}$  1 N HCL at room temperature for 10 minutes. Then, the samples were neutralized by adding 20  $\mu\text{l}$  1.2 NaOH/0.5 M HEPES. The activated GCF samples were further analysed as described previously (Kuru et al., 2004a). Briefly, an aliquot of 200  $\mu\text{l}$  of known concentrations (0, 31.2, 62.5, 125, 250, 500, 1000 and 2000 pg/ml) of the activated recombinant human TGF- $\beta$ 1 standard and of the activated samples of GCF were added to the plate, which had been pre-coated with a recombinant human soluble receptor II which specifically binds human TGF- $\beta$ 1. The plate was covered with an adhesive strip and incubated at room temperature for 3 hours. Each well was aspirated and washed 3 times with 400  $\mu\text{l}$  of the wash buffer provided, after which 200  $\mu\text{l}$  of the detecting antibody (horseradish peroxidase-conjugated polyclonal antibody against TGF- $\beta$ 1) was added. The plate was again covered with an adhesive strip and incubated at room temperature for 1.5 hour. After washing 3 times, 200  $\mu\text{l}$  of the substrate solution (tetramethylbenzidine containing  $\text{H}_2\text{O}_2$ ) was added to each well and incubated at room temperature for 20 minutes. Following the addition of 50  $\mu\text{l}$  of 2 M  $\text{H}_2\text{SO}_4$  to stop the reaction, the absorbance at 450 nm was measured using a spectrophotometer. The



absorbance was also measured at 570 nm to determine any optical imperfections between the wells, and this value was subtracted from the absorbance at 450. A standard curve was prepared for each experiment and concentrations of TGF- $\beta$ 1 in samples were then calculated from this curve. The minimum detection limit of the assay kit was 4.61 pg/ml.

## 2.5 Subgingival microbiological sampling

Subgingival microbiological samples were collected at baseline and 3 months after therapy. The mesio- or disto-buccal site of a single-rooted tooth in the upper right quadrant exhibiting probing depth  $\geq 5$  mm was selected as the microbiological sampling site. Supragingival plaque was removed from the sampling site with a sterile curette followed by isolation using a cotton roll. Subgingival plaque sample was collected with a standardized 30# sterile paper point by inserting it into the crevice, and leaving in place for 10 seconds. Paper points contaminated with saliva or blood were discarded.

## 2.6 Microbiological analysis

The proportion of anaerobic microorganisms in the total flora was determined by microbiological culture method as described previously (Kuru et al., 1999; Noyan et al., 1997; Yilmaz et al., 2002). Immediately after obtaining subgingival plaque, each sample was aseptically transferred into 4.5 ml of PBS and dispersed using a vortex mixer at maximal setting for 60 s. The dispersed samples were serially diluted and 0.2 ml portion of  $10^{-1}$ ,  $10^{-2}$ , ...,  $10^{-5}$  dilutions were spread on a solid agar medium using sterile bent glass rods. Trypticase soy agar plate (Oxoid, Oxoid Ltd., England) enriched with 0.0005 % hemin (Sigma, Sigma Chemical Co., USA), 0.00005 % menadione (Sigma), and 5 % defibrinated sheep blood, was inoculated for non-selective bacterial growth (Wolff et al., 1985). Furthermore, trypticase soy agar plate enriched with 5 % defibrinated sheep blood was used for cultivation for facultative anaerobic microorganisms.

After 7 days of incubation of the supplemented trypticase soy agar plates in Gas Pak jars (Gas generating kit, Oxoid) in an atmosphere of 95 %  $H_2$  and 5 %  $CO_2$  at  $37^\circ C$ , the total viable count was determined from the dilution giving 30-300 colonies. After 5 days of incubation of trypticase soy agar plate in air and 10 %  $CO_2$  at  $37^\circ C$ , the total number of facultative anaerobes was determined.

All the microbiological data were expressed as colony forming units/ml (CFU/ml). Obligate anaerobic bacteria was calculated as the total counts of anaerobically cultivable bacteria minus the total counts of facultatively anaerobic bacteria and expressed as a percentage of total viable count.

## 2.7 Statistical analysis

SPSS for Windows (Release 10.0, SPSS Inc., USA) was used for statistical analyses. The mean values for each periodontal measurement was calculated as the mean of whole mouth for each patient. The mean for each periodontal parameter was also calculated separately for periodontal sites selected for microbial sampling with initial probing depth  $\geq 5$  mm for each patient. Comparisons between the groups were carried out using the Mann-Whitney U test, and multiple comparisons within each group were performed using the Friedman test

followed by the Wilcoxon Sign test for comparisons of values between different time points. *P* values <0.05 were considered statistically significant. Lack of significance is indicated by not significant (NS).

3. Results

All of patients enrolled in this study completed the study protocol. None of the patients in group 2 complained of any adverse effects due to the systemic usage of β-glucan. Patient demographics are outlined in Table 1.

	Group 1	Group 2
n	10	10
Age (mean years ± SD)	44.5 ± 9.4	42.4 ± 7.7
Age range	30-56	33-52
Gender (male:female)	8:2	4:6

Table 1. Demographic characteristics of patients participated in the study.

3.1 Clinical findings

The self-performed plaque control program resulted in improved oral hygiene in all patients supported by the finding of significant reductions in full mouth plaque index scores in both groups (*p*<0.01) (Table 2). No significant difference was found between the groups regarding the plaque index values (*p*>0.05). Significant reductions were detected in full mouth gingival index and bleeding on probing parameters after therapy in both groups (*p*<0.01). However, the changes in mean gingival index and bleeding on probing values of the group 1 were not different than those of the group 2 (*p*>0.05). Probing depth measurements at sampling sites demonstrated significant reductions from baseline to 3 month in both groups (*p*<0.01). When the probing depth reduction between the groups was compared, there was no significant difference (*p*>0.05). Significant attachment gain at sampling sites was achieved in both groups (*p*<0.01) (Table 2), but intergroup comparison yielded no significant difference between the groups (*p*>0.05) (data not shown).

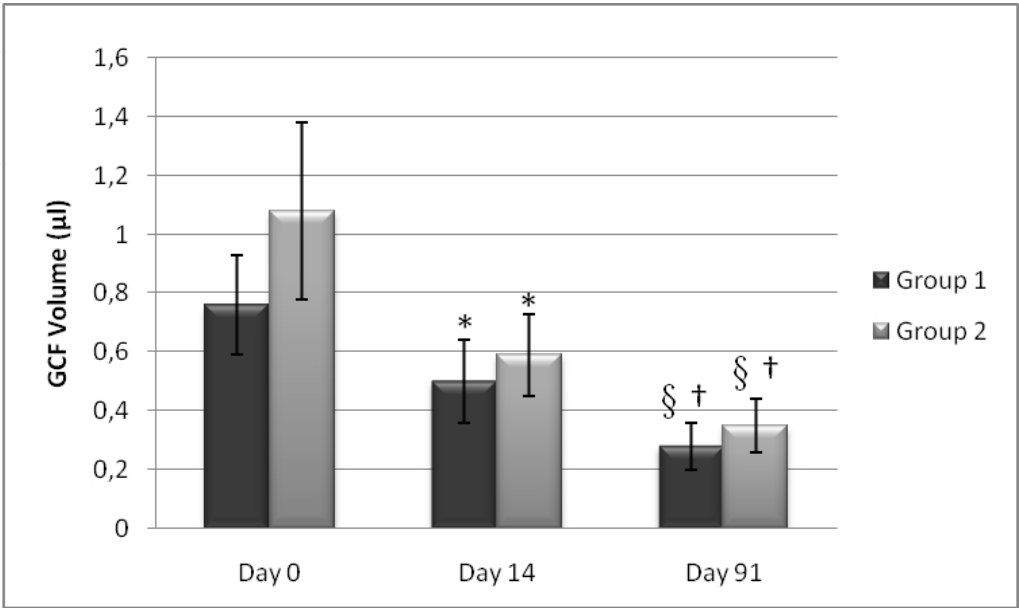
	Group 1		Group 2	
Parameter	Baseline	3 Months	Baseline	3 Months
PI(Full mouth)	2.17 ± 0.38	0.36 ± 0.23*	2.28 ± 0.31	0.44 ± 0.31*
GI(Full mouth)	2.36 ± 0.37	0.33 ± 0.21*	2.16 ± 0.47	0.45 ± 0.42*
BOP(%) (Full mouth)	88 ± 11	9 ± 4*	84 ± 14	10 ± 6*
PD (mm)(Sampling sites)	5.28 ± 0.16	3.35 ± 0.33*	5.38 ± 0.14	3.49 ± 0.15*
RAL (mm)(Sampling sites)	9.40 ± 1.14	8.18 ± 1.23*	9.56 ± 0.80	8.44 ± 0.72*

All values are expressed as mean ± standard deviation.  
\* (*p*<0.01), Wilcoxon Sign test  
PI=Plaque index, GI= gingival index, BOP= bleeding on probing, PD= probing depth,  
RAL= relative attachment level

Table 2. Clinical parameters of the study groups at baseline and 3 months after treatment.

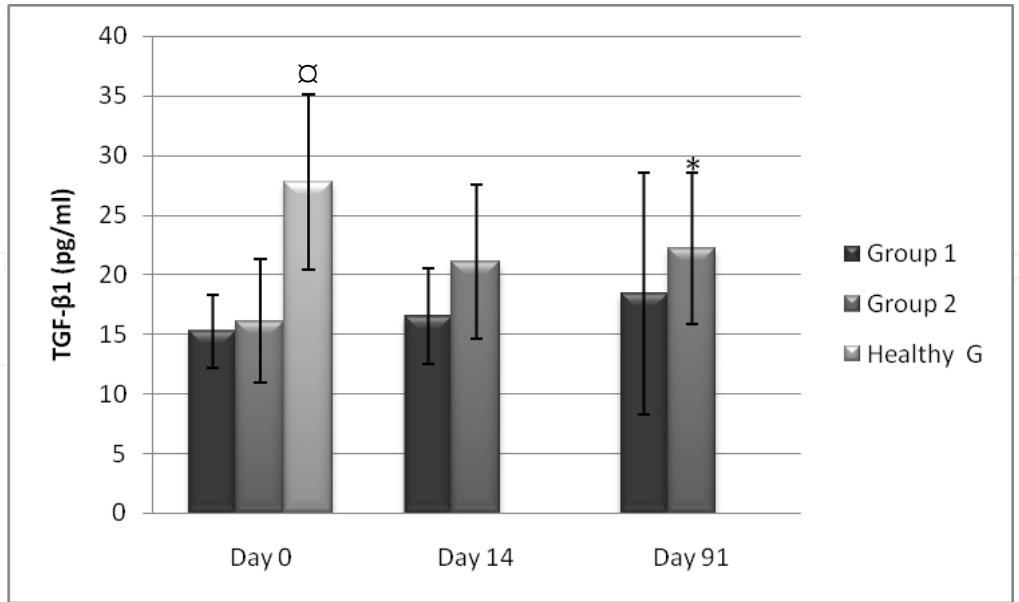
3.2 Biochemical findings

GCF volume of the groups 1 and 2 showed significant decreases during the experimental period ( $p<0.05$ ) and intragroup comparisons revealed that the decreases between days 0-14, 14-91 and 0-91 were significant in both groups ( $p<0.05$ ) (Fig. 2). However, there were no significant differences in the changes of GCF volume between the groups ( $p>0.05$ ).



\*  $p<0.05$ , between days 0-14, Wilcoxon Sign test, §  $p<0.05$ , between days 0-91, Wilcoxon Sign test.  
†  $p<0.05$ , between days 14-91, Wilcoxon Sign test.

Fig. 2. Mean GCF volume of sampling sites in study groups.



□  $p<0.01$ , compared to groups 1 and 2, Mann-Whitney U test  
\*  $p<0.05$ , respective baseline value, Wilcoxon Sign test.

Fig. 3. GCF TGF-β1 levels (pg/ml) of sampling sites in study groups at baseline and after therapy.



When baseline GCF TGF-β1 concentrations of the groups 1 and 2 and the healthy group were compared, healthy group showed a significantly higher level of TGF-β1 than that of the groups 1 and 2 ( $p<0.01$ ) (Fig. 3). At day 91, TGF-β1 concentration in GCF increased in the groups 1 and 2 when compared to their respective baseline values, but only the increase in the group 2 was found to be significant ( $p<0.05$ ) (Fig. 3). However, no significant difference was found between the groups in the GCF TGF-β1 concentration level.

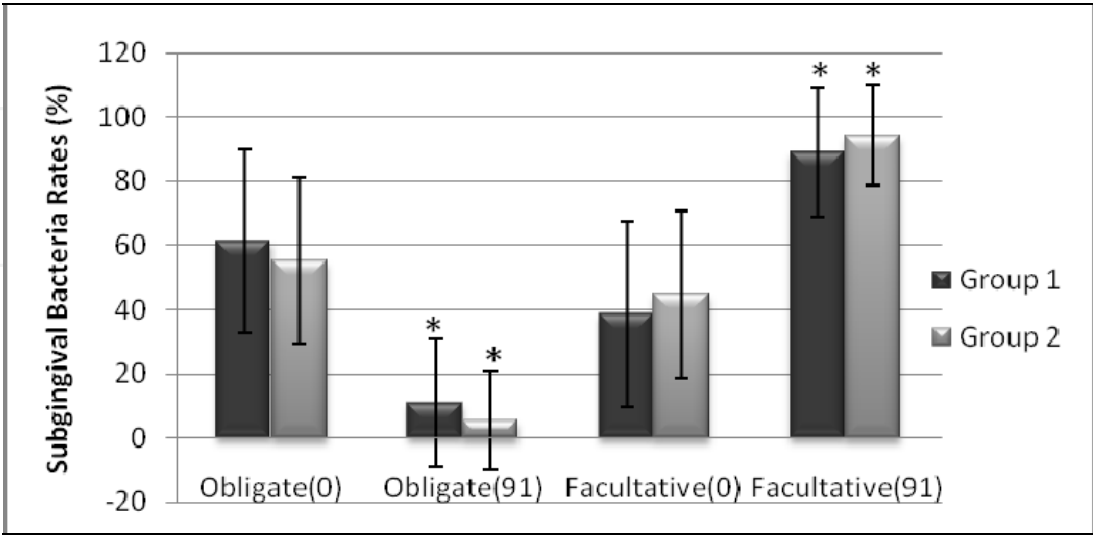
3.3 Microbiological findings

Total anaerobically grown microorganisms expressed as total viable counts in subgingival plaque samples before and 3 month after different treatment modalities, are given in Table 3. NPT applied to the group 1 resulted in a decrease in total viable counts. A similar decrease was also noted when the group 2 patients received adjunctive β-glucan. However, these reductions were not significant ( $p>0.05$ ). Furthermore, intergroup comparisons revealed no significant differences between the two groups ( $p>0.05$ ). Fig. 4 demonstrates the proportions of obligate and facultative anaerobes of the study groups. Significant reductions were detected in the percentage of obligate anaerobic bacteria along with significant increases in the percentage of facultative anaerobic bacteria in both groups after therapy ( $p<0.01$ ). However, no differences were found between the groups ( $p>0.05$ ).

		Group 1	Group 2	p <sup>‡</sup>
Baseline	mean	49	88	NS
	range	0.51-178	0.43-260	
3 Month	mean	2	14	NS
	range	0.05-6	0.06-130	
		p <sup>Ω</sup>	NS	NS

‡ Mann-Whitney U test, ΩWilcoxon Sign test, NS=not significant

Table 3. Total viable counts (x10<sup>4</sup> CFU/ml) of subgingival samples at baseline and after 3 months.



\*  $p<0.01$ , respective baseline value, Wilcoxon Sign test.

Fig. 4. Proportions of obligate and facultative anaerobes in subgingival plaque samples of the groups 1 and 2.

#### 4. Discussion

This chapter presented a randomized, controlled, parallel group clinical study which was designed to evaluate the effects of NPT with or without adjunctive use of systemic  $\beta$ -glucan on clinical, microbiological and biochemical parameters in chronic periodontitis patients over a 3-month period. NPT is the first stage of periodontal therapy that aims to reduce the number of pathogen microorganisms in periodontal pocket leading to the resolution of inflammatory response and arresting the progression of disease, resulting in probing depth reduction and attachment gain (Greenstein, 1992; Cobb, 1996; Noyan et al., 1997, Yilmaz et al., 2002). It is well known that the efficacy of nonsurgical therapy is related to the baseline probing depth, and inflammatory changes are more pronounced in deeper pockets. Therefore, the effectiveness of NPT and adjunctive  $\beta$ -glucan in this study was evaluated for only periodontal sites with baseline probing depth  $\geq 5$  mm. Significant improvements in all measured clinical parameters (plaque index, gingival index, bleeding on probing, probing depth, relative attachment level) were observed 3 months following SRP procedure, as expected. Moreover, SRP supplemented with  $\beta$ -glucan resulted in significant clinical outcome. However,  $\beta$ -glucan appears to have no additional effect on clinical parameters recorded in the present study, as evidenced by insignificant difference between the groups.

$\beta$ -glucan acts as an immunostimulant agent enhancing host-mediated immune responses to pathogens, especially by activating macrophages (Brown & Gordon 2001, Suzuki et al., 2001).  $\beta$ -glucan stimulates macrophage phagocytosis (Lee et al., 2002) and changes the balance from immunoglobulin G1 antibodies (T helper 2 dependent antibody subclasses) towards a T helper 1 dependent immunoglobulin G2a response (Suzuki et al., 2001). It also stimulates the production of T helper 1-stimulating cytokine interferon- $\gamma$  but suppresses interleukin-4 which induces T helper 2 responses (Inoue et al., 2002). Therefore,  $\beta$ -glucan skew the T helper 1/T helper 2 balance towards a T helper 1-dominated response (Suzuki et al., 2001). The presence of a T helper 2-biased response to the periodontopathogens is supported by the observation that peripheral blood cells of patients release more T helper 2 cytokines in response to periodontopathogens *in vitro* (Bartova et al., 2000, Wassenaar et al., 1998). In the present study, concentration of GCF TGF- $\beta$ 1 increased following both therapies but the increase was significant only in the group 2 which received  $\beta$ -glucan. As TGF- $\beta$ 1 is a T helper 1 stimulating cytokine, the increase of this cytokine can be explained by this mechanism.

In the present study, the level of TGF- $\beta$ 1 in GCF samples obtained from chronic periodontitis patients was investigated and compared with periodontally healthy individuals. TGF- $\beta$ 1 has a very important role in the pathogenesis of periodontal diseases and also in wound healing. To the best of our knowledge, no data is available on periodontal treatment with adjunctive use of  $\beta$ -glucan. Hence this is the first study to explore any effect of this immunomodulator agent on the treatment of chronic periodontitis patients.

In the present study we have demonstrated that the healthy group had higher GCF TGF- $\beta$ 1 concentration than the groups 1 and 2. This finding is in agreement with previous reports which found lower GCF TGF- $\beta$ 1 concentrations at inflamed sites when compared with healthy sites (Buduneli et al., 2001, Gürkan et al., 2005; Kuru et al., 2004a). Expression of

GCF constituents as concentration could result in higher levels at healthy sites where GCF volume is very low (Curtis et al., 1988, Emingil et al., 2000). Therefore, in healthy sites, high levels of TGF- $\beta$ 1 in GCF may be related to low GCF volume.

TGF- $\beta$ 1 is a key mediator in resolution of inflammation (Sodek & Overall, 1992, Steinsvoll et al., 1999). This multifunctional growth factor has both pro-inflammatory and anti-inflammatory properties (Wahl et al., 1993). Its effects on cell proliferation and differentiation suggest a key role for this cytokine in wound healing, tissue remodeling and regeneration (Sporn & Roberts, 1993). Thus, these properties of TGF- $\beta$ 1 indicate its important role in inflammatory wound healing. In the present study, concentration of GCF TGF- $\beta$ 1 increased following both therapies. After the elimination of microbial factors, as shown by decrease in the preposition of anaerobic species, there will be a rapid restoration of the periodontium; this might be the reason why the cytokine levels increased in our study. This increase was significant only in  $\beta$ -glucan group between day 0 and day 91. Since systemic  $\beta$ -glucan usage activates macrophages and neutrophils which produce TGF- $\beta$ 1 when activated (Igarasi et al., 1993), the significant increase in group 2 might have occurred due the effect of  $\beta$ -glucan. In the study of Gürkan et al. (2005), only GCF TGF- $\beta$ 1 levels of subantimicrobial dose doxycycline group was significantly higher than baseline and placebo group at 3 months. On the other hand, the GCF TGF- $\beta$ 1 concentration in the subantimicrobial dose doxycycline group decreased while it increased in the placebo group at the end of 6-month period. The authors concluded that the drug efficacy at the biochemical level may continue as long as the agent is used. In accordance with our results, Gürkan et al. (2005), have demonstrated that the level of this growth factor increased after resolution of inflammation. Thus, it could be hypothesized that antiinflammatory role of TGF- $\beta$ 1 may be more potent than its proinflammatory properties during healing after NPT. However, the levels of this cytokine at different stages of healing process needs to be clarified with further studies.

The demonstration of bacterial specificity in periodontal disease allows the clinician to direct therapy toward the elimination or suppression of the periodontopathogens in terms of antimicrobial treatment. As antibiotics have important disadvantages including side effects, drug resistance etc., recent researches have focused on new therapeutic agents alternative to antibiotics. Since  $\beta$ -glucan has been suggested to possess antimicrobial activity (Bedirli et al., 2003, Di Luzio et al., 1980, Nicoletti et al., 1992), we evaluated the microbiological effect of  $\beta$ -glucan as an adjunct to NPT. In this study, a reduction in the proportion of obligate anaerobic bacteria occurred in both groups. A decrease in the number of anaerobic bacteria is synonymous with a successful treatment of periodontal infections and reflects the antimicrobial effect following NPT, as expected (Greenstein, 1992; Noyan et al., 1997; Yilmaz et al., 2002). But as there are no significant differences between the two groups, we can assume that  $\beta$ -glucan has no additional antimicrobial effect when used systemically as an adjunct to NPT in patients with chronic periodontitis.

Regarding the duration of systemic  $\beta$ -glucan usage, no data is available so far on its systemic usage in the periodontal diseases. Treatment duration with  $\beta$ -glucan varies from 39 days to 90 days in animal and human studies according to the type and severity of the problem (Breivik et al., 2005; Kabasakal et al., 2011; Lin et al., 2011; Sarinho et al., 2009; Turunen et al., 2011). Biagini et al. (1988) documented soft tissue healing after NPT and found precisely

oriented collagen bundle fibres by 30 days to 60 days. Furthermore, Magnusson et al. (1984) reported that repopulation of subgingival microbiota occurred between 30 to 60 days. Therefore, our patients in this study were put on  $\beta$ -glucan medication for 40 days according to the combination of the aforementioned data.

## 5. Conclusion

This is the first preliminary report investigating the effects of NPT plus  $\beta$ -glucan on clinical parameters, subgingival microflora and GCF TGF- $\beta$ 1 level in patients with chronic periodontitis. Within its limits, systemic  $\beta$ -glucan used as an adjunct to NPT did not provide additional clinical and microbiological effects. However,  $\beta$ -glucan might increase the concentration of TGF- $\beta$ 1 thereby augmenting periodontal healing potential. As  $\beta$ -glucans are inexpensive and have a good margin of safety (Chan et al., 2009), their potential therapeutic value deserves further detailed investigations for clarifying the paucity of information in the literature in order to design a strategy for their possible use in clinical periodontal practice.

## 6. Acknowledgements

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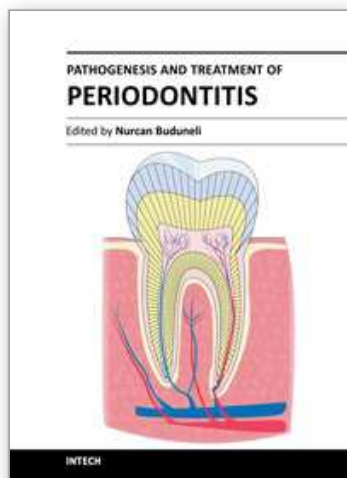
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Phone: +86-21-62489820  
Fax: +86-21-62489821

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