

## Beta<sub>2</sub>-Microglobulin Levels in Serum and Saliva of Patients with Juvenile Periodontitis

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### Abstract

Beta<sub>2</sub>-microglobulin ( $\beta_2$ -m) is a low-molecular-weight protein which has been suggested to play an important role in immune functions. The aim of this study was to determine  $\beta_2$ -m levels in serum and saliva from patients with juvenile periodontitis (JP) and to compare them with those of periodontally healthy subjects. The study was performed on 11 patients with JP and 10 periodontally healthy controls (C). Clinical measurements were recorded and serum and saliva samples were obtained from the individuals.  $\beta_2$ -m levels were determined using the ELISA technique. Serum  $\beta_2$ -m levels were significantly higher in the JP group than in the control group. In saliva, no significant difference in  $\beta_2$ -m levels between the groups was found. The higher  $\beta_2$ -m levels in serum in the JP group suggest that  $\beta_2$ -m may play a role as a systemic factor in the etiology and pathogenesis of JP.

### Introduction

Beta<sub>2</sub>-microglobulin ( $\beta_2$ -m) is a low-molecular-weight protein (Mr11,800) composed of a single 100-amino-acid peptide with one intrachain disulfide bridge<sup>[1]</sup>. Its amino acid sequence and three-dimensional structure bear a strong resemblance to the constant domains of the heavy and light chains of immunoglobulins<sup>[2,3]</sup>.  $\beta_2$ -m has been demonstrated on the surface of all normal human nucleated cells, where it is associated with the major histocompatibility complex (MHC)<sup>[4]</sup>. The MHC is located in the human lymphocyte antigen (HLA), which includes the HLA-A, B and C antigen complexes. Therefore  $\beta_2$ -m constitutes part of the HLA antigen complexes on nearly all cell types including lymphocytes<sup>[4,5]</sup>. Studies have shown that  $\beta_2$ -m is synthesized by almost all human cells, tumor cells and both B and T lymphocytes being high  $\beta_2$ -m producers<sup>[6,7]</sup>. Although the exact function of  $\beta_2$ -m is unknown, it is suggested to play an important role in immune responses, especially T-lymphocyte activation<sup>[8]</sup>.

This protein is present at low concentrations in normal serum<sup>[9]</sup>, urine<sup>[10-18]</sup>, saliva<sup>[19-21]</sup> and other body fluids such as cerebrospinal fluid<sup>[22]</sup> and synovial fluid<sup>[20,21]</sup>. Free molecules are also detectable in plasma as products of cell turnover, particularly from lymphocytes<sup>[23]</sup>. Determination of  $\beta_2$ -m levels in various biological fluids is now possible, and this has proved useful for diagnosis and treatment monitoring in many clinical situations such as renal diseases<sup>[10,11,14-18]</sup>, solid tumors and cancers<sup>[24,25]</sup>, leukemia<sup>[26]</sup>, lymphoma<sup>[27]</sup>, AIDS<sup>[28,29]</sup>, chronic inflammatory diseases such as Sjögren's syndrome and rheumatoid arthritis<sup>[20,21]</sup>, liver diseases<sup>[30]</sup>, and hyperthyroidism<sup>[23]</sup>.

Periodontitis is an inflammatory disease which causes destruction of the periodontal tissues surrounding the affected teeth<sup>[31]</sup>. Microbial plaque has been regarded as the primary factor in the etiology of periodontitis. Juvenile periodontitis (JP) is a severe type of periodontitis which occurs in young individuals, and some immunologic response defects are reported to be involved

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in its etiology and pathogenesis<sup>[32,33]</sup>. Because of its association with the HLA antigens present on the lymphocyte cell surface and the similarities of its amino acid sequence to the immunoglobulin chain, this peptide may play an important role in immune functions. Furthermore, several unexpected biological activities of free  $\beta_2$ -m related to connective tissue turnover have also been described<sup>[34]</sup>. These data suggest that  $\beta_2$ -m could be involved in the modulation of connective tissue breakdown and bone remodelling. Therefore the aim of the present study was to determine  $\beta_2$ -m levels in serum and saliva from patients with JP and to compare them with those of periodontally healthy controls.

### Materials and Methods

The study was performed using a group of patients with JP and a control (C) group. The JP group comprised 11 patients (10 females and 1 male) aged 14-23 yr (mean, 20.6 yr). The C group comprised 10 individuals (7 females and 3 males) aged 22-27 yr (mean, 24.1 yr). The subjects were selected from among individuals who presented at the Department of Periodontology, Faculty of Dentistry, University of Hacettepe, for periodontal treatment or routine checkups. In the patients, clinical and radiographic examinations revealed deep periodontal pockets and advanced vertical bone loss especially around the incisors and first molars. Clinical measurements were performed and pocket depths, gingival index (LÖE AND SILLNESS)<sup>[35]</sup>, bleeding on probing (positive or negative), plaque index (SILLNESS AND LÖE)<sup>[35]</sup> and Russell's periodontal index<sup>[35]</sup> scores were recorded. Venous blood and total saliva samples were obtained from all individuals. For determination of serum  $\beta_2$ -m levels, 5 ml of nonheparinized blood was obtained from each individual and the serum was separated. The samples were kept frozen at  $-30^\circ\text{C}$  until use. An ELISA test (Cistron Biotechnology, Pirie Brook, N.J., U.S.A.) was utilized to determine the  $\beta_2$ -m levels in serum and saliva<sup>[36]</sup>. Mann-Whitney U test was used to determine the significance of differences in parameters between the groups.

### Results

*Clinical Measurements:* The mean pocket depth scores were  $3.39 \pm 0.22$  mm in the JP group and  $1.21 \pm 0.07$  mm in the C group. The mean GI index scores were  $1.69 \pm 0.19$  in the JP group and  $0.21 \pm 0.02$  in the C group. The areas which bled on probing were  $25.81 \pm 1.15$  in the JP group and  $2.10 \pm 0.58$  in the C group. In the JP group, the mean PI score was  $1.20 \pm 0.16$ , whereas that in the C group was  $0.51 \pm 0.04$ . The mean periodontal index scores were  $5.04 \pm 0.22$  and  $0.87 \pm 0.05$  in JP and C groups, respectively. All of the clinical indices were significantly higher in the JP group than in the C group (Table 1).

*$\beta_2$ -m Levels:* The mean values of  $\beta_2$ -m in serum were  $2.86 \pm 0.13$  mg/ml in the JP group and  $2.62 \pm 0.05$  mg/ml in the C group. Serum  $\beta_2$ -m levels were significantly higher in the JP

Table 1 The median values of clinical measurements in groups

	PD	GI	BI	PI	RPI
JP	3.70	1.64	28	1.10	5.13
C	1.15	0.19	2	0.5	1
Mann Whitney U value	110*	109.5*	110*	99.5*	110*
P	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*

PD: Pocket depth

GI: Gingival index

BI: Bleeding index (number of teeth showing bleeding)

PI: Plaque index

RPI: Russell's periodontal index

\* The difference between the groups is significant.

Table 2 The median values of  $\beta_2$ -m in serum and saliva

	Serum mg/ml	Saliva mg/ml
JP	2.8	1.7
C	2.6	0.95
Mann Whitney	79*	73**
U		
P	<0.05*	>0.05**

\* The difference between the groups is significant.

\*\* The difference between the groups is not significant.

group than in the C group.

In saliva, the mean  $\beta_2$ -m values were  $2.08 \pm 0.46$  mg/ml in the JP group and  $1.29 \pm 0.28$  mg/ml in the C group. The difference between the groups was not statistically significant (Table 2).

### Discussion

Only a few studies of  $\beta_2$ -m in periodontal disease have been performed previously. SYRJÄNEN et al.<sup>[37]</sup> studied the  $\beta_2$ -m pattern in gingival biopsy samples from patients with severe chronic periodontitis and JP, and from periodontally healthy subjects. Only JP specimens showed positive  $\beta_2$ -m staining in the epithelium; the others did not. In another study<sup>[38]</sup>, significantly increased levels of  $\beta_2$ -m were found in gingival fluid from patients with severe periodontitis. MARKKANEN et al.<sup>[22]</sup> found lower  $\beta_2$ -m levels in saliva from patients with severe periodontitis than in controls, although the difference was not statistically significant. The present results indicated higher salivary  $\beta_2$ -m levels in JP patients than in controls, but again the difference was not statistically significant, thus confirming the finding of MARKKANEN et al.<sup>[22]</sup> Other studies have shown increased leukocyte migration into the gingival crevice in periodontal disease<sup>[39,40]</sup>. It has been suggested that when these cells are affected,  $\beta_2$ -m together with some other substances may be released, which would lead to elevated  $\beta_2$ -m values in affected patients<sup>[22]</sup>. Therefore it seems surprising to find similar salivary  $\beta_2$ -m levels in the JP and C groups. Markkanen et al. explained the lower salivary  $\beta_2$ -m levels by an imbalance or a decrease in the secretion of  $\beta_2$ -m by the minor salivary glands in patients with severe periodontitis<sup>[22]</sup>. However, in the present study, the difference in serum  $\beta_2$ -m levels was significant between the groups, being higher in the JP group than in the controls. This suggests the role of a possible systemic factor, perhaps  $\beta_2$ -m, in the etiology and pathogenesis of JP.

It has been reported that when no active renal disease is present, an increase in the concentration of serum  $\beta_2$ -m is due to increased synthesis of this protein<sup>[41]</sup>. Such an increase in  $\beta_2$ -m may be correlated with T-lymphocyte activity<sup>[41,42]</sup>, or partly with activation of B lymphocytes<sup>[37,41]</sup>.  $\beta_2$ -m is synthesized and released by many cells, particularly lymphocytes<sup>[23]</sup>, and its levels in serum reflect cell membrane turnover<sup>[42]</sup>. The finding of SYRJÄNEN et al.<sup>[37]</sup> that gingival fluid  $\beta_2$ -m levels were significantly higher in patients with periodontitis compared to those in controls suggests that  $\beta_2$ -m, like other proteins, is released as an exudate from serum, since a significant correlation between protein and  $\beta_2$ -m concentrations in gingival fluid was found in the same study. Other studies have demonstrated deposition of  $\beta_2$ -m in bone in dialysis patients showing elevated serum  $\beta_2$ -m levels<sup>[43]</sup>. The bone matrix includes many growth factors known to influence bone cell activity, one of which is  $\beta_2$ -m. There is evidence that  $\beta_2$ -m stimulates the proliferation of human bone cells and that these cells exhibit immunoreactivity for  $\beta_2$ -m. Osteoblast-like cells have also been shown to produce  $\beta_2$ -m.

Recently, there has been interest in the relationship between the immune system and the

regulation of bone metabolism. Since there is a strong association between  $\beta_2$ -m synthesis with MHC class I expression and the production of  $\beta_2$ -m by human bone cells, an immunomodulatory role of this peptide in bone remodelling has been suggested.  $\beta_2$ -m also regulates the synthesis of type I collagen in rat osteoblast cultures, indicating that at least in these cultures osteoblasts are responsive to  $\beta_2$ -m<sup>[44]</sup>. In addition,  $\beta_2$ -m inhibits the process of calcification, and stimulates the growth of fibroblasts and the production of collagenase<sup>[44]</sup>. It has been suggested that high circulating concentrations of  $\beta_2$ -m could substantially affect osteoblast function and consequently bone turnover<sup>[45]</sup>. Therefore these effects of  $\beta_2$ -m may be of importance in periodontal bone turnover and destruction in JP. No previous studies have evaluated the levels of serum  $\beta_2$ -m in patients with periodontitis. The significance of salivary or serum  $\beta_2$ -m in periodontitis is not yet clear, and further research is needed.

### Conclusions

1.  $\beta_2$ -m levels in serum were significantly higher in the JP group than in the control group.
2. Salivary  $\beta_2$ -m levels showed no significant differences between the groups.

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