International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-8, Issue-2, 2017, pp813-819
http://www.bipublication.com

Research Article

Expression of BCL-2 Protein in Gingival Tissue of Patients with Chronic Periodontitis

Mohammad Mohammadi MD[1], Molook Torabi Parizi MD[2], Mina Bagheri DDS[3] and Neda Khodarahmi MD[4]*

[1]Assistant Professor, Oral and Dental Diseases Research Center AND Kerman Social Determinants on Oral Health Research Center AND Department of Periodontics, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran
[2]Associate Professor, Department of Oral Pathology, Faculty of Dentistry, Kerman University of Medical Sciences, Kerman, Iran
[3]Dentist, Faculty of Dentistry, Kerman University of Medical Sciences, Kerman, Iran
[4]Postgraduate Student, Department of Periodontics, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran* (Corresponding author Email adress: neda.kh999@gmail.com)

ABSTRACT:

Introduction: Periodontal disease forms an inflammation in the tissues surrounding the teeth, which results in the increased production of inflammatory mediators, growth factors, anti-bacterial polypeptide, and apoptosis, closely related to carcinogenesis. The aim of this study was to compare expression of Bcl-2 in gingival tissue of patients with chronic periodontitis and healthy periodontium.

Materials and methods: In this study, 36 patients with moderate to severe chronic periodontitis (attachment loss≥ 3mm, probing depth≥5mm) that had not received any treatment, and 25 periodontally healthy subjects were studied. Gingival biopsies were obtained and stained with immunohistochemistry methods and the expression of Bcl-2 was evaluated. Independent t-test and chi-square test were used to compare the variables.

Results: Bcl-2 in periodontitis group showed a significantly higher expression in comparison with control group ($p<0.05$). It was shown that gender does not affect the expression of Bcl-2 in both periodontitis and control group ($p>0.05$). Spearman correlation showed a positive correlation between age and Bcl-2 expression in the control group ($r=0.44$, $P=0.01$).

Conclusion: In conclusion, our study demonstrated that chronic periodontitis had a significant effect on Bcl-2 expression. It was also shown that age and gender have no effect on the expression of BCL-2.

Keywords: periodontitis, apoptosis, Bcl-2, gingival, chronic, expression

INTRODUCTION:

Periodontitis is a chronic oral infection caused by anaerobic gram-negative bacteria in tooth surface biofilm, which leads to tissue destruction and tooth loss[1, 2]. Periodontal disease is one of the two main dental diseases with high prevalence across the world: 10% to 15% of the world's population suffer from advanced periodontal disease with deep pockets (6 mm or more)[3].

Although new evidence suggests the important role of viruses in the initiation and progression of periodontitis, bacteria and viruses can synergistically cause periodontitis[4]. Periodontal disease forms an inflammation in the tissues surrounding the teeth, which results in the increased production of inflammatory mediators, growth factors, anti-bacterial polypeptide, and apoptosis, closely related to carcinogenesis[5].

Apoptosis, programmed cell death, is one of the mechanisms contributed in the pathogenesis of periodontitis which regulates the turnover of gingival cell tissue[6]. It occurs in physiological and pathological conditions, and plays an important role in the process of homeostasis. Apoptotic cells are identified through condensing chromatin, degrading DNA into small pieces of oligonucleosomes, and forming nuclear and plasma membrane Blebs[7]. Furthermore, an impressive body of evidence supports the concept that apoptosis contributed to several cancers[8, 9].

A growing body of evidence proposed the probable positive association between periodontal diseases and the risk of cancer in different tissues, especially mouth, upper digestive system, lung, and pancreas [10–12]. Oral cancer is one of the most prevalent cancers throughout the word; it is the eighth prevalent cancer in men, 11th in women[13]. Several factors are involved in the development of oral squamous cell cancer including tobacco, alcohol consumption, herpes simplex virus (HSV) and HPV virus, poor diet, and chronic dental trauma[14].

B-cell lymphoma 2 (Bcl-2), a proto-oncogene, encodes a protein inhibiting the apoptosis[15, 16]. As an anti-apoptic factor it acts to surviving the cells by a variety of stimuli. Moreover, the Bcl-2 family proteins have a fundamental regulatory role in the development of apoptosis[16]. Several studies showed that increased expression of Bcl-2 protein in the primary phase of carcinogenesis impairs apoptosis and results in neoplastic changes[17]. Gamonal and colleagues reported overexpressed P53 and Bcl-2 in patients with chronic periodontitis than the control group[18]. Also, in another study, the overexpression of Bcl-2 was observed in patients with periodontal disease[19]. Gingival tissue is considered as physical and defensive barrier against bacterial infection through the expression of various antimicrobial peptides and cytokines. On the other hand, recognising the characteristic of apoptosis including the proto-apoptic and anti-apoptic markers in inflammatory gingival tissue of periodontitis patients may be clarify the mechanisms involved in the destruction of these tissues.

Given the few clinical studies evaluating apoptosis in patients with periodontitis, the present study aimed to compare the expression of Bcl-2 in gingival tissue of patients with chronic periodontitis and subjects with clinically healthy periodontium.

MATERIALS AND METHODS:

2.1. Study population

The present cross-sectional study investigated 36 subjects (17 men and 19 women) with moderate to severe chronic periodontitis (loss of attachment ≥3 mm) with probing depth ≥5 mm who were untreated, and 29 healthy subjects (18 men and 11 women) referred to the Department of Periodontology, School of Dentistry, Kerman, southeast Iran. Since a small tissue (3×2 mm) was taken from the gingival tissue apart from the teeth pulled out by surgery and considering the high repairing ability of the gingival tissue, therefore, entailed no complications and there was no ethical prohibitions for healthy subjects. Case and control participants were matched for age and sex. Written informed consents were obtained from all participants. This study conforms to the Declaration of Helsinki regarding research involving human subjects and approved by the ethics committee of Kerman

University of Medical Sciences (IR.KMU.REC12/93/209).

The inclusion criteria were as follows: (1) subjects with at least 14 teeth and 10 posterior teeth; (2) no consumption of drugs affecting periodontium, such as phenytoin and nifedipine; (3) no consumption of non-steroid anti-inflammatory drugs (NSAIDs) and antibiotics during the last six months; (4) no consumption of cigarettes, alcohol, and other opiates; (5) no history of cancer; (6) no history of chemotherapy and radiotherapy; (7) No family history of cancer; (8) no systemic diseases; (9) and consent to participate in the study.

2.2. Tissue processing and immunohistochemistry

Subjects with chronic periodontitis and control groups were selected among patients admitted for gingival surgery or any oral surgery adjacent to gingival tissue. A biopsy of approximately 3×2 mm was taken from the gingival (marginal or adhesive) tissue in all participants using a scalpel-15 under local anaesthesia with lidocaine-epinephrine 1/80000, and were kept in 10% formalin. Biopsies were transferred to the laboratory and after the preparation of paraffin blocks, immunohistochemistry of Bcl-2 protein was performed as follows: 1. The 3-4 micron Sections were prepared from paraffin blocks of tissue biopsies and were mounted on slides. 2. The Sections were incubated in buffer citrate (10 mmol for 20 min) in microwave. 3. Then, Sections were incubated in 0.5% hydrogen peroxide (15–20 min). 4. Sections were covered with 2% bovine serum albumin (15 min).

5. The Sections were then incubated overnight with a specific Bcl-2 antibody (Santa cruz biotechnology, Inc., Dallas, Texas, USA). The dilution of each antibody was determined in accordance with the initial test. 6. Sections were rinsed with the second antibody, conjugated to biotin, for 30 min. 7. Sections were washed with phosphate-buffered saline (PBS) three times in each step, if required. 8. Sections were incubated with 3,3'-Diaminobenzidine (DAB) chromogenic (5-10 min). 9. At the end, sections were washed with distilled water three times (5 min) and stained with hematoxylin-eosin (10 sec). 10. After washing, they were covered with special immunocytochemistry glue. 11. The sections were kept for imaging and data collection.

2.3. Immunohistochemical staining of tissue sections

To evaluate the immunohistochemistry results, the mean percentage of positive cells (stained cells) was counted by the total number of cells (positive and negative cells) in at least three microscopic fields using an optical microscope with a magnification of ×400[19]. To evaluate the expression of BCL-2, the following criteria were used:

- Staining of less than 25% of the counted cells.
- Staining of 25–50% of the counted cells.
- Staining of 50–75% of the counted cells.
- Staining of 75–100% of the counted cells[20].

2.4. Statistical analysis

All data for continuous variables were expressed as mean ± standard deviation (M±SD) and categorical variables were presented as numbers (percentages). Independent t-test and chi-square test were used to compare the variables. The correlation between age and Bcl-2 were analyzed using Pearson correlation between the two groups. The statistical analyses were performed using the SPSS software version 18.0 for Windows. *P*-values <0.05 were considered statistically significant.

RESULTS:

The data of sex, age, and Bcl-2 status in both groups are presented in Table 1. Thirty-six patients (17 men and 19 women) in periodontitis group and 29 patients (18 men and 11 women) in the control group were evaluated to compare the percentage of cells staining with Bcl-2 marker. In our study, men were more than women in both groups as well as the most of participants between 40-50 years in both groups. However,

we found no significant differences for sex (*P*=0.23) and age (*P*=0.25) between the two groups. The results showed that BCL-2 expression was less than 25% in the most of participants in both groups. However, only in case group, Bcl-2 was higher than 25% and there was significant difference of Bcl-2 expression between case and control groups (*P*≤0.0001).

Table 1: Demographic and BCL-2 index chracterisics in both groups divided by age

Variables \ Groups		Case	Control	P-value
Sex	male	17 (47.2)	18 (62.1)	0.23
	female	19 (52.8)	11 (37.9)	
Age	≤ 20 years	4 (11.1)	0 (0)	0.25
	21-30 years	2 (5.6)	5 (17.2)	
	31-40 years	22 (61.1)	14 (48.3)	
	41-50 years	6 (16.7)	7 (24.1)	
	≥ 50 years	2 (5.6)	3 (10.3)	
	total	35.92±9.34	38.41±7.59	
Bcl-2	≤ 25%	17 (47.2)	29 (100)	**<0.0001***
	26%-50%	12 (33.3)	0 (0)	
	51%75%	3 (8.3)	0 (0)	
	76%-100%	4 (11.1)	0 (0)	
	total	32.91±26.26	0.33±0.97	

* Significant difference (*P*<0.05).

In this study, results showed that men in the case (35.64±27.67) and control (0.46±1.2) groups had higher percentage of cells stained with Bcl-2 compared to women in the case (30.47±25.45) and control (0.11±0.29) groups, however, we found no significant difference (*P*>0.05 for both) (Figure 1).

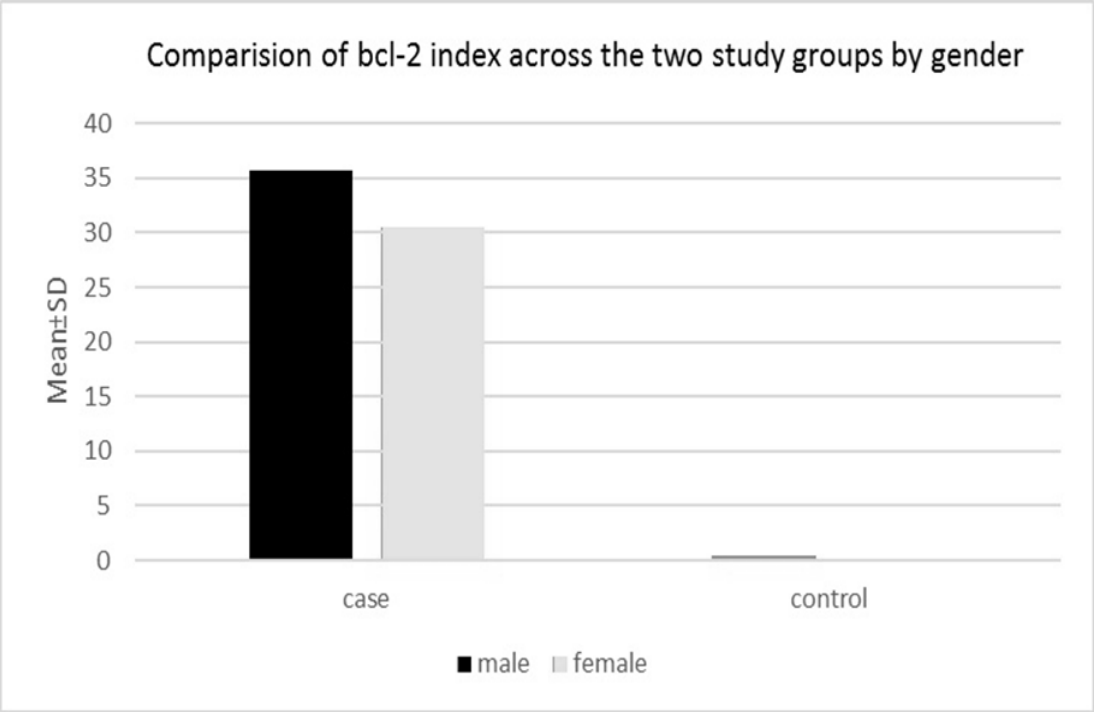

Figure 1: Comparision of Bcl-2 expression across the two studied groups by gender

Spearman correlation showed that there was no significant difference between age and Bcl-2 expression in case group (r=-0.08, *P*=0.6). However, there was a positive correlation between age and Bcl-2 expression in the control group (r=0.44, *P*=0.01).

DISCUSSION:

Findings obtained from previous reports demonstrated that apoptosis might be contributed to the destruction of periodontitis-associated gingival tissues. Although the presence of bacterial pathogens is essential to start the disease, the immune response can play an important role in the progression of periodontal disease [2,23]. Apoptosis, programmed cell death, plays a vital role in the regulation of inflammation and immune response of the host. During this process, a series of morphological and biochemical events are induced in the cells, which leads to their death [24]. Bcl-2, an anti-apoptotic protein, causes preventing the cell death induced by different stimuli[20]. Therefore, this study aimed to determine the expression level of Bcl-2 protein by immunohistochemistry in gingival tissue of patients with chronic periodontitis compared to healthy subjects.

The results of the present study suggested the presence of apoptosis in the gingival tissues of periodontitis patients. Immunohistochemical evaluations showed that the expression of Bcl-2 in all healthy periodontium was ≤25%; however, 47% of patients with chronic periodontitis had BCL-2 expression <25%, and the rest (53%) showed >25% expression levels. The mean staining percentage in periodontitis group was 32.91% and in the control group was 0.33%.

In the study by Gamonal et al., the expressions of Bcl-2, Caspase3, fas/fasl and P53 were investigated [20]. Their results indicated the presence of apoptotic cells by electron microscopy in deep areas of biopsies, taken from areas with probing depth ≥5 mm, and attachment loss ≥3 mm. Their study strongly supported strong apoptotic mechanisms in periodontal tissues of individuals with chronic periodontitis. In addition, the expression levels of Bcl-2 and P53 increased in patients with chronic periodontitis than those of the control group. In our study, the mechanism of apoptosis and increased expression of anti-apoptotic proteins was observed in patients with chronic periodontitis, like Gamonal's study [20]. In agreement with our results, the staining percentage of cells with Bcl-2 marker was reported at more than 25%. Also, the expression of Bcl-2 in the control group of Gamonal's study was reported at 0%, which was similar to Bcl-2 protein levels in our study (0.33%).

Along with Gamonal's study [20], the results of the study by Kasprzak and et al. [25] showed no difference in the expression of Caspase-3 and showed overexpression of Bcl-2 and P53 in chronic periodontitis ratio than the control group, which is in agreement with our study. The increased expression of Bcl-2 in patients with periodontitis represents the effect of chronic inflammation on cell apoptosis. Bulut and et al. [19] examined the expression of P53, Bcl-2, and Caspase-3 proteins in patients with aggressive periodontitis that showed no difference in the expression of P53 and Caspase-3 in the periodontitis patients compared with the control group. But the Bcl-2 expression in periodontitis group was in agreement with the results of our study, with the difference that Bulut's study was performed on eight patients with periodontitis, while our study was conducted on 36 patients with chronic periodontitis. This indicates that the small sample size was the limitation of Bulut's study which was widely compensated in the current study.

In the present study, the effect of sex on the expression of Bcl-2 in patients with chronic periodontitis and healthy controls was studied. The results showed no effect of sex differences in the expression of Bcl-2. The present study also investigated the effect of age on the expression of

Bcl-2 in the two groups; although most patients with periodontitis are aged between 30 to 40 years, there was no statistically significant relationship between age and staining percentage. In contrary, the Bcl-2 expression increased with age in the healthy subjects. Given that previous studies have not examined the impact of sex [18, 19, 23], so the effects of age and sex on the expression of Bcl-2 can be considered among the strengths of this study. Further studies with a larger sample size can be effective in evaluating the mechanisms involved in inflammation and apoptosis. Considering the importance of apoptosis and its role in the pathogenesis of periodontal diseases and cancer, further molecular studies are required to identify the leading mechanism proposed the relation between the apoptosis and chronic periodentitis.

CONCLUSION

The present study provides evidence that the expression of Bcl-2 in patients with chronic periodontitis are different from those with healthy periodontium. It was also shown that age and gender have no effect on the expression of Bcl-2.

REFERENCES:

[1] Smith M, Seymour GJ, Cullinan MP, (2010), Histopathological features of chronic and aggressive periodontitis, Periodontology 2000.53:45–54.

[2] Colombo APV, Magalhães CB, Hartenbach FARR, et al, (2016), Periodontal-disease-associated biofilm: A reservoir for pathogens of medical importance, Microbial pathogenesis.94:27–34.

[3] Petersen PE, Ogawa H, (2005), Strengthening the prevention of periodontal disease: the WHO approach, Journal of periodontology.76:2187–2193.

[4] Cappuyns I, Gugerli P, Mombelli A, (2005), Viruses in periodontal disease–a review, Oral diseases.11:219–229.

[5] Loesche WJ, Grossman NS, (2001), Periodontal disease as a specific, albeit chronic, infection: diagnosis and treatment, Clinical microbiology reviews.14:727–752.

[6] Calenic B, Yaegaki K, (2014), Apoptotic Pathways Triggered by Oral Malodorous Compounds in Periodontal Cells: Novel Periodontal Pathologic Cause, In: Stud. Periodontal Dis. Springer, pp 85–93

[7] Nagata S, (2000), Apoptotic DNA fragmentation, Experimental cell research.256:12–18.

[8] Radogna F, Dicato M, Diederich M, (2015), Cancer-type-specific crosstalk between autophagy, necroptosis and apoptosis as a pharmacological target, Biochemical pharmacology.94:1–11.

[9] Khan KH, Blanco-Codesido M, Molife LR, (2014), Cancer therapeutics: Targeting the apoptotic pathway, Critical reviews in oncology/hematology.90:200–219.

[10] Abnet CC, Qiao Y-L, Mark SD, et al, (2001), Prospective study of tooth loss and incident esophageal and gastric cancers in China, Cancer Causes and Control.12:847–854.

[11] Michaud DS, Joshipura K, Giovannucci E, Fuchs CS, (2007), A prospective study of periodontal disease and pancreatic cancer in US male health professionals, Journal of the National Cancer Institute.99:171–175.

[12] Rosenquist K, Wennerberg J, Schildt E-B, et al, (2005), Oral status, oral infections and some lifestyle factors as risk factors for oral and oropharyngeal squamous cell carcinoma. A population-based case-control study in southern Sweden, Acta oto-laryngologica.125:1327–1336.

[13] Neville BW, Damm DD, Chi AC, Allen CM, (2015), Oral and maxillofacial pathology. Elsevier Health Sciences

[14] Johnson N, (2001), Tobacco use and oral cancer: a global perspective, Journal of Dental Education.65:328–339.

[15] Akl H, Vervloessem T, Kiviluoto S, et al, (2014), A dual role for the anti-apoptotic Bcl-2 protein in cancer: mitochondria versus endoplasmic reticulum, Biochimica et Biophysica Acta (BBA)-Molecular Cell Research.1843:2240–2252.

[16] Hardwick JM, Soane L, (2013), Multiple functions of BCL-2 family proteins, Cold Spring Harbor perspectives in biology.5:a008722.

[17] Sulkowska M, Famulski W, Sulkowski S, et al, (2003), Correlation between Bcl-2 protein expression and some clinicopathological features of oral squamous cell carcinoma, Pol J Pathol.54:49–52.

[18] Gamonal J, Bascones A, Acevedo A, et al, (2001), Apoptosis in chronic adult periodontitis analyzed by in situ DNA breaks, electron microscopy, and immunohistochemistry, Journal of periodontology.72:517–525.

[19] Bulut Ş, Uslu H, Özdemir BH, Bulut ÖE, (2006), Expression of caspase-3, p53 and Bcl-2 in generalized aggressive periodontitis, Head & face medicine.2:17.

[20] Melcher AH, (1976), On the repair potential of periodontal tissues, Journal of periodontology.47:256–260.

[21] Hasan A, Palmer RM, (2014), A clinical guide to periodontology: Pathology of periodontal disease, British dental journal.216:457–461.

[22] Cohen JJ, (1991), Programmed cell death in the immune system, Advances in immunology.50:55–85.

[23] Kasprzak A, Hausmann M, Małkowska-Lanzafame A, et al, (2016), Immunocytochemical indicators of apoptosis in gingival tissues of patients with chronic periodontitis, Journal of Medical Science.83:37–46.