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RESEARCH ARTICLE

Relationship Between BCL-2 and Ki-67 in Chronic Sialadenitis

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Abstract

Objective: Apoptosis or programmed cell death can be triggered by a variety of physiological and pathological signals. B-cell lymphoma-2 (Bcl-2) is an important anti-apoptotic protein of apoptosis pathways, mainly localized in intracellular membranes in mitochondrial outer membrane nuclear membrane and endoplasmic reticulum. BCL-2 family molecules can be stocked by upstream with irreversible cellular damage sites and have an important role in apoptosis studies. The best known antibody for identifying proliferating cells is Ki-67. There is a clear correlation between Ki-67 and the number of mitoses. Ki-67 is a nuclear protein that is believed to play a role in the early stages of rRNA synthesis ex- pressed in the G1, S, G2 and M phases except for the G0 phase of the cell cycle. In this study, we evaluated the expression of BCL-2 and Ki-67 in the pathogenesis of chronic sialadenitis.

Methods: This study was included 18 cases of chronic sialadenitis. The immunohistochemistry BCL-2 and Ki-67 antibodies was performed in cases.

Results: Statistically, there were significant correlation between BCL-2 and Ki-67 expression in acinar cells (p= 0.016). There was significant correlation between BCL-2 and Ki-67 expression in ductus and epimyoepithelial islands (p= 0.017). There was significant correlation between ductal and acinar cells on account of Ki-67 expression (p= 0.010).

Conclusion: In this study, it was seen that decrease of BCL-2 and Ki-67 expression in acinar cells was higher than ductal epithelial cells.

Key words: BCL-2, Ki-67, chronic sialadenitis

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Introduction

Apoptosis is a process of cell death and is proved to contribute to cell damage in many diseases (Nagata and Golstein, 1995). In addition, it plays a critical role in immune response and the regulation of inflammations (Cohen, 1991). Salivary gland conditions include inflammatory, bacterial, viral, and neoplastic etiologies. Sialadenitis may present itself in acute, chronic, and recurrent forms. Recurrent and chronic sialadenitis tend to be inflammatory, rather than infectious. Examples of inflammatory salivary gland conditions include recurrent parotitis and sialolithiasis in childhood. Inflammation is mostly
caused by duct stenosis or calculi (stones) (Wilson et al., 2014). Along with aggregates with lymphoid

infiltration, ductal epithelial proliferation and dilatation or glandular atrophy were found in the majority of patients with Sjogren's syndrome and chronic sialadenitis (Tarpley et al., 1974; Carracedo et al., 2010). Tissue damages such as apoptosis lead to chronic inflammation and physiological function loss (Ramos-Casals and Font, 2005; Sisto et al., 2007). Tissue damage-induced apoptosis is observed in chronic inflammation. BCL-2 is the first gene to play a critical role in regulating apoptosis. This important proto-oncogene is located at chromosome 18q21 (Tsujimoto et al., 1984). BCL-2 protein tends to inhibit the apoptosis, which is a process of programmed cell death (apoptosis) regulating cell division and facilitating independent cell life (Sasiet al., 2009). An increase in BCL-2 expression is observed during cancer and is thought to cause resistance against classic cancer treatment (Cotter, 2009). Moreover, various types of cancers and the onset and progression of tumors depend on the anti-apoptotic effect of BCL-2 (Certo et al., 2006). The Ki-67 protein is strictly associated with cell proliferation. Ki-67 is an excellent marker for cell proliferation and present during all active phases (Xia et al., 2002). In this study, we aimed to examine the BCL-2 and Ki-67 expression in chronic sialadenitis cases.

Methods

This study is a retrospective study approved by the ethical board of our university. The study population consisted of 18 chronic sialadenitis cases. BCL-2 and Ki-67 antibodies were injected to patients using an immunohistochemical method.

Immunohistochemical Study:

An immunohistochemical staining was performed using BCL-2 primary antibody (BCL-2 antimouse (100), monoclonal antibody (Biogenex) and Ki-67 primary antibody (rabbit monoclonal antibody [50mg/ml], BioGenex) on 3 micrometers of unstained sections of paraffin blocks. The AEC chromogen was used in the immunohistochemical procedure. Then, these slayts were examined under a light microscope. Acinus (A), ductus (D), and epimyoepithelial islands (EI) of each case were examined. BCL-2 staining was classified into four levels. These are; Level 0: no staining, level 1: up to 25% staining, level 2: 26-50% staining, level 3: above 50% staining (figure 1-4). Ki-67 index was evaluated on a percentage basis (Tables 1 and 2).

Table 1. Levels of BCL-2 staining on acinus, ductus, and epimyoepithelial islands.

Levels	Acinus	Ductus	Epimyoepithelial Islands
0	9	0	0
1	9	9	9
2	0	9	3
3	0	0	6

Table 2. Percentages of Ki-67 staining on acinus, ductus, and epimyoepithelial islands.

%	Acinus	Ductus	Epimyoepithelial Islands
0	9	0	0
1	0	6	0
5	3	3	0
10	6	6	0
20	0	3	0
50	0	0	6
60	0	0	6
80	0	0	3
90	0	0	3

Findings were statistically analyzed using IBM SPSS Statistics 20. Pearson and Spearman correlation methods were used for the study. P< 0.05 values were assumed meaningful.

Results

A meaningful correlation was detected between BCL-2 and Ki-67 in A (p=0.016). The comparison between BCL-2 staining on A and EI, and EI and D, also revealed a significant correlation (p= 0.016, p= 0.016). A significant correlation was observed between BCL-2 and Ki-67 in D and EI (p=0.017). A significant correlation was found when Ki-67 positivity was evaluated between in D and A (p= 0.010). No meaningful correlation was found between BCL-2, in A and D (p= 0.176). No meaningful correlation was found between BCL-2 in A and Ki-67 in D (p= 0.764). No meaningful correlation was found in between BCL-2 positivity in A and Ki-67 in EI (p= 0.661). No meaningful correlation was found between BCL-2 and Ki-67 in D (p= 0.616). No meaningful correlation was found between BCL-2 and Ki-67 in EI (p= 0.807).

No meaningful correlation was found between BCL-2 in EI and Ki-67 in D (p= 0.838). No meaningful correlation was found in terms of Ki-67 in D and EI (p= 0.868).

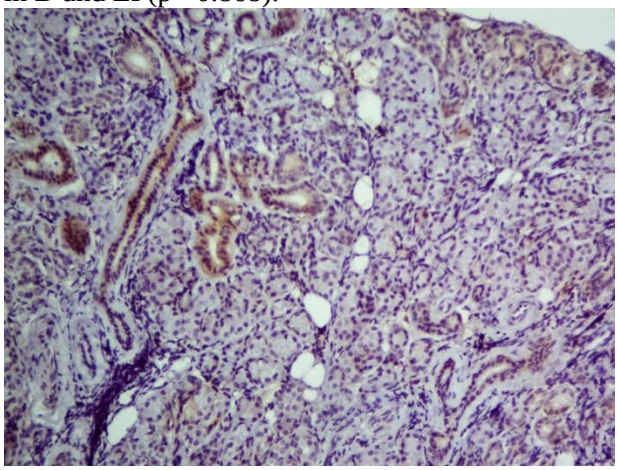

Figure 1. BCL-2 staining on ductus, Level-2 (x200)

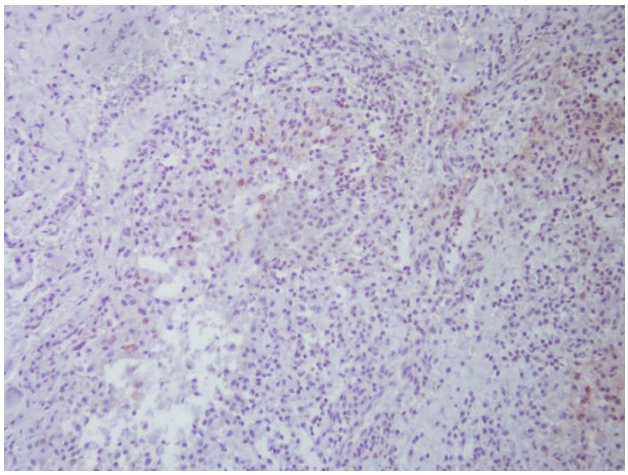

Figure 2: Ki-67 staining on EI, mild staining (x200)

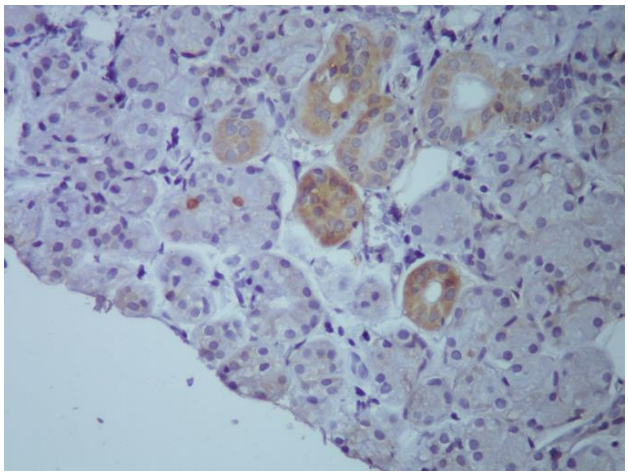

Figure 3. BCL-2 staining on A, Level-1 and Level-2 (x400)

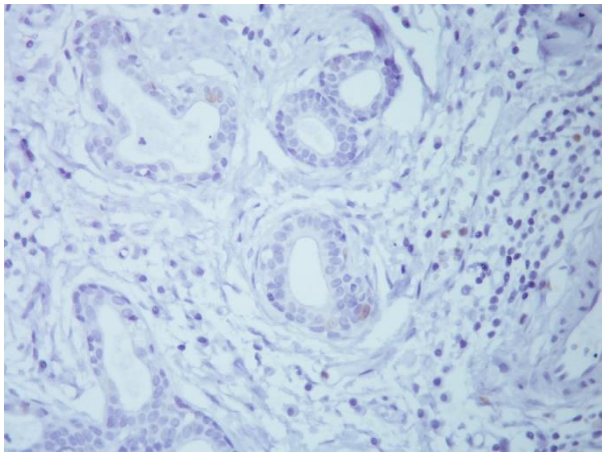

Figure 4. Ki-67 staining on A and D, mild staining (x400)

Discussion

Apoptosis, a process of programmed cell death, plays a significant role in the regulation of inflammation and immune response of the host. This process includes a series of coordinated morphological and biochemical events of cell death and their removal by the phagocytes (Bulutet al., 2006). Proliferation and apoptosis disorders, which are important changes in early carcinogenesis, may reveal the risk of neoplastic growth in histologically normal tissues, suggesting that increased expression of BCL-2 and Ki-67 may play a role in the pathogenesis of cyclosporin-A induced gingival overgrowth (AbouElkhier et al., 2014). The proliferative response of D and EI was more prominent than that of acinus in this study.

Sclerosing polycystic adenosis study by Ogasawara et al, the proliferative index (Ki-67 / MIB-1) was found to be low (Ogasawara et al., 2015). In this study, it was observed that the proliferative index of Ki-67 was different according to the region. This variability may be related to the response to inflammation and the ability of the cell to proliferate.

Apoptosis is also an important phenomenon in regulating the inflammatory response against chronic bacterial accumulation. This process has an impact on the prevalence of cellularity increase and inflammatory infiltration (Basconeset al., 2004). Cell death regulation plays an essential role in controlling the immune system. Normal cells are damaged by apoptosis either after the acute phase of the inflammation or in the progression of some diseases (Senturk et al.,2001). Bulut et al. compared the BCL-2 staining levels of periodontitis patients with a control group. They

observed that the intensity of BCL-2 staining was higher in patients compared to the control group (Wang et al., 2011). In this study, acinus, ductus, and endomysial tissue were compared. The expression of BCL-2 was found to be lower in the acinus than in the other regions. It may be considered that the acinus is less affected than the inflammatory process.

The role of apoptosis in the removal of inflammatory cells was observed in many inflammatory diseases such as Behcet's disease or leukocytoclastic vasculitis (LCV) (Senturk et al., 2001). In B lymphocytes, the overexpression of BCL-2 has been associated with anti-nuclear activity, such as occurs in lupus (Senturk et al., 2001). This oncogenic overexpression was shown to play an important role in the malign transformation and development of autoimmune diseases (Senturk et al., 2001). Infiltrating lymphocytes expressing BCL-2 protein have a tendency to enhance their survival by escaping apoptosis. However, Alvin et al. have not able to explain whether the increase in BCL-2 expressions was caused by etiological factors or a primary anomaly (Arreaza et al., 2014). Qi et al. showed that BCL-2 and procaspase-3 expressions are less present in submandibular gland acinar cells in non-obese diabetic groups compared to the control group (Qiet al., 2007). Even though no significant statistical correlation was found in this study, when Table 1 is examined, it was found that the expressions of BCL-2 and Ki-67 are higher in D and EI. On the other hand, A revealed a lesser amount of these expressions. Polihroniset. al showed that BCL-2 expression decreased in both acinal and ductal cells in minor salivary gland biopsies from patients with Sjogren's syndrome. Contrarily, they also observed that BAX expression had increased (Polihronis et al., 1998).

In this study, it was found that BCL-2 expression was higher in D cells compared to A cells. On the other hand, Gerstenbluth et al. found that BCL-2 expression had increased in the patients with proctitis (Gerstenbluth et al., 2002). This study may also refer to an increase in bcl-2 and Ki-67 as secondary to inflammation.

In conclusion, this study revealed that the expressions of BCL-2 and Ki-67 are correlated with each other in acinar, ductal, and epimyoepithelial islands. In this study, the ductal epithelial cells were thought to be more resilient to apoptosis compared to acinar cells.

Ethics Committee Approval: Ethics committee approval was received for this study from Ordu Clinical Research Ethics Committee of Ordu University (Ethic No: 2015/6).
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