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Role of Bcl-2 and Bax in parotid gland atrophy

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Abstract. – OBJECTIVE: To investigate the expression and correlation of B-cell lymphoma-2 (Bcl-2) and Bax in the parotid gland after leading duct ligation in rat.

MATERIALS AND METHODS: Atrophy of the right parotid was induced by ligating the right Stensen's duct of rats. Immunohistochemical labeling was performed to study the changes in number and distribution of Bcl-2 and Bax in each step of glandular atrophy, and every group at 1, 3, 5, 7, 14, 21, 30, 60, 90, 150, 180 days after ligation.

RESULTS: Bcl-2 and Bax showed a low level of expression in normal glandular tissues. At different time points after the ligation of the main duct, Bcl-2 was highly expressed in the duct cells, and the absorbance value reached a peak value at 21 day (3.02+0.10). The 1 D expression of Bax was found in some of the cells in the 3 D, and the expression of Bax reached the peak (1.99+0.10), and the expression of Bcl-2 and Bax were decreased in some cells. Bcl-2/Bax ratio increased at 1-day and 21-day, and then decreased and stabilized.

CONCLUSIONS: The expression of Bax and Bcl-2 after ligation of the parotid gland is closely related to the process of the parotid gland atrophy.

Key Words:
Parotid gland, Apoptosis, Bcl-2, Bax.

Introduction

Cell apoptosis is orderly death of the cell by genes controlled, which is the stability of the internal environment. Recent studies[1,2] have found that apoptosis is closely related to the occurrence and development of many diseases. B-cell lymphoma-2 (Bcl-2) family proteins play an important role in cell apoptosis. In the Bcl-2 family, Bcl-2 and Bax are the main proteins regulating the process of apoptosis. Bcl-2 regulates mitochondrial apoptosis pathway[3]. Bax is the main factor in regulating the activity of Bcl-2[4]. The primary purpose of this study was to detect the expression of Bcl-2 and Bax in the animal model of parotid gland atrophy, to explore the role of Bcl-2 and Bax in the parotid gland atrophy, to investigate the molecular mechanism of the parotid gland atrophy, and to provide a theoretical basis for the clinical treatment of salivary gland diseases.

Materials and Methods

Animal Model Establishment

A total of 72 adult healthy Wistar rats (250-300 g) was purchased from the Animal Experimental Center of Binzhou Medical College, which also granted the Ethics Committee. 72 rats were randomly divided into 12 groups, 6 rats in each groups, the 0 day group was negative, without ligation of the parotid duct. The experimental groups were 1, 3, 5, 7, 14, 21, 30, 60, 90, 150, 180 days after ligation of the parotid duct, respectively. The negative group was simulated without ligation of the main pipe, and then the incision was made. 4% hydrazine hydrate was injected intraperitoneally for anesthesia. The right parotid gland was completely removed and placed in 4% formalin fixed solution to make the paraffin samples.

Main Reagents

Bcl-2 rabbit anti mouse polyclonal antibody and Bax rabbit anti rat monoclonal antibody were obtained from Abcam (Cambridge, MA, USA), StreptAvidin-Biotin Complex (SABC) immunohistochemical staining kit was purchased by (Wuhan Boster Biological Engineering Co., Ltd., Wuhan, China).

Immunohistochemical Staining Method

Bcl-2 polyclonal antibody and Bax monoclonal antibody were used for the parotid gland tissues immunohistochemical staining and SABC staining

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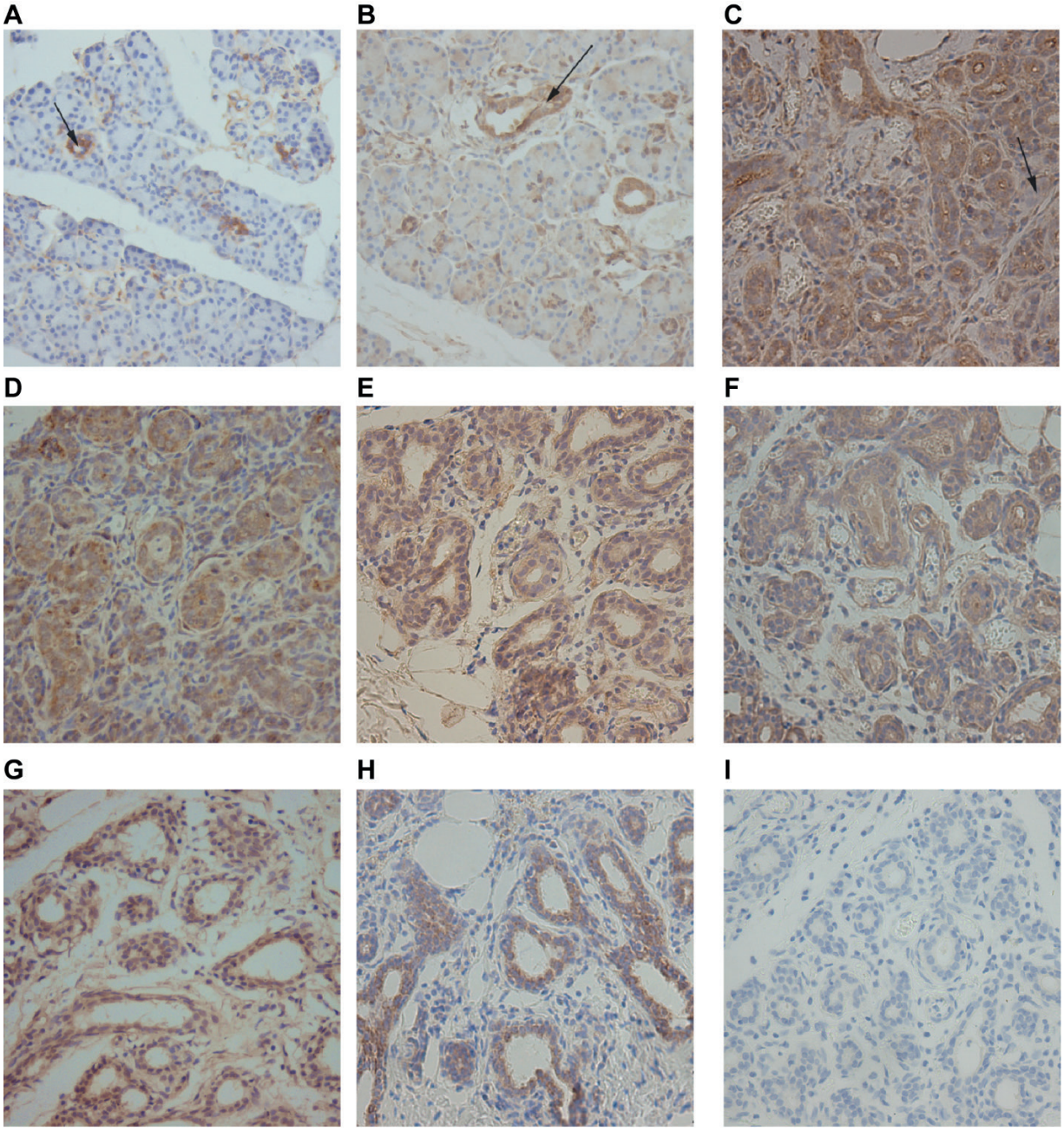

Figure 1. Bcl-2 expression of myoepithelial cells in different time points before and after duct ligation (IHC×400). **A,** Control; **B,** At day 1; **C,** At day 5; **D,** At day 7; **E,** At day 21; **F,** At day 30; **G,** At day 90; **H,** At day 180; **I,** PBS negative control group.

method was applied. Antibody dilution was Bcl-2 (1:100), Bax (1:300), diaminobenzidine (DAB) color, and hematoxylin staining. Phosphate-buffered solution (PBS) acted as a negative control.

Statistical Analysis

Specimens were observed under microscope Olympus BX51 (FN 22, Olympus, Tokyo, Japan). Image-Pro Plus 6.0 image analysis software (Media Cybernetics, Rockville, MD, USA) was applied for quantitative analysis on the expression of Bcl-2 and Bax proteins. 5 non-overlapping HPF in each section were selected. Bcl-2 and Bax positive reaction average absorbance values in each field were measured. The data were analyzed by SPSS 17 statistical software (SPSS Inc., Chicago, IL, USA). All the data were analyzed by mean ± standard deviation. Comparison between groups

was done using One-way ANOVA test followed by Least Significant Difference (LSD). Correlation analysis between Bcl-2 and Bax expression and apoptosis by Spearman rank correlation. $p<0.05$ was statistically significant.

Results

Expression of Bcl-2 in the Parotid Gland

Compared with the control group (Figure 1A), Bcl-2 expression in the leading duct ligation 1 day group (Figure 1B) was significantly increased. There were remarkably more visible striated duct cells and less intercalated duct cells. 5-day group (Figure 1C) ductal cell expression was significantly increased. There was significant difference of 5 day group with group 1 day and 0 day ($p<0.01$). In 7-day (Figure 1D) group, the expression of ductal cells increased, and most of the alveolar cell atrophy disappeared, only a few of the alveolar cells showed Bcl-2 positive expression. Ligation of 21-day (Figure 1E) positive expression of ductal cells significantly increased (3.02 + 0.01). Almost all acinar cell apoptosis, catheter dilation and vessel disappeared. 30-180 day (Figure 1F-H) residual ductal cells showed positive expression (Table I).

Expression of Bax in the Parotid Gland

Bax is expressed in normal parotid gland with low expression level (Figure 2A). The main duct ligation, and the ratio of expression of Bax in 1-day group (Figure 2B) significantly increased, ligation of 3-day (Figure 2C) reached the peak. There was a significant expression of 7-day and 3-day group ligation ($p<0.001$) (Figure 2D). Then, the amount of expression was decreased. The expression of Bax in 21-day group was gradually stabilized, and no significant difference was observed compared with normal parotid gland ($p>0.05$). 21-day (Figure 2E) after ligation acinar cells disappeared. 30-day – 180-day (Figure 2 F-H) Bax protein was expressed at low levels in some ductal cells, but no obvious cell apoptosis was found (Table I).

Table I. Bcl-2, Bax expression in acinar cell at the atrophy of the parotid glad after rat parotid duct ligation (mean± standard deviation).

Ligation time (d)	N	Bcl-2	Bax
0	6	0.45 ± 0.03^{b}	0.40 ± 0.02^{d}
1	6	0.54 ± 0.03^{b}	0.53 ± 0.14^{cd}
3	6	0.60 ± 0.02^{ab}	1.99 ± 0.10^{c}
5	6	0.77 ± 0.03^{ab}	1.50 ± 0.07^{cd}
7	6	0.96 ± 0.06^{ab}	0.95 ± 0.01^{cd}
14	6	2.07 ± 0.13^{ab}	0.68 ± 0.01^{cd}
21	6	3.02 ± 0.01^{a}	0.54 ± 0.03^{cd}
30	6	2.10 ± 0.09^{ab}	0.475 ± 0.03^{d}
60	6	1.63 ± 0.09^{ab}	0.46 ± 0.02^{d}
90	6	1.30 ± 0.02^{ab}	0.41 ± 0.02^{d}
150	6	1.15 ± 0.06^{ab}	0.35 ± 0.02^{d}
180	6	1.08 ± 0.00^{ab}	0.34 ± 0.02^{d}

Note: Bcl-2: B cell lymphoma/leukemia-2; (a) Compared with the ligation 0 day ($p<0.01$); (b) Compared with the ligation 21 day ($p<0.01$); with statistical significance. Bax: Bcl-2 associated X protein; (c) Compared with the ligation 0 day ($p<0.01$); (d) Compared with the ligation 3 day ($p<0.01$) with statistical significance.

Analysis of Bcl-2/Bax Ratio in the Process of Apoptosis of Gland Atrophy

Both Bcl-2 and Bax in normal salivary gland tissue are low-expressed (Bcl-2/Bax=1). The expressions were increased after the two main duct ligation. 3-day expression of Bax was the highest. Bcl-2 expression reached a peak at 21 days, and then the expression decreased and stabilized (Figure 3A). There were statistically significant in the ratio of Bcl-2/Bax between 1 day-7 day and 0 day groups ($p<0.01$). Bcl-2/Bax ratio began to increase at 3 days, when 21 day the ratio of Bcl-2/Bax reached the highest (Figure 3B), and then decreased and stabilized. There was not statistically significance in the ratio of Bcl-2/Bax among 90-180 day groups ($p>0.05$).

Discussion

Gland atrophy is a common clinical problem. To avoid the occurrence of salivary fistula, we sometimes need artificially boost gland atrophy, such as in oral malignant tumors. Sometimes, it is expected that the gland atrophy can be suppressed, and the function of the gland can be recovered, but the molecular biological mechanism of gland atrophy is not clear. Our results confirmed that the expression of Bax significantly increased after ligation of the parotid gland. The expression was mainly expressed in the pancreatic gland at 3 day after ligation. Takahashi et al[5] studied the submandibular gland main duct ligation can induce apoptosis of acinar cells and the expression of Bax reached to peak at 3 day after ligation, which was consistent with our research. Duct ligation of 1 day showed that a small amount of Bcl-2 was expressed in the cells at 1

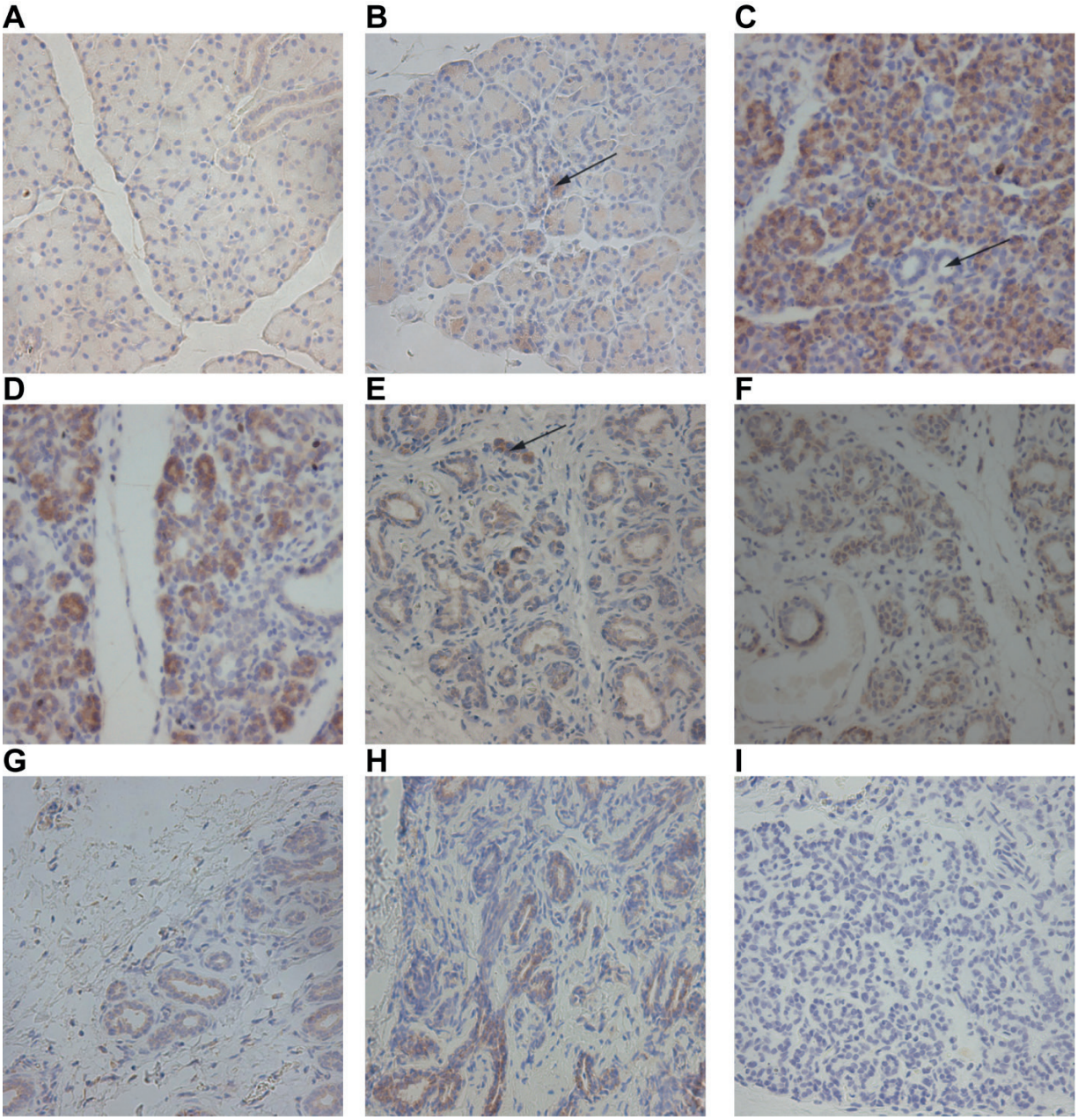

Figure 2. Bax expression of myoepithelial cells in different time points before and after duct ligation (IHC×400). ***A,*** Control; ***B,*** At day 1; ***C,*** At day 5; ***D,*** At day 7; ***E,*** At day 21; ***F,*** At day 30; ***G,*** At day 90; ***H,*** At day 180; ***I,*** PBS negative control group.

day after duct ligation. These cells resisted apoptosis in glandular tissue. The expression mechanism of these cells is not clear. The expression of Bcl-2 was mainly expressed in ductal cells, and the expression of 21 day was the highest, which may be related to the decrease of Bcl-2 expression and the inhibition effect on the expression of Bax[6,7]. Ligation of 1 day showed that a small amount of Bax expression in the duct cells, and some of the ductal structure, disappeared. In the ductal cells, the expression intensity of Bcl-2 was significantly higher than that of Bax, and its effect mechanism needs to be further explored. These studies showed that Bcl-2 inhibited the apoptosis of ductal cells and Bax promoted the apoptosis of the alveolar cells, which suggested that the atrophy of parotid gland was mainly the apoptosis of alveolar cells. Our research group has discussed myoepithelial cells (MEC) proliferation changes[8]. The expression of Bcl-2 in

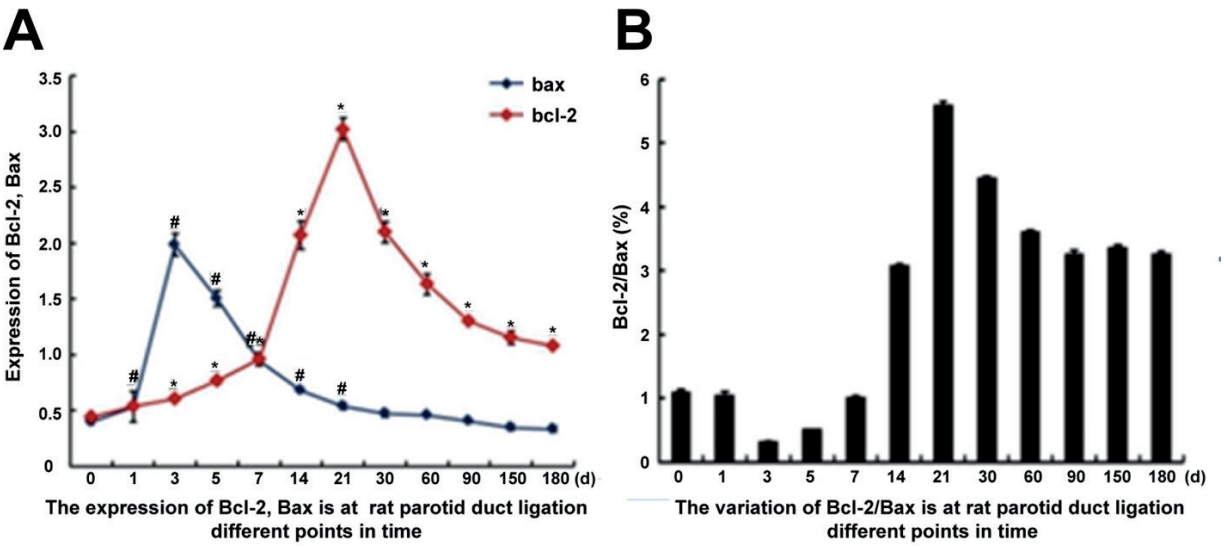

Figure 3. Bcl-2, Bax expression and Bcl-2/Bax variation in acinar cell at the atrophy of the parotid glad after rat parotid duct ligation. Figure ***A,*** *Compared with the ligation 0 day (p<0.01); #Compared with the ligation 0 day (p<0.01); with statistical significance. Figure ***B,*** Bcl-2 was negatively interrelated with Bax (r=-0.329, p=0.297).

the elongated cells of ligation 5 day and 7 day groups around the residual ductal and acinar cell surface was increased. Therefore, the expression of Bcl-2 in the process of glandular atrophy inhibited myoepithelial cells apoptosis.

Current investigations have indicated that the ratio of Bcl-2 and Bax plays a key role in determining cell apoptosis[6]. Shirali et al[9] confirmed Hetero dimer of Bax and Bcl-2 can promote cell apoptosis, which suggested Bcl-2-Bax plays an important role in promoting the gland cell apoptosis. Therefore, the parotid duct ligation leads to Bcl-2/Bax ratio, which regulates gland atrophy during apoptosis. Siqueira et al[10] proved that apoptosis can inhibitor the expression of heat shock protein 27 (Hsp27) in pleomorphic adenoma accompanied by increased Bcl-2/Bax ratio. It also confirmed that Bcl-2/Bax ratio has the correlation with apoptosis. Some researches showed that Bcl-2 and Bax are also expression in other exocrine glands atrophy, such as in pancreas and breast[11-13]. Bax promotes the apoptosis of acinar glands, which leads to gland atrophy. In the parotid gland atrophy and other exocrine glands atrophy, Bcl-2 and Bax play an important role in the regulation of the process. In addition, Marković et al[14] found high expression of Bax in thyroiditis, and no expression in normal thyroid. Therefore, inflammatory cell infiltration may also exist in the process of parotid gland atrophy, but it still needs further validation.

Conclusions

The expression of Bcl-2 and Bax has certain regularity after duct ligation. Regulation of glandular atrophy and apoptosis are closely related. Therefore, the expression of apoptosis factor can be regulated in clinical practice to prevent and treat the disease of salivary gland. The molecular mechanism of the apoptosis of the gland is not clear and further study is needed in the future.

Acknowledgments

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Conflict of interest

The authors declare no conflicts of interest.

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