

비염에서 세포고사체 및 Bax와 Bcl-2의 발현에 관한 연구

가
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Expression of Apoptosis, Bax and Bcl-2 in Nasal Polyps

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-ABSTRACT-

Background and Objectives : Apoptosis is a programmed cell death and an active regulatory response by physiologic stimuli. Eosinophils are predominant cells in the nasal polyp. However whether the apoptosis of the eosinophils could contribute to the increased numbers of eosinophils in the nasal polyp is not completely known. We have studied to reveal the relationship between the increase in the number of eosinophils and apoptosis.

Materials and Methods : Subjects are 15 non-allergic nasal polyps and 10 normal inferior turbinates. We have used TUNEL method for apoptosis study and RT-PCR was used for the Bax and Bcl-2. **Results** : The apoptotic cell ratio (apoptotic cell numbers/total cell numbers) was 0.147 in normal turbinate compared to 0.05 in nasal polyp ($p=0.056$). The apoptotic cell ratio was higher in normal turbinate compared to that of nasal polyp. The apoptotic bodies of eosinophils in nasal polyp were very scanty. The RT-PCR showed that Bax was increased in normal turbinate, however Bcl-2 was increased in nasal polyp. These results suggest that the apoptosis of eosinophils in nasal polyp was decreased compared to the normal turbinate. **Conclusion** : Apoptosis may be one of the contributing factors for the increased numbers of eosinophils in nasal polyp. (J Clinical Otolaryngol 2005; 16:264-269)

KEY WORDS : Nasal polyps · Apoptosis · Bax · Bcl-2.

서 론

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가
1)2)
3)4)
가가
(apoptosis)
(programmed cell death)
가
5)6)

: Bax Bcl - 2
 NEL (In Situ Apoptosis Detection Kit, ApopTag™ Kit. Oncor) . Labeling target DNA 3'-OH DNA
 TUNEL (terminal deoxynucleotidyl transferase (TdT) - mediated exogenous nucleotide (dUTP - digoxigenin) nick end - labeling)
 Xylen 100%, 95%, 70% ethanol , 3% H₂O₂ 3 blocking
 Bcl - 2 Bax . Bcl - 2 proteinase K 30
 family (Bcl - 2, Bax) equalibration buffer 가 .
 Bcl - 2 , Bax working strength reaction buffer 2 drop
 TdT enzyme 1 drop
 가 .
 working strength stop/wash buffer
 30 anti - digoxigenin - peroxidase
 PBS diaminobenzidine 30
 methyl green 1 .
 H - E 가 TUNEL
 1998 5 9 가 CT
 11 24 55 14 H - E (fragmented nucleus) (prototype)
 . 1 RAST TUNEL
 (× 400)
 , 10 15 10 (apoptotic cell ratio)
 방 법
 H - E
 4 μm
 H - E , RNA cDNA
 H - E H - E H - E TRIZOL® (GIBCOBRL, Grand Island, NY) RNA RNA 2 μg
 TUNEL . H - E Moloney murine leukemia virus (Life Technologies, Gaithersberg, MD) , 5x Reverse transcription buffer, 10 mM dNTPs, RNase inhibitor, random hexamer 가 37 1
 TUNEL TUNEL .⁸⁾ TU- 94 5 cDNA

(polymerase chain reaction, PCR)
 5 µl cDNA 10x PCR reaction buffer, 25 mM
 MgCl₂, 2.5 mM dNTPs, Bax, Bcl-2 GAPDH
 sense antisense primer 20 mol, *Taq* poly-
 merase,
 (Table 2). Gene Amp PCR sys-
 tem 2400(Perkin Elmer) 94 5
 94 45 , 55 30 , 72 1
 35 72 7

Table 1. Apoptotic cell counts in nasal mucosa

	Apoptotic cells/total cells (ratio)	Apoptotic cells/HPF (× 400)
Control turbinate	0.147	8.2 ± 2.5*
Nasal polyp	0.050	2.1 ± 0.2

* : p < 0.05

Table 2. Primer sequences for PCR

Bax	Sense	5' ATG GAC GGG TCC GGG GAG CAG 3'
	Antisense	5' CAG TTG AAG TTG CCG TCA GA 3'
Bcl-2	Sense	5' AGC TGC ACC TGA CGC CCT TCA 3'
	Antisense	5' AGC CAG GAG AAA TCA CAG AGG 3'
GAPDH	Sense	5' ATA GGA TCC GTG GAC ATT GTT GCC ATC AAC GAC CCC 3'
	Antisense	5' ATA GGA TCC GCC CCA GCC TTC TCC ATG GTG GT 3'

GAPDH : glyceraldehyde-3-phosphate dehydrogenase

cDNA
 2% agarose gel ethi-
 dium bromide 30
 RNA
 GAPDH(glyceraldehydes - 3 - phosphate de-
 hydrogenase)
 SPSS 7.5 for Windows(version 7.5,
 SPSS Inc., Chicago, USA) paired t - test
 p 0.05

결 과

세포고사체의 검출

HPF, 8.2 ± 2.5/HPF
 2.1 ± 0.2/
 ()
 0.05, 0.147
 (Table 1).

H - E TUNEL

(Figs. 1 and 2).

Bax와 Bcl-2 mRNA의 발현

가 Bax mRNA
 Bcl - 2

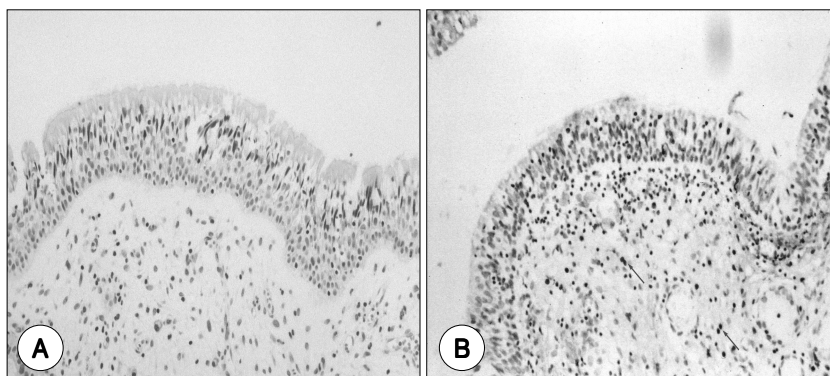


Fig. 1. In Situ apoptosis detection in normal turbinate. A : Inferior turbinate shows some inflammatory cells in epithelium and stroma (H-E, × 200). B : Many apoptotic bodies are shown (arrows) in epithelial and stroma area (TUNEL stain, × 200).

Fig. 2. In Situ apoptosis detection in nasal polyp. A : Nasal polyp shows many eosinophils (arrows), (H-E, $\times 200$). B : A few apoptotic bodies are present in subepithelial and stroma area (arrow), (TUNEL stain $\times 200$).

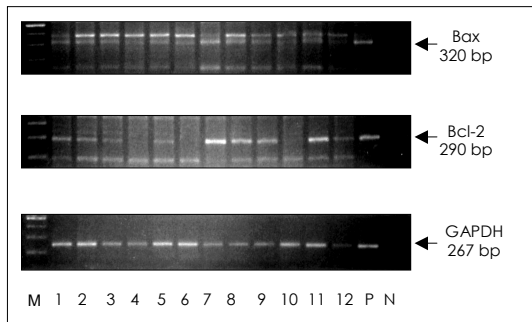
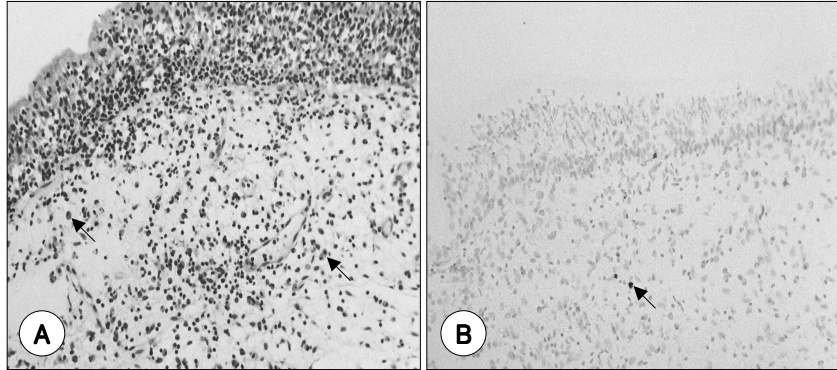


Fig. 3. RT-PCR of Bax, Bcl-2 in nasal mucosa. Bax was increased in control inferior turbinate, Bcl-2 was increased in nasal polyp (Normal inferior turbinate : lane 1 - 6, Nasal polyp : lane 7 - 12). GAPDH : glyceraldehyde-3-phosphate dehydrogenase.

Table 3. Densitometry of Bax and Bcl-2 mRNA expression

	Bax/ G	Bcl-2/ G	Bax/ Bcl-2
Control turbinate	0.79 ± 0.17	0.35 ± 0.11	$1.4 \pm 0.43^*$
Nasal polyp	0.47 ± 0.15	0.50 ± 0.12	0.96 ± 0.11

* : $p < 0.05$, G : GAPDH (glyceraldehyde-3-phosphate dehydrogenase)

cells/hr)

(Fig. 3). densitometer
 hosue keeping gene GAPDH
 Bax/G control Bcl - 2/G
 Bax/Bcl - 2 가
 가 (Table 3).
고 찰
 가 가
 (apoptosis) 가
 (2.5 x 10⁹)

가 가 가
 가 가 가
 가
 1)
 7)
 7
 12 50%
 3
 가
 anti - IL5 mAb 가
 Bcl - 2가
 IL - 5가 cytokine
 가
 IL - 3, IL - 5, GM - CSF cytokine
 가
 가
 가
 가
 가
 가
 가
 가
 가
 가

BAL(bronchoalveolar lavage)
가 가
2 가 가

가 가
가 가

4)

결론

15)

가

중심 단어 : Bax · Bcl - 2.

Bcl - 2
Bcl - X_L가
Bax
16)
RT - PCR
Bcl - 2가 가 가
Bax 가
가 (fragmentation)
H - E 가 TUNEL 가
8)
TUNEL (cell necrosis)
NEL TU-가 H - E TUNEL 가
가 H - E TUNEL 가
가 가
가

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