# Differential Diagnosis of Gingival Hyperplasia Based on IFN-γ-stimulated Gene Expression Using RT-PCR

( epulus / gingival squamous cell carcinoma / RT-PCR / IFN-γ / apoptosis / proliferation )

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**Abstract. Epulus is a benign gingival tumour of unknown aetiopathogenesis. Classification is inconsistent, and standard management strategies are lacking. Epuli are generally believed to be inflammatory rather than neoplastic lesions. The literature does not present any molecular analysis of the tumour characteristics. The purpose of the present study was to compare benign (epulus) and malignant (cancer) gingival hyperplasias with regard to the activity of the genes of apoptosis, proliferation, and inflammation using RT-PCR. The investigation involved 70 patients with epuli and 15 patients with gingival squamous cell carcinoma. Each subject had specimens collected from the tumour, tissue margin (incision line), and healthy tissue. Molecular investigations by RT-PCR were used to evaluate expression levels of the genes associated with apoptosis (*Bcl-2, Bax, Bcl-2/Bax*), proliferation (H3 histone), and inflammatory processes (*IFN-γ, IFNγ-R1, IFN-γR2, IFN-γR1/IFN-γR2*). Correlations have been disclosed between apoptosis and proliferation genes expression in giant cell epuli and high-differentiated gingival squamous cell carcinoma. In RT-PCR molecular analysis, giant cell epulus shows characteristics of a neoplastic lesion, while other epulus types seem to be inflammatory tumours.**

## Introduction

Epuli (granulomas) are the most frequently observed gingival tumours. The aetiopathogenesis of the hyperplasia is not yet clear, and classification inconsistent (Axhausen, 1940; Bernier and Cahn, 1954; Anderson and Jones, 1970; Demetrion, 1973). Literature on the subject presents considerable discrepancies regarding the tumour origin (Anderson and Jones, 1970; Demetrion, 1973; Eversole and Rovin, 1972,1973; Stones, 1962; Thoma and Goldman, 1960, Thoma, 1963; Thyldesley, 1974). Based on the results of clinical, histological, and immunohistochemical studies, epuli are generally believed to be inflammatory rather than neoplastic lesions (Pammer et al, 1998). However, the literature does not present any molecular profile of the tumours.

The purpose of the present study was to compare benign (epulus) and malignant (cancer) gingival hyperplasias with regard to the activity of the genes associated with apoptosis, proliferation, and inflammation using RT-PCR.

## Material and Methods

Molecular investigations involved 70 patients with epuli (Group I) and 15 patients with gingival squamous cell carcinoma (Group II). Based on the histopathology result, Group I was further divided into three subgroups, i.e., giant cell epulus (Subgroup I/1 – 11 subjects), fibrous epulus (Subgroup I/2 – 34 subjects), and inflammatory epulus (Subgroup I/3 – 25 subjects). Molecular investigations were carried out on intraoperative specimens collected from three sites: the lesion, tissue margin (incision line), and healthy tissue (opposite to lesion site; in the tables referred to as Control).

Among Group II subjects, GI (high-differentiated carcinoma) was diagnosed in 7 cases, GII in 6, and GIII in 2 cases (due to a low number of cases, GII and GIII patients were further treated as one group of low-differentiated cancer). Healthy tissue was sampled from the gingiva opposite to the epulus or cancer site. Informed consent was obtained from the patients; the investigations were approved by the Bioethic Committee of the Medical University of Silesia (L.dz.NN-013-313/I/03).

This phase of molecular analysis used RT-PCR to determine the expression profile of genes encoding IFN-γ receptor subunits, H3 histone (proliferation marker), and Bcl-2, Bax (apoptosis markers); the analysis was carried out in 210 biopsies from the epulus group, and 45 biopsies from gingival squamous cell carcinoma. Molecular analysis (RT-PCR) consisted of three stages: RNA extraction, amplification and quantification of amplification products, and, finally, evaluation of amplification product specificity.

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Statistical analysis used the Shapiro-Wilk, Mann-Whitney, Friedman, and Wilcoxon tests.

## Results

Bcl-2 median was higher in the epulus than in the cancer group. For epuli, the highest values were noted in the healthy tissue sample (17,495), followed by tissue margin (10,532), and the lesion (10,215). For cancer, BCL-2 median was the highest in the lesion (6,183), and the lowest in tissue margin (3,401).

In the epulus group, Bax median decreased from healthy tissue (9,546), tissue margin (8,508) to the lowest in tumour section (7,505). For cancer, the highest BAX expression was found in the lesion (17,478), and the lowest in the tissue margin (7,336).

The values of H3 histone median were lower in the epulus than in the cancer group. For epuli, the highest H3 histone median was noted in the tissue margin (5,417), and the lowest in healthy tissue (3,144). For cancer, the highest H3 histone median was observed in healthy tissue specimens (12,242), and the lowest in the lesion (2,915).

The values of IFN-γR1 median were markedly higher in the cancer than in the epulus group. In the latter, the values decreased from healthy tissue (990), tissue margin (650) to the lowest in the tumour sections (615). For cancer, IFN-γR1 median values went down from tissue margin (5,968) to the tumour (5,297) and to healthy tissue (3,685).

The values of IFN-γR2 median were also higher in cancer subjects. In the epulus group, the highest median was noted in the tissue margin (3,945), and the lowest in the lesion (2,236). For cancer, the highest IFN-γR2 median was found in tumour sections (15,297), and the lowest in the tissue margin (6,746).

IFN-γ median reached highest values in the healthy tissue specimens from both study groups (epulus – 8,072; cancer – 14,032). The lowest values were found in tumour sections of the epulus group (4,009), and in the tissue margin of the cancer subjects (1,722). The median of the IFN-γR1/IFN-γR2 quotient was low in the epulus group (did not exceed 0.26), whereas it ranged between 0.43 and 0.77 in cancer patients.

### *Comparison of gene expression levels between the epulus and cancer groups, and their subgroups*

Significant difference in Bcl-2/Bax expression was found in tissue margin between inflammatory, fibrous, and giant cell epuli, and high-differentiated carcinoma (GI). Tumour expression of Bcl-2/Bax was significantly different for fibrous and inflammatory epuli ($P = 0.007$), and low-differentiated carcinoma ($P = 0.02$). Differences were also disclosed in IFN-γ expression between inflammatory and giant cell epuli, and high-differentiated carcinoma. With regard to the proliferation process (H3 histone), and expression of both IFN-γ receptor subunits, differences were disclosed between all epulus types and low-differentiated carcinoma. The IFN-γR1/IFN-γR2 quotient was not significantly different between particular epulus types, and between giant cell epulus and high-differentiated carcinoma (GI). Significant differences were found between inflammatory and fibrous epuli, and cancers, irrespective of malignancy grading (Table 1).

## Discussion

Based on molecular analysis by RT-PC, epulus types showed significant differences regarding IFN-γ expression. Proliferation and apoptosis gene expression was weaker in giant cell epulus when compared to other epulus types; however, the gene expression levels were similar to those observed for high-differentiated carcinoma. Another recognized proliferation marker, apart from PCNA, Ki 67, is H3 histone (Isenberg, 1979; Baumach et al, 1987; Bosch et al, 1993). Sakamoto et al. (2004) found high-, medium-, and low-level H3 expression in squamous cell carcinoma, dysplasia/hyperplasia, and normal lingual epithelium, respectively. In hyperplastic epithelium, H3 was only disclosed in the areas of inflammation (Bosch et al,1993). Since H3 histone is absent in normal epithelium and abundant in neoplastic tissue, it was selected as a marker differentiating between specific disease entities originating from the same tissue. Different H3 histone expression patterns were determined for inflammatory and fibrous epuli. The fact of H3 histone median reaching higher values in inflammatory than in fibrous epulus seems to confirm our previous suggestions that H3 histone expression is characteristic of inflammatory conditions. Thus, it could be concluded that H3 histone is not sensitive enough to differentiate between benign and malignant processes OR that the processes are characterized by a similar proliferation course.

*Bcl-2* belongs to the family of *Bcl-2* genes, which involves pro-apoptotic (Bax, Bad, Bak, Bik, Bid), and anti-apoptotic (Mcl-1, Bcl-X, Bcl-2) proteins. The function of Bcl-2 consists in inhibiting apoptosis, as seen in both physiological and neoplastic processes (Isenberg, 1979). However, the mechanism of its action is not clear. Differences in Bcl-2 expression between epuli and gingival carcinoma found in our study were more pronounced in healthy tissue and tissue margin, and more distinct in the epulus than in the cancer group.

In gingival squamous cell carcinoma, Bcl-2 expression levels decreased from high-differentiated through low-differentiated carcinoma, and were the lowest in healthy tissue, which is in accordance with the results of Drenning et al. (2001). Molecular investigations carried out have changed the attitude towards the so far accepted epulus classification. Molecular characteris-

*Table 1. Comparison of gene expression profiles between Group I and II subgroups (statistically significant variables)*

| Parameter | Site | General | Comparison between subgroups | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | gce-fe | gce-ie | gce-hdc | gce-ldc | fe-ie | fe-hdc | fe-ldc | ie-hdc | ie-ldc | hdc-ldc |
| Bcl-2 | Lesion | NS (P = 0.72) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | Margin | NS (P = 0.37) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | Control | NS (P = 0.34) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Bax | Lesion | NS (P = 0.29) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | Margin | NS (P = 0.74) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | Control | NS (P = 0.54) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Bcl-2/Bax | Lesion | **P = 0.03** | NS (P = 0.31) | NS (P = 0.80) | NS (P= 0.90) | NS (P = 0.22) | **P = 0.007** | NS (P = 0.31) | **P = 0.02** | NS (P = 0.58) | NS (P = 0.37) | NS (P = 0.15) |
| | Margin | **P = 0.01** | NS (P = 0.32) | NS (P = 0.95) | **P = 0.03** | NS (P = 0.34) | NS (P = 0.19) | **P = 0.003** | **P = 0.04** | **P = 0.01** | NS (P = 0.13) | NS (P = 0.67) |
| | Control | **P = 0..006** | NS (P = 0.19) | NS (P = 0.34) | NS (P = 0.13) | NS (P = 0.15) | **P = 0.003** | NS (P = 0.52) | NS (P = 0.43) | **P = 0.002** | **P = 0.01** | NS (P = 0.78) |
| Histone H3 | Lesion | **P = 0..03** | NS (P = 0.69) | NS (P = 0.11) | NS (P = 0.91) | NS (P = 0.43) | **P = 0.002** | NS (P = 0.94) | NS (P = 0.19) | NS (P = 0.07) | NS (P = 0.67) | NS (P = 0.25) |
| | Margin | NS (PP = 0..44) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | Control | NS (P = 0..29) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| IFN-γR1 | Lesion | **P = 0..03** | NS (P = 0.33) | NS (P = 0.65) | NS (P = 0.64) | **P = 0.04** | NS (P = 0.33) | NS (P = 0.15) | **P = 0.008** | NS (P = 0.13) | **P = 0.009** | NS (P = 0.09) |
| | Margin | **P = 0.02** | **P = 0.03** | NS (P = 0.34) | NS (P = 0.16) | **P = 0.001** | NS (P = 0.28) | NS (P = 0.94) | **P = 0.004** | NS (P = 0.88) | NS (P = 0.08) | NS (P = 0.25) |
| | Control | **P = 0.003** | **P = 0.02** | **P = 0.03** | NS (P = 0.13) | **P = 0.001** | NS (P = 0.35) | NS (P = 0.89) | **P = 0.02** | NS (P = 0.73) | **P = 0.001** | **P = 0.05** |
| IFN-γR2 | Lesion | **P = 0.03** | NS (P = 0.57) | NS (P = 0.20) | NS (P = 0.13) | **P = 0.002** | NS (P = 0.28) | NS (P = 0.43) | **P = 0.01** | NS (P = 0.84) | **P = 0.02** | **P = 0.03** |
| | Margin | NS (P = 0.17) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | Control | **P = 0.02** | NS (P = 0.07) | NS (P = 0.10) | NS (P = 0.72) | **P = 0.001** | NS (P = 0.70) | NS (P = 0.43) | **P = 0.02** | NS (P = 0.39) | **P = 0.009** | **P = 0.03** |
| IFN-γR1/ IFN-γR2 | Lesion | **P = 0.03** | NS (P = 0.36) | NS (P = 0.63) | NS (P = 0.29) | NS (P = 0.27) | NS (P = 0.66) | **P = 0.02** | **P = 0.03** | **P = 0.01** | **P = 0.02** | NS (P= 0.48) |
| | Margin | NS (P = 0.11) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | Control | NS (P = 0.23 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| IFNγ | Lesion | **P=0.005** | NS (P=0.21) | **P=0.003** | NS (P=0.41) | NS (P=0.06) | **P=0.01** | NS (P=0.15) | NS (P=0.24) | **P=0.024** | NS (P=0.28) | **P=0.02** |
| | Margin | **p=0,02** | NS (P=0,46) | **p=0,001** | NS (P=0,10) | NS (P=0,79) | NS (P=0,07) | NS (P=0,08) | NS (P=0,79) | **P=0,008** | NS (P=0,31) | NS (P=0,25) |
| | Control | NS (P=0,34) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

**gce** – giant cell epulus; **fe** – fibrous epulus; **ie** – inflammatory epulus; **h-dc** – high-differentiated carcinoma; **l-dc** – low-differentiated carcinoma

tics of giant cell epulus reminds of a neoplastic process; thus, more radical intervention seems recommended than in the case of inflammatory and fibrous forms.

## Conclusions

In RT-PCR molecular analysis, giant cell epulus shows characteristics of a neoplastic lesion, while other epulus types seem to be inflammatory tumours.

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