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Immunohistochemical expression of p53, KI-67 and Bcl – 2 in Premalignant and Malignant Oral Lesions

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Abstract

Background: p53 protein is a product of TP53 tumor Suppressor gene. TP53 mutations leading to loss of function are the commonest type of genetic damage found in human cancers and oral squamous cell carcinomas, often preceding recognizable histological alterations. Ki-67 has been shown to be excellent for the estimation of the growth fraction in both normal and malignant human tissue and this antibody is now used as the usual standard for the assessment of cell proliferation. Aim: To study the expression of p53 and Ki-67 in premalignant and malignant lesions of oral cavity. Material and Methods: The immunohistochemical expression of Ki-67 and p53 was studied on 110 cases of premalignant and malignant lesions of oral cavity during a period from July 2017 to June 2018. Results: The immunohistochemical expression of Ki-67 and p53 showed similar trends, and increased with the degree of dysplasia. On analyzing statistically, a high significant association was found between p53 expression and higher grades of tumour differentiation. However expression of Ki67 did not show significant association with various grades of differentiation of oral squamous cell carcinoma. Conclusion: p53 and Ki-67 are useful biomarkers of malignant transformation in oral precancerous lesion and may serve as intermediate points for cancer prevention programmes.

Keywords: Ki-67; p53; premalignant; malignant; oral lesions

Introduction

There is an increased awareness of the importance of apoptosis in cancerogenesis.1 Studies of human B-cell. lymphomas (Bcl) led to the discovery of proto-onco- gene bcl-2, which is situated on chromosome 18. This 25-kd protein is also located at the mitochondrial outer membrane and in the nuclear envelope, plasma membrane, and endoplasmic reticulum.2 bcl-2 expres- sion was first described in follicular lymphomas with a chromosomal translocation; later studies also found it in lymphomas and epithelial tumors without this translocation.2 Overexpression of bcl-2 results in an alteration of programmed cell death with persistence of cells that fail to die.3 In normal proliferating epi- thelium, bcl-2 is expressed in stem cell zones such as the basal layers, where it acts to prevent the death of cells in the regenerative compartment.3,4 Bcl-2 pro- tein overexpression has been found in the early phase of epithelial

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cancerogenesis.1 Bcl-2 was mainly ex- pressed in well-differentiated transitional cell carcinomas, whereas it was absent in high-grade transitional cell carcinomas.5 This loss may reflect a deregulation of the mechanisms that control bcl-2 expression.5 Little is known about the presence of bcl-2 in normal, dysplastic, or neoplastic oral epithelium.6,7 Loss of normal p53 function is correlated to the progression of several preneoplastic lesions to neoplasms and with a shortened survival in several malignancies.5 Moreover, p53 expression showed a statistically significant correlation with tumor grade and stage.5

The detection of p53 in preinvasive areas adjacent to squamous cell carcinoma and in dysplastic oral epithelium suggests that p53 abnormalities may constitute an early event in the natural history of oral cancer.8-11 p53 safeguards genomic stability by blocking the entry of a DNA-damaged cell from the G1 phase to the S phase and by activating apoptosis through a down-regulation of bcl-2.12

The p53 gene is thus involved in the apoptotic pathway.1 The majority of oral squamous cell carci- nomas (SCCs) (up to 80%) carry p53 mutations, and this fact shows that oral mucosa is one of the most common targets for p53 mutations.13 A strong corre- lation of Ki-67 with high tumor grade has been re- ported,5 and MIB-1 is regarded as a reliable marker of proliferating cells.14 The aims of our study were: 1) to investigate, in normal oral epithelium, leukoplakia, dysplasia and oral SCC, the prevalence of p53, bcl-2, and Ki-67 immunoreactivity and of apoptosis; and 2) to determine the relationship between them.

Materials and Methods

A total of 70 biopsy samples were analyzed. The following formalin-fixed, paraffin-embedded tissues were used in this study: normal oral mucosa obtained during third molar removal (10 cases), leukoplakia (12 cases), epithelial dysplasia (12 cases: 6 mild and 6 severe dysplasia and carcinoma in situ), invasive car- cinoma well differentiated (G1) (12 cases), invasive carcinoma moderately differentiated (G2) (12 cases), and invasive carcinoma poorly differentiated (G3) (12 cases). The age of the patients ranged from 48 to 67 years (mean, 55 years). All selected samples had been routinely fixed in 10% neutral formalin (24 to 48 hours). dehydrated in graded alcohols, cleared in xy- lene, and embedded in paraffin. The hematoxylin- eosin slides were all reviewed, the diagnosis was confirmed, and the slides for the quantitative evalua- tion were selected. Epithelial dysplasia was diagnosed according to the criteria and the definition proposed by Pindborg et al15: mild dysplasia was considered mild expansion of the proliferative zone, with mitotic activity, hyperchromatism, and nuclear variability; moderate dysplasia indicated these same features were present in up to half of the thickness of the epithelium; and severe dysplasia indicated the atypi- cal proliferative zone encompassed up to three fourths of the epithelium.

Immunohistochemical staining for bcl-2 protein was performed using the following antigen retrieval system. Sections were deparaffinized in 2 changes of xylene for 10 minutes each and then were rehydrated through graded alcohols and immersed in 0.3% hydro- gen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Sections were then washed in phosphate-buffered saline (PBS). The tissue sections were put in a microwave oven

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(Cooktyronic M720, 700 W; Philips, Eindhoven, The Netherlands) in a plastic Coplin jar filled with 10 mmol/L sodium citrate buffer (pH 6.0) at 5-minute intervals for a total of 10 minutes. At each 5-minute interval, the fluid in the Coplin jar was removed from the microwave oven and allowed to cool. Slides were incubated overnight in a 1:60 dilution of the primary mouse anti-human bcl-2 monoclonal antibody (124; DAKO, Glostrup, Denmark). A biotin streptavidin detection system was used with diaminobenzidine as the chromogen.

Slides were washed twice with PBS and incubated with the linking reagent (biotinylated anti-immuno- globulins) for 15 minutes at room temperature. After being rinsed in PBS, the slides were incubated with the peroxidase-conjugated streptavidin label for 15 minutes at room temperature. The sections were again rinsed in PBS and incubated with diaminoben- zidine for 10 minutes in the dark. After chromogen development, slides were washed in 2 changes of water and counterstained with a 1:10 dilution of he- matoxylin. The sections were then dehydrated, cleared in xylene, and mounted. A negative control was performed in all cases by omitting the primary antibody, which in all instances resulted in negative immunoreactivity. Sections from a lymph node with follicular lymphoma were used as positive controls. Normal lymphocytes present in the tissues repre- sented an internal positive control for bcl-2 immuno-staining. In all positive cases, immunoreactivity was restricted to the cytoplasm. The percentage of bcl-2– positive cells was evaluated from a minimum of 1000 cells in each case, and bcl-2 immunostaining was scored using a 3-tiered scale in which minus indicates less than 5% cells, plus indicates 5% to 50% cells, and double-plus indicates more than 50% cells.

Descriptive statistical analysis was performed for each group of lesions.

Immunohistochemical staining for p53 protein was performed using the following antigen retrieval system. Sections were deparaffinized in 2 changes of xylene for 10 minutes each and then were rehydrated through graded alcohols and immersed in 0.3% hydro- gen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Sections were then washed in PBS. The tissue sections were put in a microwave oven (Cooktyronic M720, 700 W; Philips) in a plastic Coplin jar filled with 10 mmol/L sodium citrate buffer (pH 6.0) at 5-minute intervals for a total of 10 minutes. Slides were incubated overnight with a 1:50 dilution of the primary mouse anti-human p53 monoclonal antibody (DO-7; DAKO). A biotin-strepta- vidin detection system was used with diaminobenzi- dine as the chromogen. Slides were washed twice with PBS and incubated with the linking reagent (bi- otinylated anti-immunoglobulins) for 15 minutes at room temperature. After being rinsed in PBS, the slides were incubated with the peroxidase-conjugated streptavidin label for 15 minutes at room tempera- ture. The sections were again rinsed in PBS and incu- bated with diaminobenzidine for 10 minutes in the dark. After chromogen development, slides were washed in 2 changes of water and counterstained with a 1:10 dilution of hematoxylin. The sections were then dehydrated, cleared in xylene, and mounted. A negative control was performed in all cases by omitting the primary antibody, which in all instances resulted in negative immunoreactivity. p53 expression and location were evaluated on histo- logic sections using a Leitz Orthoplan microscope (Wetzlar, Germany) equipped with a ×63 objective and an eyepiece graticule. Only nuclear staining of epithelial cells was observed, and the nuclei with a clear brown color, regardless of staining intensity,

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were regarded as p53 positive. The percentage of p53-positive cells was evaluated from a minimum of 1,000 cells in each case, and p53 immunostaining was scored using a 3-tiered scale, as for bcl-2 immunostain- ing. The cells were evaluated in both the basal and parabasal layers. Descriptive statistical analysis was performed for each group of lesion, and the $\chi 2$ test was used to determine statistically significant differ- ences between the groups.

For MIB-1 immunostaining, the slides were pretreated with 3-aminopropyltriethoxysilane (APES; Sigma Chem- ical Co, St Louis, MO), which avoided the separation of the section from the slide during the incubation in the microwave oven. For each case, a 5-µm section was cut and placed onto a pretreated slide. The stain- ing protocol of this slide consisted of the applications of a series of reagents in the following manner: over- night drying at 37°C, dewaxing and rehydration, im- mersion in a plastic box containing 0.01 mol/L citrate buffer at pH 6.0, incubation for 5 minutes in a micro- wave oven initially at 750 W until boiling began and then at 350 W for the remaining time, incubation for 5 minutes in a microwave oven at 350 W, cooling for 20 minutes at room temperature, washing in running water and then in distilled water for 5 minutes, wash- ing in Tris-buffered saline (TBS) for 5 minutes, re- moval of any excess TBS, addition of primary mono- clonal mouse anti– ki-67 antibody (Immunotech, Marseille, France) diluted 1:25 in TBS, and overnight incubation at 4°C in a humidified room. Next, the protocol consisted of washing in TBS for 5 minutes (3 times), addition of secondary prediluted biotinylated anti-mouse antibody (LSAB; DAKO) and incubation for 10 min at room temperature, washing in TBS for 5 minutes (3 times), addition of prediluted streptavidin-peroxidase complex (LSAB; DAKO) and incubation for 10 minutes at room temperature, and washing in TBS for 5 minutes (3 times). The protocol continued with immersion in 0.05% DAB and 0.01% H2O2 in TBS for 2 to 3 minutes at room temperature, washing in running water and then in distilled water for 5 min- utes, counterstaining with ethyl green for 30 minutes, washing in distilled water for 30 seconds, washing in buthanol I for 5 seconds, washing in buthanol II for 30 seconds, and dehydration and mounting in Per- mount.

The positivity to MIB-1 was evaluated by counting the number of positive cells per 1000 cells, and the values were expressed in an percentage. The pres- ence of apoptotic bodies was evaluated in a random fashion in the parabasal layer in 20 high-power fields. Mann-Whitney U, Kruskal-Wallis, and $\chi 2$ tests were used to evaluate the presence of statistically signifi- cant differences. Simple regression analysis was used to evaluate the correlation between features.

Results

p53

p53 was present in the basal layer in normal oral epithelium; in the basal and parabasal layers in leuko- plakia, dysplasia, and carcinoma in situ; and in central and peripheral regions in invasive carcinoma. In nor- mal oral epithelium in only 1 sample, the positivity was between 5% and 50%, whereas all of the other specimens showed a positivity of less than 5% (Fig 1). In leukoplakia, in 9 samples, the positivity was less than 5%, and in 3 samples, it was between 5% and 50%. In mild dysplasia, in 4 samples, the positivity was less than 5%, and in 2 samples, it was between 5% and 50%. In severe dysplasia, 5 samples had a positivity between 5% and

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50% (Fig 2), and 1 sample had more than 50%. In well-differentiated carcinoma, 6 samples had a positivity of less than 5%, 5 samples had be- tween 5% and 50%, and 1 sample had more than 50%. In moderately differentiated carcinoma, 5 samples had a positivity of less than 5%, 5 samples had be- tween 5% and 50%, and 2 samples had more than 50%. In poorly differentiated carcinoma, 5 samples had a positivity of less than 5%, 4 samples had be- tween 5% and 50%, and 3 samples had more than 50%.

bcl-2

In normal oral epithelium and in leukoplakia, bcl-2 protein expression was seen in the basal layer, and in dysplasia, it was evaluated in the basal layer and rarely in the parabasal layer. In carcinoma, it was present in the central and peripheral areas of the tumor. In normal oral epithelium, in 3 samples, the positivity was less than 5%, in 6 samples, it was between 5% and 50%, and in 1 sample, it was more than 50% (Fig 3). In leukoplakia, 8 samples showed a positivity of less than 5%, and 4 samples had between 5% and 50%. In mild and moderate dysplasia, 3 samples had a positiv- ity of less than 5% (Figs 4, 5), and 3 samples had between 5% and 50%. In severe dysplasia and carci- noma in situ, in all 6 samples, the positivity was less than 5%. In well-differentiated carcinoma, in 8 sam- ples, the positivity was less than 5%, in 3 samples, it was between 5% and 50%, and in 1 sample, it was more than 50%. In moderately differentiated carci- noma, in 9 samples, the positivity was less than 5%, in 2 samples, it was between 5% and 50% (Fig 6), and in 1 sample, it was more than 50%. In poorly differenti- ated carcinoma, in 6 samples, the positivity was less than 5%, in 2 samples, it was comprised between 5% and 50%, and in 4 samples, it was more than 50%.

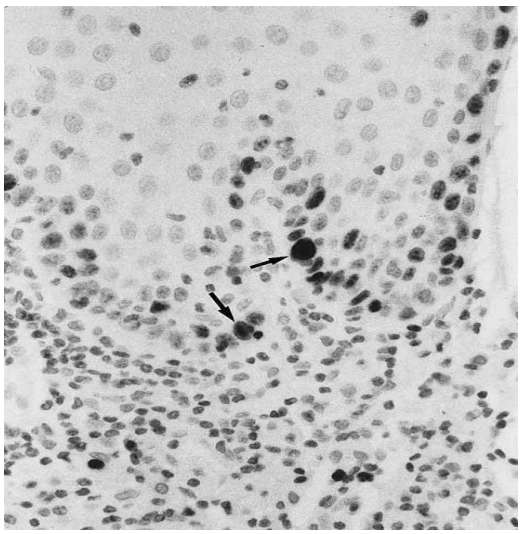

FIGURE 1. Normal oral mucosa, showing a few basal cells positive to p53 (*arrows*) (p53 immunostaining alkaline phosphatase antialka- line phosphatase, original magnification ×40).

MIB-1

The expression of MIB-1 was very similar to that of p53. In normal oral mucosa, the value was 5.2% (±2.20%); in leukoplakia, 9.0% (±3.89%; in mild dys- plasia, 15.3% (±5.61%); in severe

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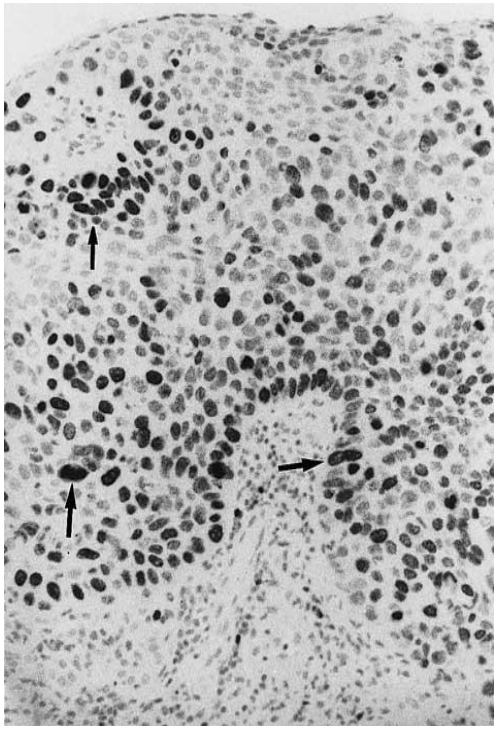
dysplasia and carci- noma in situ, 28.6% (±6.21%) (Fig 7); in well-differentiated carcinoma, 18.8% (±6.20%); in moderately differentiated carcinoma, 20.8% (±6.06%); and in poorly differentiated carcinoma, 24.2% (±6.0081%) (Figs 8, 9)

The mean values for all types of carcinoma was 21.6% (±6.81%). With the Mann-Whitney U test, sta- tistically significant differences were found between normal oral mucosa and leukoplakia (P = .0161), normal oral mucosa and mild dysplasia (P = .0017), normal oral mucosa and severe dysplasia (P = .0011), and normal oral mucosa and carcinomas (P < .0001). Statistically significant differences were found, more- over, between leukoplakia and mild dysplasia (P = .039), leukoplakia and severe dysplasia (P = .0007), and leukoplakia and carcinoma (P < .0001). Statisti- cally significant differences were also found between mild and severe dysplasia (P = .0065), mild dysplasia and carcinoma (P = .0455), and severe dysplasia and carcinoma (P = .0455). Kruskal-Wallis test showed no statistically significant differences among well-differ- entiated, moderately differentiated, and poorly differ- entiated carcinoma (P = .1209).

FIGURE 2. Severe dysplasia, showing strong positivity to p53 is seen in about 5% to 10% of epithelial cells (*arrows*) (p53 immunostaining alkaline phosphatase antialkaline phosphatase, original magnification ×20).



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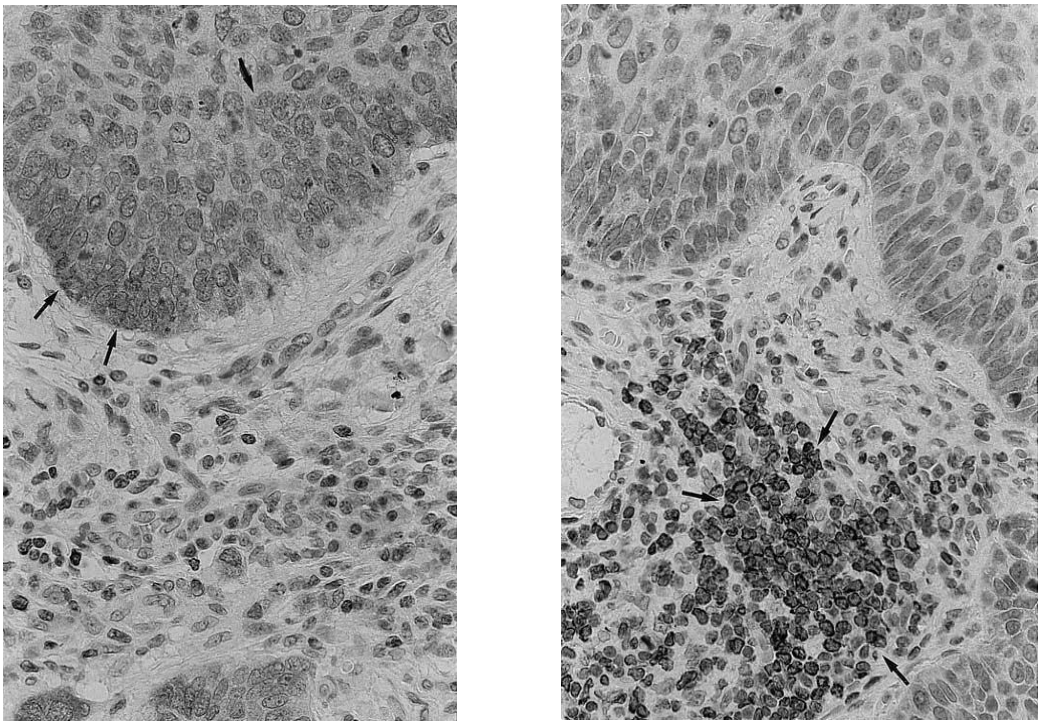

FIGURE 3. Normal oral mucosa, showing diffuse positivity of the basal and parabasal cell layers to bcl-2 protein (*arrows*) (bcl-2 immu- nostaining alkaline phosphatase antialkaline phosphatase, original magnification ×40).

APOPTOTIC INDEX

In normal oral mucosa, the apoptotic index (AI) was 0.09 (±0.07); in leukoplakia, 0.083 (±0.08); in

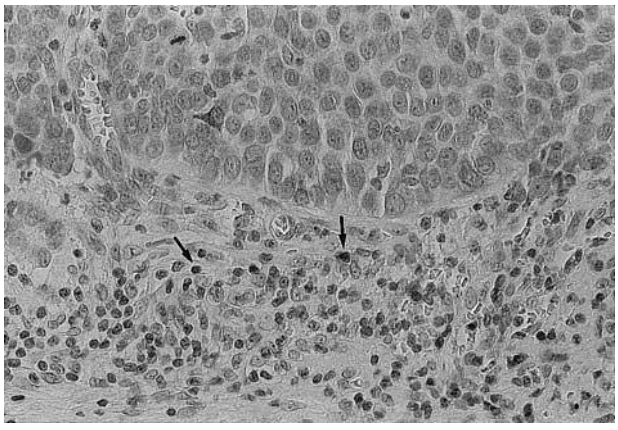

FIGURE 4. Mild dysplasia, showing negativity of the basal cell layers to bcl-2 and positivity of the lymphocytes (arrows) (bcl-2 immunostain- ing alkaline phosphatase antialkaline phosphatase, original magnifi- cation ×40).

FIGURE 5. Moderate dysplasia, showing complete negativity to bcl-2 of the basal cell layer and positivity of the lymphocytes (arrows) (bcl-2 immunostaining alkaline phosphatase

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antialkaline phosphatase, original magnification ×40). mild dysplasia, 0.133 (±0.10); in severe dysplasia and carcinoma in situ, 0.317 (±0.12); in well-differenti- ated carcinoma, 0.242 (±0.12); in moderately differ- entiated carcinoma, 0.367 (±0.23); and in poorly dif- ferentiated carcinoma, 0.433 (±0.25) (Fig 10). With the Mann-Whitney U test, statistically significant differences were found between normal oral mucosa and severe dysplasia (P = .0024), leukoplakia and severe dysplasia (P = .0020), and mild and severe dysplasia (P = .025). Moreover, a statistically signifi- cant difference was found between well-differenti- ated carcinoma and normal oral mucosa (P = .002), leukoplakia (P < .001), and mild dysplasia (P =.0103). Kruskal-Wallis test has been used to evalu- ate the differences of the AI of well-differentiated, moderately differentiated, and poorly differentiated carcinoma: these differences were not statistically significant (P = .1286). Moreover, no statistically dif- ferences were found between normal oral mucosa and leukoplakia (P = .8175), normal oral mucosa and mild dysplasia (P = .4477), leukoplakia and mild dys- plasia (P = .3490), and severe dysplasia and carci- noma (P = .8939).

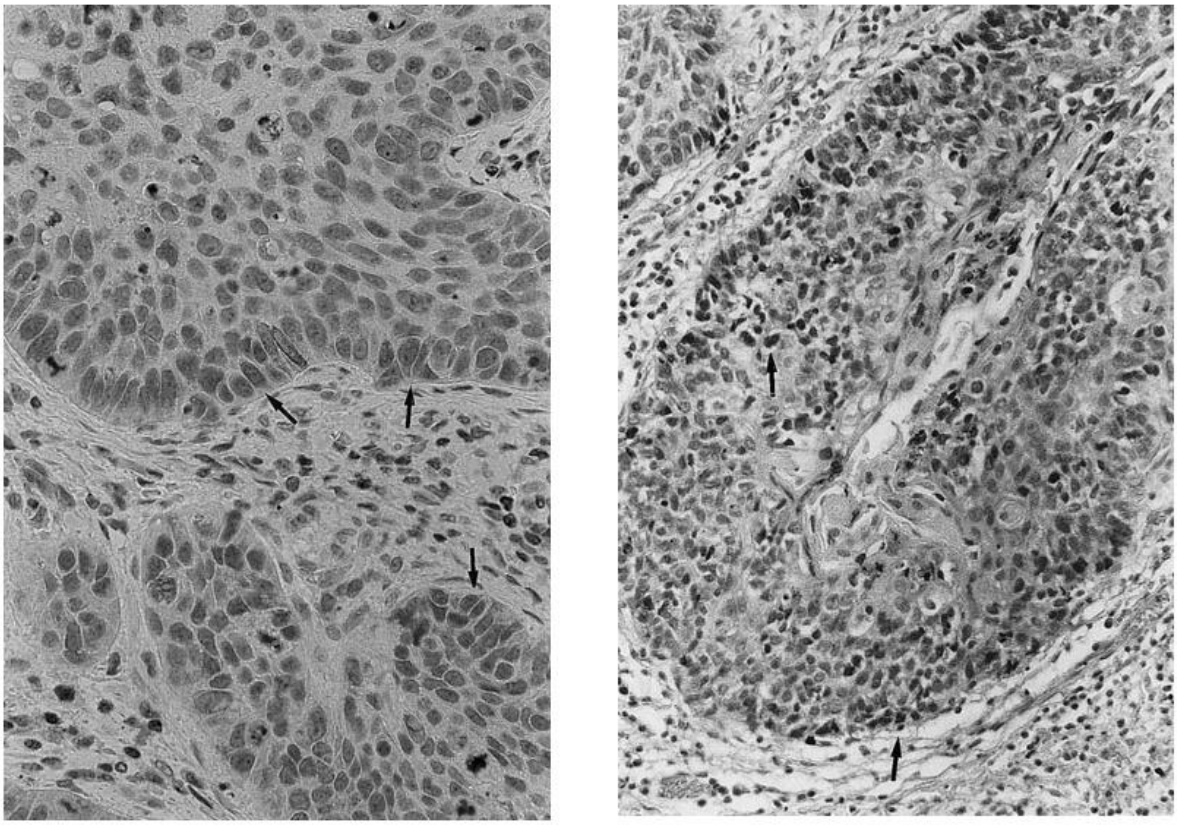

FIGURE 6. Moderately differentiated carcinoma, showing nonhomo- geneous, diffuse (<50%) positivity of the neoplastic cells to bcl-2 (arrows) (bcl-2 immunostaining alkaline phosphatase antialkaline phos- phatase, original magnification ×40).

FIGURE 8. Poorly differentiated carcinoma, showing positivity (<50%) of the neoplastic cells (arrows) (MIB-1 immunostaining alkaline phosphatase antialkaline phosphatase, original magnification ×20).

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CORRELATION BETWEEN FEATURES

p53 protein was negative in 16 bcl-2–negative cases (42.1%), in 16 bcl-2–positive (+) cases (42.1%), and in

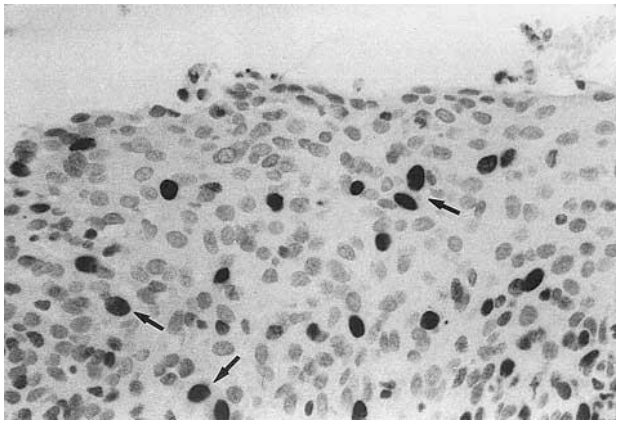

FIGURE 7. Severe dysplasia, showing strong positivity of basal, parabasal, and superficial layers to MIB-1 (arrows) (MIB-1 immuno- staining alkaline phosphatase antialkaline phosphatase, original mag- nification ×40). 6 bcl-2–positive (++) ones (15.8%). p53 was positive (+) in 20 bcl-2–negative cases (80%), in 4 bcl-2– positive (+) cases (16%), and in 1 bcl-2–positive (++) case (4%). All of the 7 p53-positive (++) cases were bcl-2 negative. There was a negative significant statis- tical correlation between p53 and bcl-2 protein (P =.001). In the 43 bcl-2–negative cases, the mean value of AI was 0.314 (±0.217), whereas in the 27 bcl-2– positive (+ and ++) cases, the mean value was 0.133 (±0.130); the difference between the 2 groups was statistically significant (P < .0001). A good, but neg- ative, correlation between these 2 features was present. Finally, considering all of the 70 cases, the simple regression analysis (Fig 11) showed that there was a good positive correlation (r = 0.694, P < .0001) between AI and MIB-1 expression.

Discussion

Ramsay et al3 showed that compared with nevi, in which the bcl-2 protein immunoreactivity was present in all cells, melanomas exhibited a progres- sive loss of the protein expression with increased levels of malignancy. These data suggest, perhaps, that bcl-2 loss is associated with or reflects an in- creased malignant potential3. The presence of bcl-2 seems to be associated with a better prognosis in some tumors but not in others.2,3,16 High levels of bcl-2 protein correlated with lower rates of complete remission and shorter survival in patients with acute myeloid leukemia.17,18 On the other hand, the bcl-2 expression in breast cancer seems to be predictive of a positive response to endocrine therapy and corre- lates with improved survival, whereas in prostate can- cer, bcl-2 expression is related to androgen-indepen- dent tumor growth and chemoresistance.12,16,17 In thyroid cancer, bcl-2 expression seems to be correlated with the differentiation of the tumors, and indif- ferentiated tumors do not express the protein.16,18

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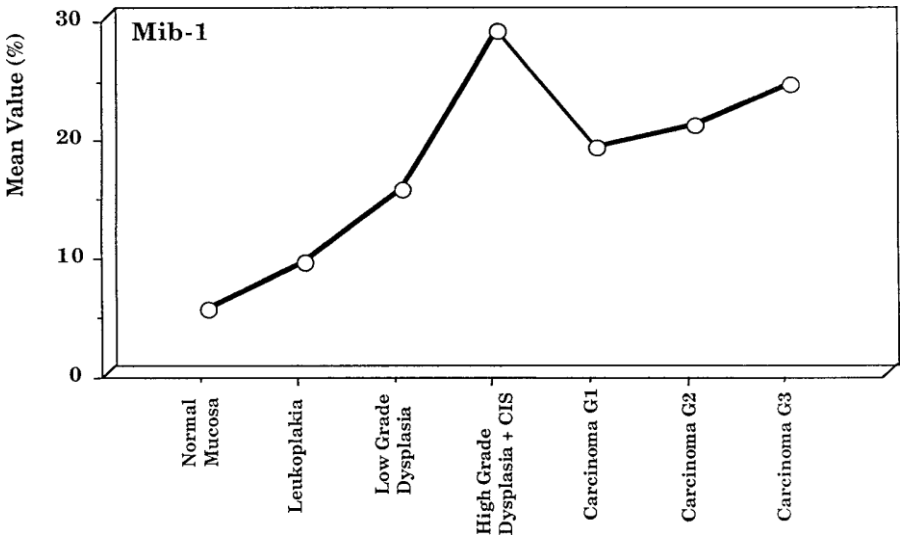

FIGURE 9. Mean values of the MIB-1–positive cells. (CIS, carci- noma in situ.)

In neuroblastoma, bcl-2 expression does not seem to influence prognosis.16 Tjalma et al2 found a strong relationship between bcl-2 expression and prognosis in patients with carcinoma of the uterine cervix. Flo- hil et al,16 in a study that compared hyperplastic polyps, adenomas, and carcinomas of the colon, found that most carcinomas did not present a bcl-2 immunoreactivity.

The expression of bcl-2 may provide data about individual tumor cell dynamics that in the future will most probably be very important for therapy and prognosis.17 A significant inverse relationship between bcl-2 and p53 was found in our specimens: similar data were reported by Harn et al1 in breast carcinoma and by other researchers in carcinomas of the thyroid, colorectum, stomach, and esophagus and in gastric lymphoma.12 Studies in human breast can- cer and in cancer cell lines have shown that p53 can down regulate bcl-2 expression4 and that apoptosis induced by p53 can be blocked by bcl-2 in cultured cancer cells.4 p53 has been shown to down-regulate bcl-2 via binding to a negative regulatory element outside the bcl-2 gene promoter.19 A significant in- verse relationship was found between bcl-2 expres- sion and the pathologic stage of esophageal tumors, and bcl-2 was found more frequently in early than in advanced stages.12 Our results also provide evidence of an interaction between bcl-2 and MIB-1: the highest expression of MIB-1–positive cells was observed in severe dysplasia and carcinoma in situ, where the bcl-2 protein expression was lowest or even absent. A possible influence of bcl-2 expression in the down- regulation of a proliferation marker was reported by Konstantinidou et al,20 who found that bcl-2–positive meningiomas presented a lower growth fraction rate and had a significantly higher proportion of proliferating cell nuclear antigen (PCNA)–strongly positive nuclei than the bcl-2–negative subgroup.

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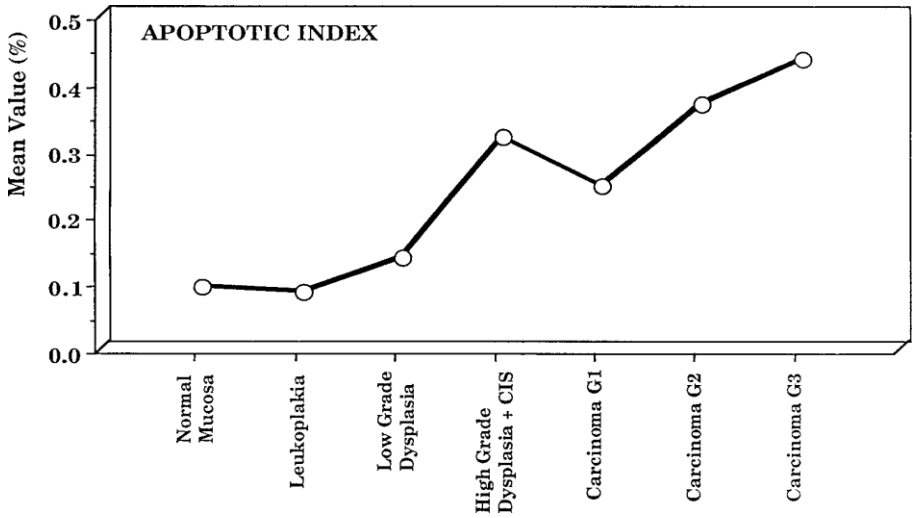

FIGURE 10. Mean values of the apoptotic index. (CIS, carci- noma in situ.)

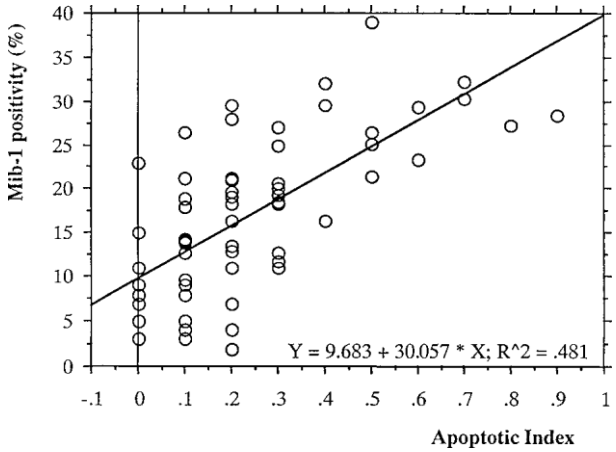

FIGURE 11. Simple regression analysis considering apoptotic index and MIB-1 positivity. A good, positive correlation is evident ($r = 0.694$, $P < .0001$).

We found, on the contrary, an inverse relationship between bcl-2 and MIB-1, and the loss of bcl-2 expression in our specimens of severe dysplasia and carcinoma in situ probably reflects a deregulation of the mechanisms that control bcl-2 expression.5 Our data show that a bcl-2 down-regulation was strongly associated with peaks in MIB-1 and AI in these lesions. Ravi et al9 reported an increased bcl-2 expression in oral dysplasia and carcinoma. bcl-2 expression inversely correlated with the degree of differentiation between CIN I/II and III in tumors of the uterine cervix.21 We found that p53 was expressed in supra- basal layers in leukoplakia, dysplasia, and carcinoma in situ; Cruz et al13 demonstrated that p53 suprabasal expression was significantly associated with the development of carcinoma. Our results are fully in accordance with the results reported by Murti et al,8 who found that the overexpression of p53 protein was significantly more common in severe than in mild epithelial dysplasia and that p53 expression peaked close to the time of transition from the precancer state to cancer rather than earlier in the

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natural his- tory of oral precancer. A fairly close relationship be- tween p53 and AI was found in our specimens. Birchall et al6 found that apoptosis increased from normal through dysplastic epithelium to reach a maximum in carcinoma in situ, whereas in invasive SCC AI fell to normal values6; it also increased with increasing degrees of dysplasia. A strong relationship was found in our specimens between AI and the degree of dysplasia and the onset of SCC; completely different results were reported by Birchall et al,6,7 who found no such association.

A diminution of apoptosis was reported in gastric carcinoma, whereas esophageal SCC, prostatic adeno- carcinoma, brain tumors, and non-Hodgkin lympho- mas showed an increased AI in relationship to less tumor differentiation and increased mitotic activity.22,23 Our results show a close correlation between AI and MIB-1, whereas Birchall et al6,7 found that the AI was highly correlated with mitotic index but not with PCNA: these authors failed to demonstrate an increased expression of PCNA in dysplastic epithelium. Apoptosis can be important in tumor growth and prognosis; in colorectal carcinoma, Langlois et al24 found that tumors with higher apoptotic counts seemed to have a good prognosis, and this may reflect that neoplasms with higher levels of apoptosis are slower growing. In renal cell carcinoma, Hindermann et al23 found a decrease in cells undergoing apoptosis in less-differentiated tumors with an increase in the number of tumor cells and of tumor growth. This decrease in apoptosis was correlated with an increase in the proliferative activity. The AI was much higher in the most aggressive type of prostatic carcinoma.1 The presence of Ki-67 closely coincided with p53 protein,10 and a close relationship between PCNA and p53 protein was found in some oral tumors.25 In nasopharyngeal carcinoma, the patients with a high PCNA had a poorer disease-free survival.26

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