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Original Research

Evaluation of P53 gene expression by immunohistochemistry to diagnosis oral precancerous lesions

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Abstract: Oral Precancerous lesions include leukoplakia, erythroplakia, and mucosa palate changes due to reverse smoking. Assessing the prevalence of these lesions in a cross-sectional study can be effective in the timely prevention and treatment of lesions, in any community. Hence, in the present study, evaluation of P53 gene expression was done by immunohistochemistry method to diagnosis oral precancerous lesions. For this purpose, 111 Chinese patients (54 women and 57 men) were selected for examination. The age range of these patients was 22 to 69 years, and their average age was 32.6 years. All patients were examined by one physician. Oral mucosa was used for immunohistochemical evaluations. All samples taken from patients' mucosa were evaluated by one pathologist under a light microscope. 80 cases of the 111 patients were smokers and 27 were non-smokers. Among the 80 smokers, 56.25% had leukoplakia, 3.75% had erythroplakia, and 40% had mucosa palate changes. Regarding non-smokers, 74.07% had leukoplakia and 25.93% had erythroplakia. None of the non-smokers had mucosa palate changes. In terms of the lesion location, in patients with leukoplakia 89.23%, and patients with erythroplakia 90% of the lesion was located in the cheek mucosa and buccal vestibule. Also, in patients with leukoplakia 9.23%, and patients with erythroplakia 10% of the lesion was located in the lips vestibular mucosa. Only 1.54% of leukoplakia had a lesion in the vermilion border, and none of the erythroplakia patients had a lesion on the vermilion border. 76 patients (68.46%) showed positive expression of the P53 gene. The expression level of the P53 gene did not show a significant relationship with age, and the genders did not have a statistically significant difference in terms of gene expression. The expression level of the P53 gene was 59.8% in leukoplakia, 70% in erythroplakia, and 40% in Mucosa palate changes. The present study showed that the evaluation of P53 gene expression was well able to detect oral precancerous lesions and their severity by increasing their expression rate.

Key words: Erythroplakia; Immunohistochemistry; Leukoplakia; Mucosa palate changes; P53 gene.

Introduction

Oral cancer sometimes results from clinically visible lesions that are not cancerous, called precancerous lesions (1). Precancerous lesions include erythroplakia, leukoplakia, and palate changes by reverse smoking (2). The presence of some oral diseases can be a predisposing factor for oral cancer. These diseases are called precancerous conditions (3). Clinical diagnosis of leukoplakia based on clinical observation is a white plaque that has not been removed and does not resemble any other white lesion (4). Erythroplakia is also a red area that is stable and does not look like other lesions (5). Palate changes between soft and hard palates, which are induced by reverse-smoking, are detected by changes in texture, color, and structure (6). Histopathologic diagnosis of precancerous lesions is based on the observation of tissue changes such as hyperkeratosis, acanthosis, and morphological changes of the basal layer, and can range from non-dysplastic cell states to severe cell dysplasia (7).

In previous studies, the prevalence of erythroplakia has been reported to be 0.02% to 0.1% (8, 9). The prevalence of leukoplakia is reported to be 2% to 11.7% and the prevalence of palate changes by reverse smoking is 0.1% to 2.2%. Although oral cancer is the sixth most common malignant neoplasm in developed countries, it accounts for 20% to 48% of all reported cancers in Southeast Asia and countries such as India and Sri Lanka (9, 10).

Non-lethal genetic damage plays an important role in carcinogenesis (11). Genetic damage (mutations) may be caused by a variety of factors, such as chemicals and radiation, viruses, or they may be inherited in a germ cell line (12). Three classes of natural regulatory genes are the main targets of genetic damage. Growth-promoting proto-oncogenes, cancer-suppressor genes of growth-suppressing, and genes that regulate programmed cell death (13).

The P53 gene is a known tumor suppressor gene located on the short arm of chromosome 17 and plays an important role in regulating the cell cycle (14). This gene is one of the main targets of genetic changes in human tumors (15). Studies have shown that more than 50% of human tumors contain mutations in this gene (16, 17). The P53 protein is a product of this gene, which plays a role in protecting against tumor formation through apoptosis and prevents the spread of gene-

tically damaged cells (18). Disruption or inactivation of the P53 protein due to a mutation in this gene can lead to uncontrolled cell growth, resulting in cancer (19).

Increased expression of the mutated P53 protein or P53 gene has been shown in many human malignancies, including gastrointestinal, prostate, breast, and oral tumors (19). Patient survival analysis in various studies has shown that overexpression of P533 is associated with the histological type of oral tumors and prognosis of patients and its role as a carcinogen has been suggested in oral cancer (20).

Because it is epidemiologically important to know the prevalence of oral lesions, and we can use it to evaluate other variables and treat patients in a timely manner, we decided to examine the prevalence of precancerous oral lesions (erythroplakia, leukoplakia, and mucosa palate changes induced by reverse-smoking) by assessing the expression of P53 gene, immunohistochemically.

Materials and Methods

This study is a cross-sectional descriptive study, and its sample size was calculated 111 patients by considering the minimum prevalence of 3%, based on similar studies, and considering the maximum confidence range of 95% and error of 1%. Patients were selected for examination based on a random number table and 54 women and 57 men were examined. The age range of the examined people was 22 to 69 years and their average age was 32.6 years. All patients were examined by one physician. A questionnaire containing demographic information and smoking habits was completed for each of them. People who had smoked for more than six months were considered smokers. The amount of smoking in the study population was calculated based on the following formula (pack/year):

$$\frac{\text{Number of cigarette sticks used every day}}{20} \times \frac{\text{Number of months of use}}{12}$$

The examination was performed in natural light with the help of a flashlight and using a tongue stick, a mirror and a standard dental catheter. Oral mucosa was used for immunohistochemical evaluations. DAKO kit was used to stain P53 protein. All samples taken from patients' mucosa were examined and interpreted by one pathologist under a light microscope. In P53 protein staining, grading was performed as follows:

0	<10%
+1	10-25%
+2	26-50%
+3	51-75%
+4	>75%

After entering the data into the computer through SPSS 16 software, the Chi-square test was used to determine the relationship between different variables, and the significance level of the test was considered 0.05.

Results

Clinical and demographical results

On oral mucosal examination, all patients had red or white lesions, with clinically 65 cases of leukoplakia (58.5%), 10 cases of erythroplakia (0.09%), and 32 cases of mucosa palate changes (28.82%). The last four cases had safe lesions. The age range of patients with leukoplakia was 24-57 years and their mean age was 37.6 years. The age range of patients with erythroplakia was 22-63 years and their mean age was 36.1 years. The age range of patients with mucosa palate changes was 50-69 years and the mean age was 63.2 years. 80 cases of the 111 patients were smokers and 27 were non-smokers. Among the 80 smokers, 56.25% had leukoplakia, 3.75% had erythroplakia, and 40% had mucosa palate changes (Table 1). Regarding non-smokers, 74.07% had leukoplakia and 25.93% had erythroplakia. None of the non-smokers had mucosa palate changes. The average smoking rate in those patients who smoked at the time of the study was 45 packs per year.

In terms of the lesion location, in patients with leukoplakia 89.23%, and patients with erythroplakia 90% of the lesion was located in the cheek mucosa and buccal vestibule (Table 2). Also, in patients with leukoplakia 9.23%, and patients with erythroplakia 10% of the lesion was located in the lips vestibular mucosa. Only 1.54% of leukoplakia had a lesion in the vermilion border, and none of the erythroplakia patients had a lesion in the vermilion border.

P53 gene expression results

As mentioned before, 65 patients had leukoplakia (58.5%), 10 patients had erythroplakia (0.09%) and 32 patients had mucosa palate changes (28.81%). The last four cases had safe lesions. 76 patients (68.46%) showed positive expression of the P53 gene. The degree of staining of oral precancerous lesions for P53 is shown in Table 3. The expression level of the P53 gene did not show a significant relationship with age, and the genders did not have a statistically significant difference in terms of gene expression. The expression level of the P53 gene was 59.8% in leukoplakia, 70% in erythroplakia, and 40% in Mucosa palate changes.

Discussion

Precancerous lesions are lesions that are more likely to develop cancer than healthy tissue (7). These include leukoplakia, erythroplakia, and changes in the palate due to smoking (5). Because a significant association has been observed between smoking and oral cancers, in areas where smoking is high, the need to assess precancerous lesions is greater and more important (21). Therefore, by examining and diagnosing these lesions in a timely manner, cancer can be prevented in these patients. The frequency distribution of patients showed

Table1. Frequency distribution of precancerous lesions by smoking.

	Leukoplakia		Erythroplakia		Mucosa palate changes		Total	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Smoker	45	56.25	3	3.75	32	40	80	100
Non-smoker	20	74.07	7	25.93	0	0	27	100

Table2. Clinical distribution of leukoplakia and erythroplakia frequency according to the location of the lesion.

The lesion location	Leukoplakia		Erythroplakia	
	Number	Percent	Number	Percent
Cheek mucosa and buccal vestibule	58	89.23	9	90
Lips vestibular mucosa	6	9.23	1	10
Vermilion border	1	1.54	0	0
Total	65	100	10	100

Table3. Frequency distribution of staining degree of oral precancerous lesions in terms of P53 expression.

	0 (No gene expression)		+1		+2		+3 and more		Total	
	number	percent	number	percent	number	percent	number	percent	number	percent
Leukoplakia	31	47.69	10	15.38	15	23.08	9	13.85	65	100
Erythroplakia	1	10	0	0	3	30	6	60	10	100
Palate changes	3	9.38	13	40.62	7	21.88	9	28.12	32	100
Safe lesions	4	100	0	0	0	0	0	0	4	100
Total	39	35.13	23	20.73	25	22.52	24	21.62	111	100

that 65 patients (58.5%) had leukoplakia (22). It is noteworthy that the patients were only accustomed to smoking cigarettes and did not use any other smoking (21).

The prevalence of leukoplakia in other studies in South Asia has been between 0.2-1.7 percent (23). The reason for this high number can be found in the predisposing factors of precancerous lesions (5). In many studies that have been done so far, tobacco use in any form has been considered as one of the most important predisposing factors for precancerous lesions (23).

Out of 80 smokers, 45 (56.25%) had leukoplakia, which was statistically significant compared to the group of non-smokers (P=0.01). In other words, smokers had more than twice as many oral leukoplakias as non-smokers. The results of similar studies conducted so far are consistent with the results of this study (24, 25). In the prognosis of precancerous lesions, the location of infection is important, although the lesions can infect any area in the mouth (26).

Involvement in a particular area of the mouth depends on the different habits of smoking in that area. A study on 441 people in Saudi Arabia found that all 187 people who used a substance called "Shammah" had leukoplakia. 75% of this lesion was seen in the lower lip or buccal mucosa of these patients, a location in which Shammah was kept according to their habit (27). The patients of the current study used only cigarettes and the most location of leukoplakia (58 cases, 89.23%) were observed in the cheek mucosa and buccal vestibule.

In most studies of precancerous lesions, the incidence of men was reported to be 87-55%. This may be due to the higher consumption of tobacco and other predisposing factors such as alcohol in men compared to women (28). But in societies where women have more smoking habits than men, there are different reports. For example, in a study on 10,000 people, 4,700 people smoked cigarettes in reverse, of which 69% were women and 31% were men. Of the 413 reported cases of leukoplakia, 66% occurred in women and 34% in men (29). In the present study, 41 women had precancerous lesions, of which 32 cases of them smoked.

Precancerous lesions mainly occur from the age of 30 years. In the present study, the age range of patients with leukoplakia was 24-57 years and their mean age was 37.6 years. The age range of patients with erythroplakia was 22-63 years and their mean age was 36.1 years. The age range of patients with palate mucosa changes was 50-69 years and the mean age was 63.2 years.

In the present study, the frequency of P53 gene expression was 58.5% in patients with leukoplakia, 0.09% in patients with erythroplakia, and 28.81% in patients with mucosal changes. No association was found between the expression of this gene and any of the individual variables and histological characteristics. In various studies, the expression of P533 by the immunohistochemical method has been seen in 43% to more than 60% of patients with oral cancer (30). Shun *et al.* (31) reported that the immunohistochemical expression of P53 was significantly associated with some histopathological phenotypes and adenocarcinoma and intestinal-type had the highest expression (76.5% and 69.8%) of P53 gene expression, respectively, but no correlation was observed between individual variables (age and sex). In a study by Kyokane *et al.* (32), they found that P53 expression was associated with tumor morphology (mostly with differentiated adenocarcinomas). In a review of 178 patients with primary gastric cancer, they reported that there was a significant difference between the intestinal and diffuse gastric tumors in terms of the expression time of P53 so that in the intestinal type most cases of increased expression of P53 in the early stages and the diffuse type often occur in the advanced stages.

There are conflicting theories about the relationship between P53 expression and patients' prognosis. In some studies, a positive correlation has been found between P53 expression and tumor invasion to surrounding tissues, resulting in tumor stage and prognosis in cancer patients. But other studies have not proven this theory (33-39). The present study showed that the evaluation of P53 gene expression was well able to detect oral precancerous lesions and their severity by increasing their expression rate.

Since the study of the oral precancerous lesions is one of the research priorities, the present study was performed to identify and prevent these lesions promptly through clinical studies and evaluating P53 gene expression, to solve the economic and psychological problems of patients who suffer from more advanced lesions due

to lack of timely diagnosis and treatment. The results showed that the evaluation of P53 gene expression, as an effective biomarker, plays an important role in the diagnosis of oral precancerous lesions. Therefore, it is suggested that in all health centers, in addition to the oral mucosal examination, the expression of the P53 gene should be evaluated, in order to treat patients with oral precancerous lesions faster and more accurately.

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