

## **Usefulness of “mapping” technique, including dispersive X-ray analysis EDX, in the diagnosis of gingival squamous cell carcinoma**

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Early detection of carcinomatosis is still the main problem of modern medicine. The scanning microscope used in medical diagnostics has offered new possibilities of recognizing various pathologies of human body. The authors presented a mapping technique for carcinous tissue, including dispersive X-ray analysis EDX. 36 preparations obtained from the samples collected from people of both sexes with gingival squamous cell carcinoma diagnosed histopathologically were analyzed. The concentrations of calcium, sulphur and phosphorus within the area of carcinous cells were determined.

*Key words: EDX analysis, squamous cell carcinoma, evident mineralization, hidden mineralization*

### **1. Introduction**

Carcinomas usually develop very rapidly with a very dramatic course of the disease. The surveys show that more than 91% of malignant cancers occurring in oral cavity are squamous cell carcinomas (SCC) of differentiated advancement [1], [2], [4], [6], [9], [12], [15]. Many scientific centres in Poland deal with carcinomas. For many years, Oncology Institute, Central Cancer Register (CRNIO) in Warszawa has been collecting the data. Zatoński and Tyczyński, who have analyzed the CRNIO issues since 1998, draw attention to a constant increase of middle-age men mortality due to carcinomas, the highest figure in the world [19]–[23]. It is important that many patients apply for treatment at a very advanced stage of disease development; some of these cannot be treated at all. Late diagnosis causes treatment problems and seriously decreases the number of the cured [3]–[5], [9], [10], [13].

Structures of oral cavity, and specially cheeks and gingival mucous membrane, due to their contact with food and fluids of different temperature as well as the exposure to ionizing radiation are imperilled by numerous, repeated injuries. These injuries and co-occurring inflammatory changes seem to be responsible for carcinomatous lesions in oral cavity [4], [8], [11].

Recently, an essential improvement in diagnosing and treatment of cancers has been made. However, histological assessment is most important in tumor determination, choice of treatment and prognosis on curing the squamous cell carcinoma in oral cavity. Modern study techniques that enable re-use of tissue material taken from the patients during clinical diagnosing become a material for further studies. These studies not only aim at confirming the histological diagnosis in the pre-operative diagnosis, but they also can answer a question whether tissue margins are accurately determined after the operation. The aim of the study was to determine the usefulness of squamous cell carcinoma gingival tissue 'mapping' in relation to sex, age and degree of carcinoma differentiation. Sensitivity and specificity of the method in monitoring a stage of carcinous tissue mineralization in relation to the site where the material was taken were evaluated.

## 2. Material and method

The study is based on 36 paraplasm blocks containing samples of histopathologically diagnosed gingival squamous cell carcinoma of various degree of differentiation. Control samples consisted of fragments of oral cavity mucous membrane collected from 20 persons after surgeries at Teaching Hospital of Maxillofacial Surgery in Zabrze. Tissue material for evaluation in scanning microscope was prepared in a typical way; the tissue was examined under 500 and 5000 $\times$  magnification. Chemical analyses by EDX method were performed using a scanning microscope JEOL JSM 5400 made in Japan equipped with a device for EDX chemical analyses.

The analysis of superficial distribution of changeability zones of chemism in the form of maps showing concentrations of the elements determined was performed near the sites of intensive carcinous process. The results obtained are of semi-quantitative character and show the areas of higher and lower element content. The application of EDX analysis with mapping of the chosen zones of a preparation enables an evaluation of quantitative changeabilities of chemical parameters in tissues, whose element concentration, i.e. mineralization, can be evaluated.

## 3. Results

On the basis of the analysis it was found that in the group of 36 patients with gingival squamous cell carcinoma there were 30 men and 6 women. In most cases, the carcinomatous lesion was located in the upper gingiva (table). Superficial analysis

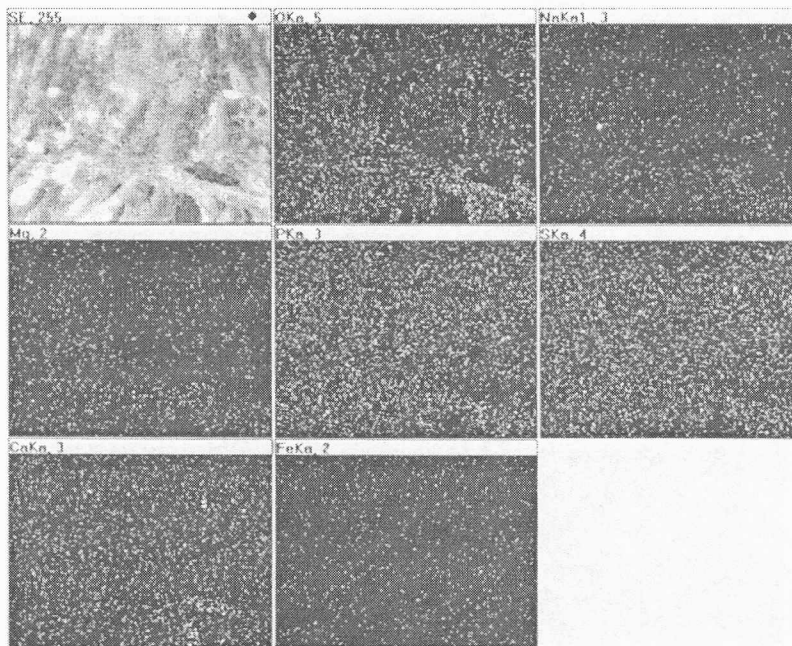


Fig. 1. Superficial distribution of chemical components in carcinomatous area. Woman with cancer of the gingiva. Magnification approx. – 500x

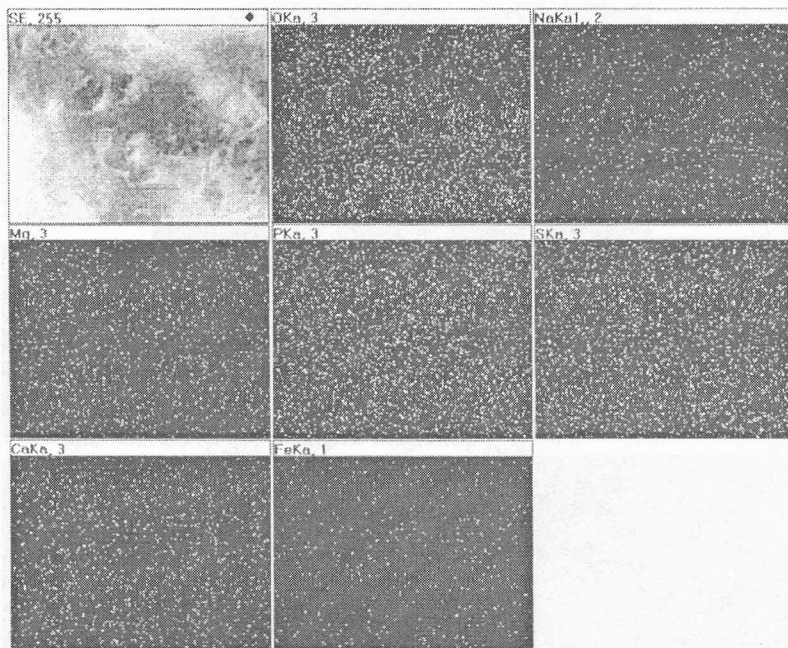


Fig. 2. Superficial distribution of chemical components in carcinomatous area. Woman with cancer of the gingiva. Magnification approx. – 5000x

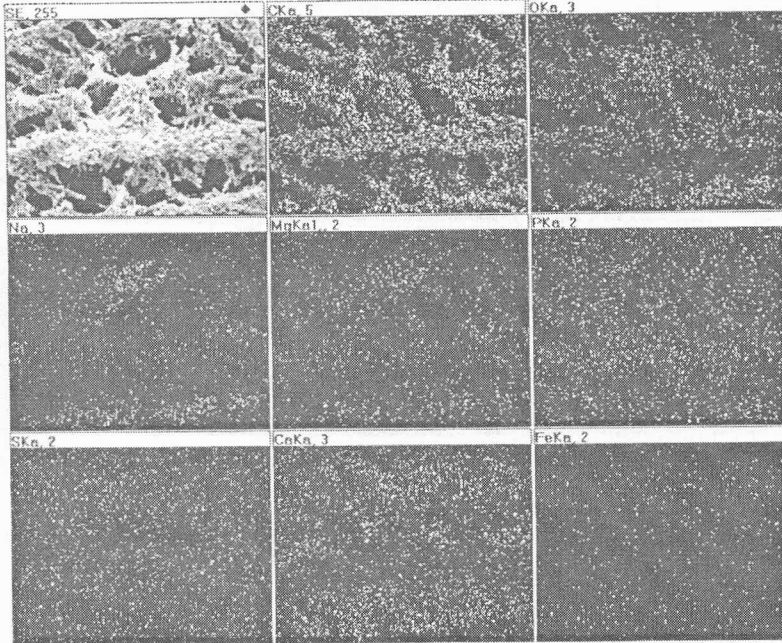


Fig. 3. Superficial distribution of chemical components in carcinomatous area. Man with cancer of the gingiva. Magnification approx. – 500×

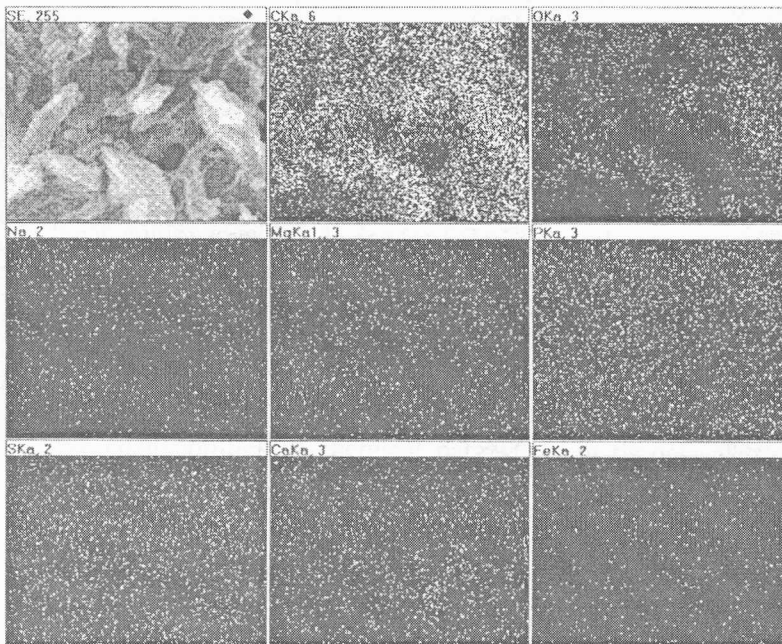


Fig. 4. Superficial distribution of chemical components in carcinomatous area. Man with cancer of the gingiva. Magnification approx. – 5000×

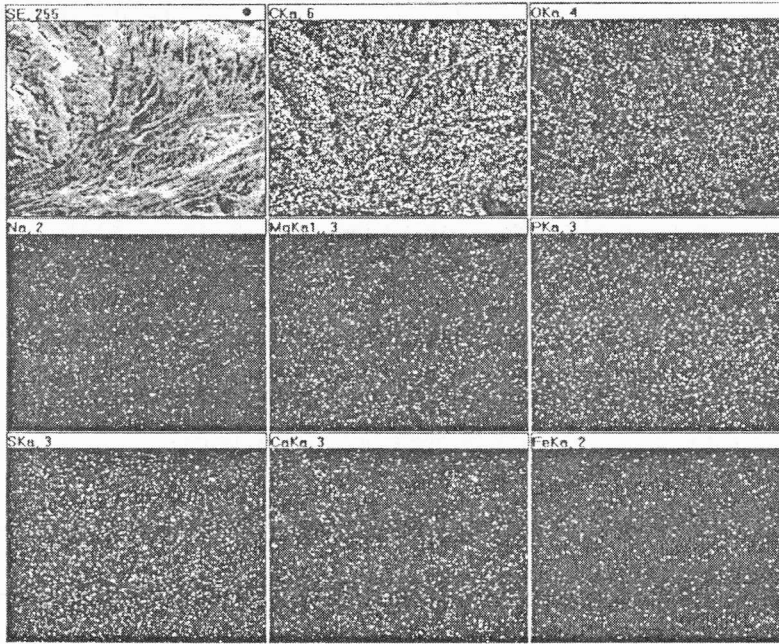


Fig. 5. Superficial distribution of chemical components in comparative group of healthy people. Man with cancer of the gingiva. Magnification approx. – 500×

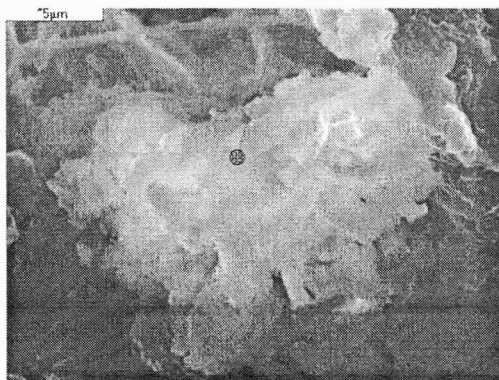


Fig. 6. Conglomeration of calcium in mineral granule in carcinomatous area. Man with cancer of the gingiva. Magnification approx. – 5000× (SEM)

Table. Existence of squamous oral epithelium carcinoma

Sex	Gingiva		Total
	Upper	Lower	
Men	4	26	30
Women	2	4	6
Total	6	30	36



of mineral substances' distribution in the group examined showed that regardless of the degree of cancer differentiation, the distribution of the elements determined in the area of the cells infected by a disease was irregular. However, it was noticed that the higher the degree of cancer differentiation, the higher the levels of phosphorus, sulphur and calcium. This statement, however, is false in the case of patients representing the age group of 60–69 years. While in the women's group the distribution of all elements was regular (figure 1). The changes in distribution are visible more clearly at 5000× magnification (figure 2). In the men's group, the irregularity of element distribution was observed only for iron (figure 3). This was confirmed by examinations at 5000× magnification (figure 4). In most preparations, high concentrations of calcium were determined. It occurred in the form of grains of evident concentrations (figure 6). In the control group, regular distributions of mineral substances were observed (figure 5), with the exception of irregular calcium distribution in both sexes in the age group over 70 years.

#### 4. Discussion

There are individual reports discussing mineralization of carcinous tumours. Examining lung cancers by means of chemical analysis with an electronic microprobe, some authors observed important quantitative differences in proportions of calcium, phosphorus, magnesium and sodium. The differences in distribution of those elements were also noticed within grains in individual patients. Dubious results show that this problem requires further studies [18]. Other authors claim, on the basis of their own examination, that in breast cancer tissue in women, the level of magnesium was raised five times [7]. In our studies, we found a minor increase of magnesium concentrations in favour of sodium and potassium concentrations. Marcinkowska-Suchowierska analyzing the phosphor disorders underlined its role in proper body functioning. She found that the body homeostasis disorder caused changes of the phosphorus content in intracellular fluid, which led to the metabolism disorders [14]. This was also confirmed by our investigations – an increased phosphorus level was found around the carcinous cells. They also proved that in mineralization tests, evaluation of calcification of carcinous tissue was particularly important. This was also confirmed by many authors [17], [16]. Our results encouraged our interest in this problem. Ambiguity of the results obtained might be caused by the patient's age, since in elderly people pathological over-calcification of soft tissue occurred.

#### 5. Conclusions

- The zones in the areas closest to carcinous cells are highly enriched with calcium that occurs in the form of grains of evident concentrations.
- Regardless of the site of a sample collection and together with an increase in the degree of differentiation of gingival squamous cell carcinoma, the higher levels of

phosphorus, sulphur and calcium were observed. A hidden mineralization of carcinous tissue seems to be responsible for this process.

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