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TISSUE FLUORESCENCE IMAGING FOR QUICK NON-INVASIVE DIAGNOSIS IN ORAL AND MAXILLOFACIAL SURGERY

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Abstract

Significant vital functions take place in the oral cavity and oropharynx, primarily mastication, as the initial function of the gastrointestinal system, swallowing, respiration and speech.

All these vital biological functions can be endangered, ie aggravated, and even disabled by the appearance of neoplasms in that anatomical space. In the maxillofacial region, neoplasms can originate from a variety of tissues, from the mucous membranes of the oral cavity to the jaws, salivary glands, and even tumors of odontogenic origin.

However, the most common tumor in the oral cavity is squamous cell carcinoma (OSSC), which originates from the oral mucosa.

To determine the accuracy, sensitivity (Se) and specificity (Sp) of the Velscope screening method in the detection of premalignant and initial malignant lesions compared to the gold standard surgical biopsy.

The study group consisted of 60 patients divided into two groups. The first group was formed by 30 patients with potentially malignant oral lesions (PML). Another 30 patients with preliminary diagnosis - oral cancer (OC) were included in the second group of examinees.

The high sensitivity value of 92.86% and the accuracy of the method of 86.67% largely confirm the reliability and efficacy of the Velscope method in patients with highly suspected oral cancer lesions (OC), significantly more, comparing to the group of premalignant lesions.

Key words: Velscope, oral cancer, premalignant lesions, tumor.

Introduction

The maxillofacial area, which includes the oral cavity, is a specific environment composed of different types of tissues, such as skin, mucous membranes, connective, muscular and bone tissue, blood, lymph vessels, nerves, and salivary glands.

All of these tissues are susceptible to infection, trauma, degenerative changes, malignant alterations or other diseases.

Oral carcinoma most commonly belongs to the group of squamous cell carcinoma of the lips, oral cavity and oropharynx. Few researchers previously predicted that worldwide, oral cancer will be diagnosed in more than 600,000 cases, making it the sixth most common malignant disease in the world, while in terms of mortality malignancies are positioned as the second most common disease after cardiovascular disease.[1,2].

The American Cancer Association emphasizes that tobacco and alcohol use are considered major risk factors for oral squamous cell carcinoma, while human papillomavirus (HPV) infection is the leading risk factor in cases of oropharyngeal cancer [3].

The most common type of oral cancer is squamous cell carcinoma, which accounts for 96% of all oral cancers.

Over 350,000 new incident cases and 150,000 deaths were reported in 2018, while in 2019, oral cancer confirmed its position as sixth most common cancer worldwide, detected in 450,000 cases[4].

Worldwide, an estimated 19.3 million new cancer cases (18.1 million excluding nonmelanoma skin cancer) and almost 10.0 million cancer deaths (9.9 million excluding nonmelanoma skin cancer) occurred in 2020 [5].

Adhering to the WHO Classification of Head and Neck Tumors 4th edition, Oral squamous cell carcinoma (OSCC) is the principal malignant surface epithelial tumor of the oral cavity and mobile tongue [6,7].

Accordingly, OSCC may be preceded by oral potentially malignant disorders (OPMD), initially benign lesions, associated with a statistically increased risk of developing oral cancer, such as forms of leukoplakia or erythroplakia [8,9].

Despite numerous advances and innovations in the treatment of malignancies in the last 5 decades, only 50% of cases achieve a five-year survival [10,11].

This unfavorable statistic is probably due to several factors.

Oral cancer develops on the mucous membrane in which genetic mutations occur that over time lead to the clinical manifestation of cancer [11].

Slaughter proposed a theory known as the "field of carcinoma" which suggests the possibility of mucous membranes in the oral cavity being genetically mutated, so chronic exposure to carcinogens can lead to the development of cancer [12].

Although this theory is not accepted by all authors, patients with oral cancer who survive five years after diagnosis and treatment have an up to 35% chance of developing at least one new primary tumor in that period [12].

However, the most common cause of failure treatment and death in patients with oral cancer are local recurrences and cervical metastases.

Second, the low survival rate of patients with oral cancer can be attributed to the advanced stage of disease at the time of diagnosis. More than 60% of patients present for examination in the III and IV clinical stages of the disease.

These poor statistical indicators seem overwhelming knowing that the disease mainly begins in the superficial layers of the oral epithelium which is readily available for direct visual and tactile investigation. The conclusion that some lesions are ignored or missed by patients, healthcare professionals, or both is inevitable. In part, this may be due to insufficient knowledge or awareness that even small asymptomatic lesions may have significant malignant potential.

One approach to solving this problem is to improve the ability and willingness of physicians to detect oral cancer early by recognizing potentially malignant lesions or cancerous lesions at their earliest possible or initial stage. Such a goal can be achieved by increasing public awareness of the importance of regular screening procedures or procedures for identifying small but also asymptomatic cancerous lesions and precancerous lesions[13].

Another strategy will be the development and use of diagnostic tools that can help the general dentist and specialist to more easily identify or assess the presence of oral lesions of unknown origin [14,15].

Mehrotra et al., indicate that there are two approaches to detecting oral dysplasia and cancer: oral cancer screening programs that identify asymptomatic patients with suspected lesions and specific diagnostic tools to identify dysplasia and initial oral cancer in asymptomatic patients with oral abnormalities[16].

The occurrence of oral cancer is most often noted at the age of over 40, with a peak at the age of 60, when it affects men twice as much as women. Recently, several studies have suggested that worldwide, head and neck cancers, and especially tongue cancer, are on the rise in young people[17].

The factors contributing to this increase are still unknown, but as suspected etiological agents are included chewing tobacco, various forms of drug abuse, environmental factors and HPV[18].

There is a general consensus that the clinical stage of the disease at the time of diagnosis is the most important predictor of treatment success in patients with oral cancer. The moment of diagnosis is crucial, but most often the right moment, ie. early detection of cancer is absent in everyday practice. It is a result of lack of information of the population, poor health culture and education, irregular visits to the dentist and doctor but sometimes fear of facing a poor diagnosis.

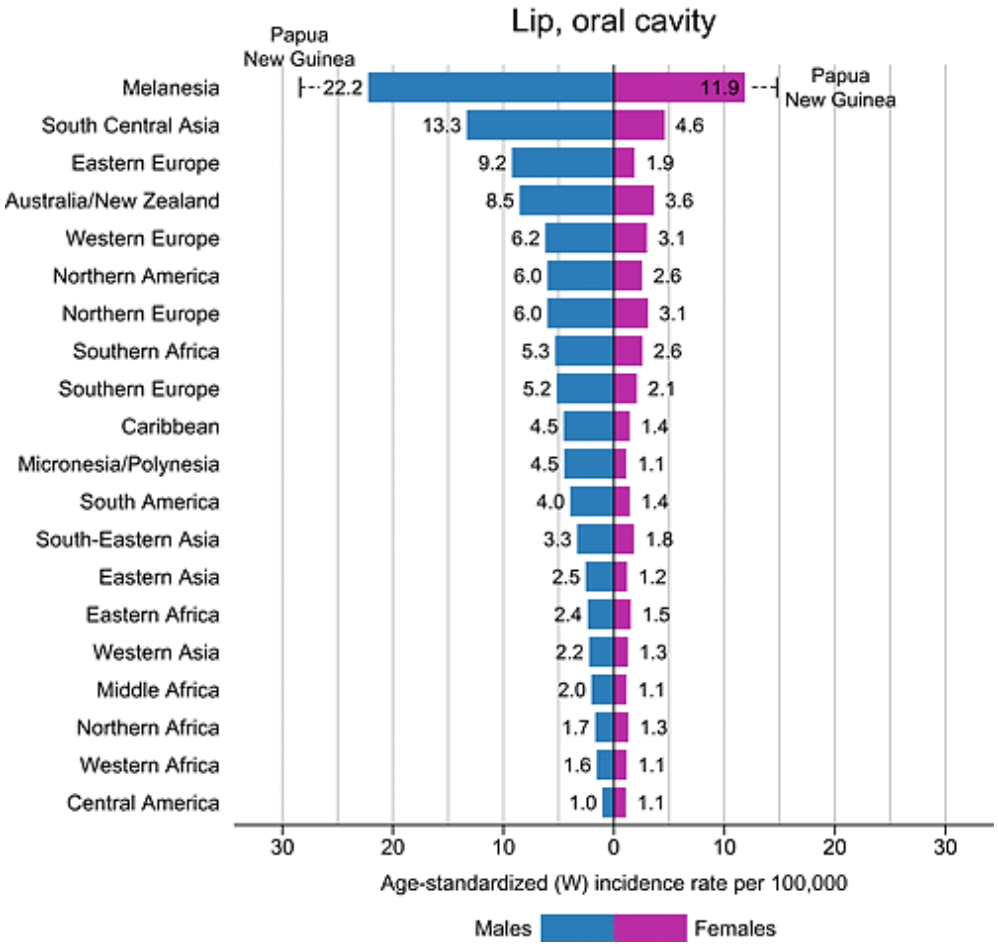

Figure. 1. Region-Specific Incidence Age-Standardized Rates by Sex for Cancers of the Lip and Oral Cavity in 2020. Rates are shown in descending order of the world (W) age-standardized rate among men, and the highest national rates among men and women are superimposed. Source: GLOBOCAN 2020.

Oral cancer screening involves the detection of oral precancerous and carcinogenic lesions, usually before the first symptoms appear. Numerous established and so far applicable screening programs for various malignancies have shown a significant reduction in patient morbidity and mortality.

Aim

The aim of the study was to evaluate the accuracy/efficacy of the easy to perform and fully noninvasive light-assisted oral cancer screening method – Velscope imaging system, as well as its specificity and sensitivity, the positive and negative predicted values according to the diagnostic gold standard – tissue biopsy in two different groups of examinees with suspicious oral tissue changes.

Material and Methods

The study group consisted of 60 patients divided into two groups. The first group was formed by 30 patients with potentially malignant oral lesions (PML). Another 30 patients with preliminary diagnosis - oral cancer (OC) were included in the second group of examinees.

All selected patients were followed by the American Joint Commission on Cancer Diagnosis Protocol, with diagnostics, pre-operative preparation, surgical excision, and postoperative clinical follow-up.

The selection of patients in the study was made according to certain inclusion and exclusion criteria.

- Inclusion criteria:
- Have not received antibiotic therapy for the last two months
- Have not undergone periodontal treatment for the last two months
- Have not been / or have not undergone radiotherapy or chemotherapy for the last three months
- Exclusion criteria:
- Inability and unwillingness to participate in the study protocol
- Gravity

All participants who agreed to take part in the study signed a consent form for voluntary participation in the study.

- For achievement of research purposes, selection of participants in the study group was conducted at the University Clinic for Maxillofacial surgery at the University "Ss. Cyril and Methodius" and the Clinic for Oral pathology and periodontology at the University Dental Clinical Center "St. Pantelejmon" in Skopje, North Macedonia.

- The histopathological analysis of the specimens of the examined group was performed at the Institute of pathological anatomy at the Faculty of Medicine, University "Ss. Cyril and Methodius" in Skopje, North Macedonia.

Clinical examination

- Anamnestic data, clinical examination analysis of digital orthopantomographic X-rays
- Clinical evaluation of the condition of the oral epithelium through standardized procedures (conventional oral examinations - extra oral and intraoral examination with inspection and palpation)
- By the clinical examination – inspection, we recorded the size, shape and color of the lesion, the depth of the lesion, as well as epithelial desquamation, the presence of erosions, ulcers or rashes. During the clinical examination, additional signs such as bleeding, loss of sensitivity and burning of the oral mucosa were noted. The palpation of changes determines the following characteristics: lesion hardness, induction of surrounding structures and tissues, and lesion fixation for the underlying tissues.
- After the inspection, the oral mucosa in both groups of patients was screened with the Velscope system, and the recordings were compared and evaluated with the previously achieved results.

Oral examination in all patients was double performed (by two independent examiners) and in most of the cases, they both presented same interpretation of the oral screening. In those where they reported similar or fully different findings, eminent specialists for oral medicine or maxillofacial surgeons were engaged for confirmation. Examiners were supported by a histological report and based to the clinical examination, a working diagnosis was made.

Complete blood tests in all participants in the study were done and an incisional or excisional biopsy was performed for histopathological verification of the biopsy specimens, as the current gold standard of the research procedure.

The histopathological finding, defined as a negative specimen, means that no pathological changes were found outside the edges of the biopsy material. A positive sample indicates the presence of pathological change (epithelial dysplasia, Ca in situ and oral carcinoma) and requires treatment.

Sensitivity measures the percentage of subjects with the disease that were tested positive, while specificity determines the percentage of subjects without the disease tested negative. Predictive values determine the percentage of people with positive or negative test results who have or do not have the disease. There are no defined values for an ideal screening test, but it is highly desirable to have both high specificity (several false positives) and high sensitivity (few false negatives).

Light-assisted detection systems - The VELscope®

In the present study, the discrimination of physiological mucosa, non-malignant lesions and oral squamous cell carcinoma (OSCC) using VELscope® system were evaluated. Conceptualized by the British Columbia Cancer Agency and LED Dental, the product was released in 2006 as the very first tissue fluorescent device commercially available to dentists.

The VELscope® system uses oral tissue autofluorescence as a new and completely non-invasive method for screening mucosal changes, and enables early detection of potential precancerous lesions, oral cancer and other oral diseases, thus positioning itself as an advanced level of preventive care. It is performed easily, quickly (1-2 minutes), safely and completely painless, it is easy to manipulate, non-invasive and non-toxic. When using a wavelength between 375 and 440 nm, normal, intact mucosa emits a pale green light when viewed through a selective narrow-band filter. Areas of reduced autofluorescence are suspected of epithelial dysplasia or oral cancer and present as dark areas, while normal mucosa is seen as a light green area. No prior preparation of the tissue to be screened is required.

Statistical analysis

- The statistical series, according to the defined variables of interest, are tabulated and graphically presented;
- distribution of numeric statistical series (correct / incorrect) is tested with Kolmogorov Smirnov test, Lilliefors test and Shapiro-Wilk's W test;
- the numeric / quantitative series structure is analyzed with central tendency measures (averages) and dispersion measures (standard deviation);
- the structure of attribute / qualitative series is analyzed by means of relationships and proportions;
- Testing the significance of the difference between both arithmetic environments in the independent samples, with correct distribution being performed by One-way ANOVA, and testing the significance of the difference between the two arithmetic median values with the Turkey honest significant difference Test;
- The accuracy / diagnostic value of the test methods is determined by the Sensitivity and Specificity Test;
- Significance level for $p < 0.05$ at CI = 95% is taken as statistically significant;
- The database is analyzed with the statistical programs STATISTICA 7.1 and SPSS for Windows ver. 20.

Results

In the first group, which consisted of patients with potentially malignant lesions, the result of the histopathological finding (biopsy) was positive in 6 patients and negative in 24 patients. According to the

VELscope screening method, of all 30 examinees, 14 were classified as positive (noted changes of the oral mucosa not specified for a certain disease or disorder), from which 12 were true positive, 2 were false positive, 12 were false negative, and 4 were classified as true negative. The analysis showed that in the group of potentially malignant lesions (PML), the sensitivity for the VELscope method is 50%, the specificity is 66.67%, the positive predictive value is 85.71%, and the negative predictive value is 25%. Accuracy, ie the general probability that the patient will be correctly classified by the VELscope method is 53.33%. (Tables 1 and 2 and Charts 1 and 2)

Table 1. Crosstabulation of the results in examinees with potentially malignant lesions (PML) by Velscope method and histopathological results.

VELscope			
		+	-
+	14	12	2
-	16	4	12
		Histopathological results	
Total	30	6	24

Table 2. Sensitivity and specificity of Velscope system within potentially malignant lesions (PML).

VELscope	Value	CI = 95%
Sensitivity (Se)	50%	29.12% to 70.88%
Specificity (Sp)	66.67%	22.28% to 95.67%
Positive predictive value (PPV)	85.71%	64.37% to 95.22%
Negative predictive value (NPV)	25%	14.29% to 40%
Accuracy	53.33%	34.33% to 71.66%

Table 2 shows the values of sensitivity, specificity, positive predictive value, negative predictive value and method accuracy, together with the lower and upper limit of the confidence interval of 95% (CI = 95%).

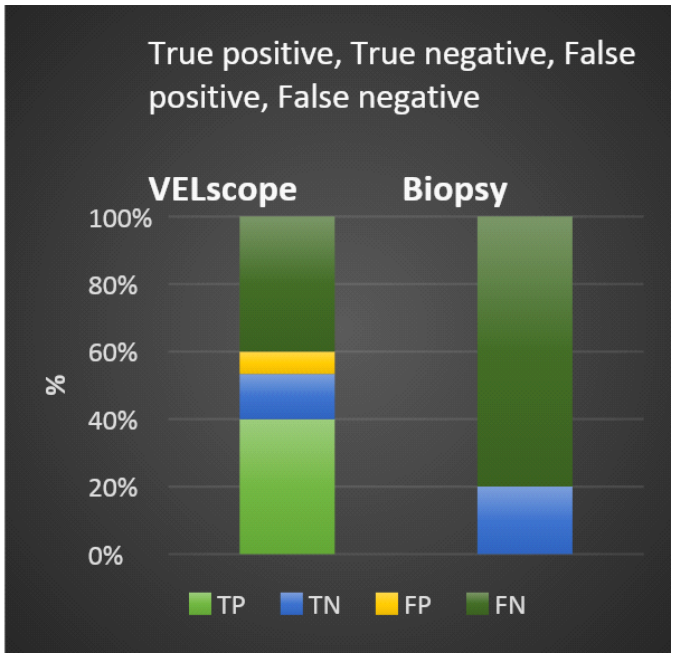

Chart 1. Distribution of examinees with potentially malignant lesions (PML) by histopathological finding and Velscope screening method.

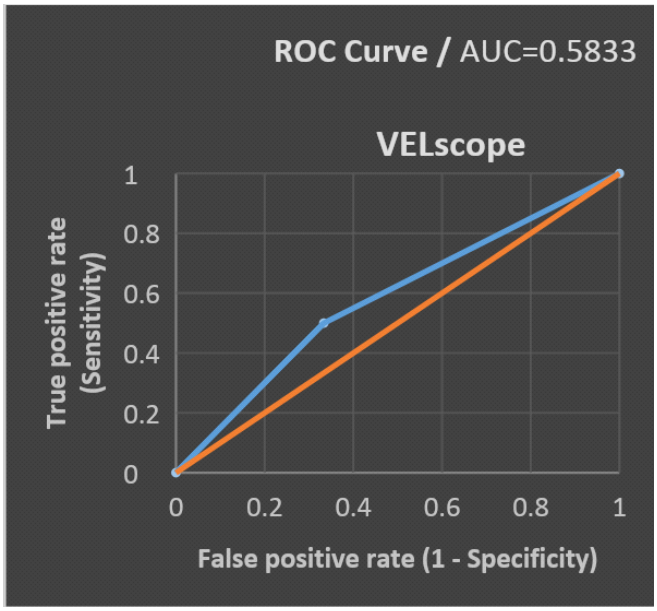

Chart 2. ROC curve - Sensitivity and specificity of VELscope system in potentially malignant lesions (PML).

AUC	Standard error	Lower bound (95%)	Upper bound (95%)
0.5833	0.0000	0.5833	0.5833

In the second examined group of patients with lesions with a highly suspected malignant potential - oral cancer (OC), the result of the biopsy was positive in 28 patients and negative in 2 patients. By VELscope, 28 patients were classified as positive, 26 of them were true positive, 2 were false positive and 2 cases came out false negative. We measured sensitivity value for the VELscope method of 92.86%, specificity is 0%, positive predictive value is 92.86% and negative predictive value is 0%.

The accuracy of this method that the patient will be correctly classified by clinical examination / inspection is 86.67%. (Tables 3 and 4, Charts 3 and 4)

Table 3. Crosstabulation of the results in examinees with highly suspected malignant potential – oral cancer (OC) by VELscope and pathological results.

VELscope			
		+	-
+	28	26	2
-	2	2	0
		Histopathological results	
Total	30	2	28

Table 4. Sensitivity and specificity of VELscope within lesions with highly suspected malignant potential – oral cancer (OC).

VELscope	Value	CI = 95%
Sensitivity (Se)	92.86%	76.50% to 99.12%
Specificity (Sp)	0%	0.00% to 84.19%
Positive predictive value (PPV)	92.86%	92.15% to 93.51%
Negative predictive value (NPV)	0%	
Accuracy	86.67%	69.28% to 96.24%

Table 4 presents the values of sensitivity, specificity, positive predictive value, negative predictive value and accuracy of VELscope, together with the lower and upper limit of the confidence interval of 95% (CI = 95%).

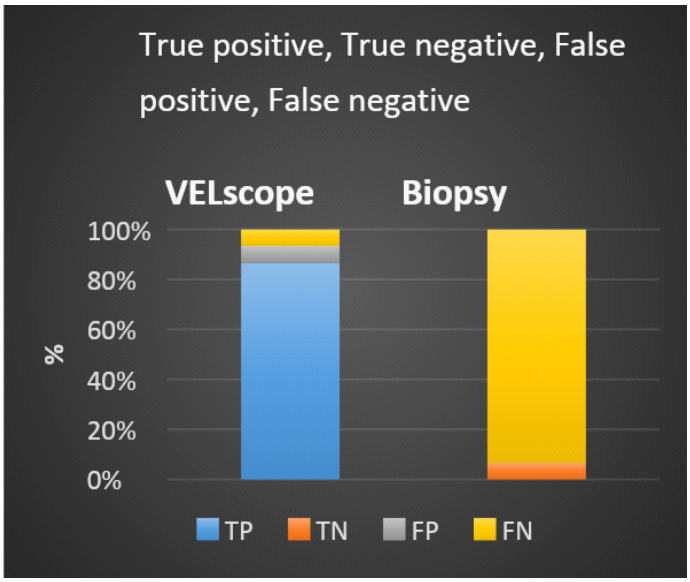

Chart 3. Distribution of examinees with highly suspected malignant potential – oral cancer (OC) by histopathological finding and VELscope screening.

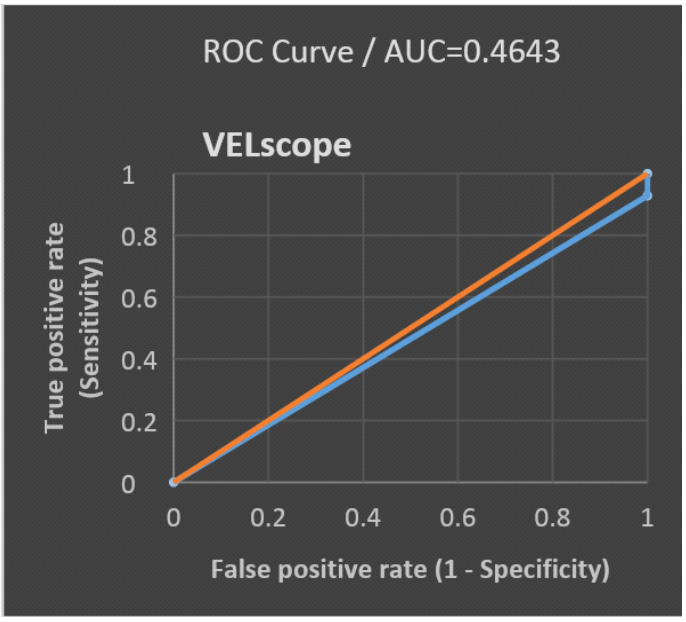

Chart 4. ROC curve - Sensitivity and specificity of VELscope screening in lesions with highly suspected malignant potential – oral cancer (OC).

AUC	Standard error	Lower bound (95%)	Upper bound (95%)
0.4643	0.0000	0.4643	0.4643

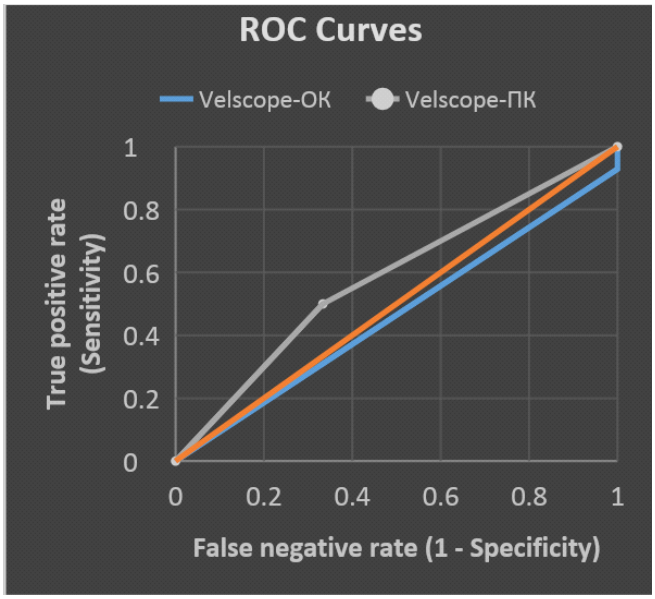

Chart 5. ROC curve - Sensitivity and specificity of VELscope screening in both study groups –PL group and OC group.

Chart 5 presents the ROC curves - Sensitivity and specificity of the method of VELscope in both study groups; the first with the potentially malignant lesions - precancerous lesions (PL) and the second study group with examinees with highly suspected malignant potential - oral cancer (OC).

Discussion

The use of autofluorescence as a diagnostic tool for cancer detection was first described in 1924 and has been under intensive evaluation for another 30 years. Autofluorescence uses natural fluorophores found in the epithelium and submucosa (for example, collagen, elastin), which radiate at different wavelengths resulting in excitation.

When irradiated at wavelengths between 375 and 440 nm, fluorophores show fluorescence in the green spectral range, and the normal, intact mucosa emits pale green autofluorescence when viewed through a selective, narrow band. Adequate filtration is required due to the intense light used to excrete fluorophores. Without proper filtration, it would be impossible to visualize the faint and narrow autofluorescent signal.

However, dysplastic tissues lose fluorescent emission power due to impaired fluorophore distribution and are noted as darker zones compared to surrounding healthy tissue. The main criticism of autofluorescence in the diagnosis of cancer is the lack of ability to distinguish high-risk lesions from low-risk lesions[19].

Among the first studies to use this device was that of Lane et al., which explored VELscope's ability to identify precancerous or cancerous lesions [20]. Following a conventional oral examination (COE), the oral cavity was examined with a VELscope to identify areas that would show loss of autofluorescence.

In addition, histopathological analyzes of the lesions were performed. Using biopsy as the gold standard, the device showed 98% sensitivity and 100% specificity for distinguishing dysplasia and cancer from normal oral mucosa.

Confirmation of the role of fluorescent imaging in detecting the surgical boundaries of oral cancer during surgery in the operating room, presents the results of a small study, where the loss of

autofluorescence extended 25 mm away from the visible tumor and that 89% which subsequently excised in these regions showed either dysplasia or cancer [21].

Our analysis showed that in the group of respondents with precancerous lesions, for the Velscope method, the sensitivity was 50%, the specificity reached 66.67%, the positive predictive value was 85.71%, and the negative predictive value was 25%. Accuracy, ie, the general probability that the patient will be correctly classified by the Velscope method is 53.33%.

Our results are in contrast to the results of Awan et al. and Rana et al., whose values are higher and range from 95% for the sensitivity and 77% for the specificity of the method [22.23].

Sawan and Mashlah present a sensitivity that is higher and does not correspond to the value we obtained in our study[24].

About 9.4% of the lesions detected were abnormal lesions and in 83.09% there was a loss of the effect of fluorescent light. Based on the use of surgical biopsy, the VELscope method showed a sensitivity of 74.1% and a specificity of 96.3%. According to the statistical analysis, with a 95% confidence level, there was a significant compliance between the VELscope results and the biopsy results.

Our result obtained after the histopathological processing of the biopsy material was positive in 28 respondents and 2 persons came out negative. The analysis showed that in this examined group, for the Velscope method, the sensitivity is 92.86%, the specificity is 0%, the positive predictive value is 92.86% and the negative predictive value is 0%. The accuracy that the patient will be properly classified with the Velscope screening method is 86.67% (Table 3 and 4, Chart 3 and 4).

Our results are in line with those of Marzouki et al., who found an almost identical sensitivity of 92% in their prospective study [25].

A high sensitivity of 94.44% of the VELscope system that is in accordance to our values, is presented by Canjau et al.[26], when differentiating normal mucosa from invasive cancer, and close values with the previous ones are also published by Moro et al [27], in their prospective study from 2010, examining 32 entities suspected for oral cancer, which receive a value for sensitivity of 100% and 93% specificity.

Particularly low values for the specificity of this method, which strongly correlate with our results, are presented by Koch et al., In their prospective study from 2010, the reported 15% are closest to our obtained value of 0%.[28].

The sensitivity is completely equated with our results in the amount of 93% and is another trump card in support of this auxiliary diagnostic method.

Conclusion

The high sensitivity value of 92.86% and the accuracy of the method of 86.67% largely confirm the reliability and efficacy of the Velscope method in patients with highly suspected oral cancer lesions (OC), in contrast to the values which we received in the first group of respondents (PML).

In group (PML) - subjects with oral potentially malignant lesions - precancerous, these values were lower, with measured sensitivity of 50%, and accuracy of the method of 53.33%, which makes this method preferable for use in patients classified in the second group (OC).

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