

**VICTOR BABEȘ UNIVERSITY OF MEDICINE
AND PHARMACY TIMIȘOARA
FACULTY OF DENTAL MEDICINE
DEPARTMENT I**

MOGA ALEXANDRA



PhD THESIS

NON-INVASIVE SCREENING AND GENETIC MARKERS IN ORAL CANCER

A B S T R A C T

Scientific Coordinator
PROF.UNIV.DR.LAURA-CRISTINA RUSU

**Timișoara
2022**

TABLE OF CONTENTS

List of published papers	VI
List of abbreviation	VII
Figure index	IX
Table index	XI
Dedication	XII
Acknowledgments	XIII
INTRODUCTION	XV

GENERAL PART

Chapter 1. ORAL CANCER – EPIDEMIOLOGY CURRENT INSIGHTS	1
1.1. Epidemiological trends	1
1.2. Oral cancer demography and evolution	5
1.3. Risk factors and their dynamics	9
1.3.1. Tobacco and alcohol	10
1.3.2. Betel quid	12
1.3.3. Human Papilloma Virus	13
1.3.4. Dietary habits	14
1.3.5. Oral hygiene and bacteria	15
Chapter 2. GENETIC ALTERATIONS ASSOCIATED WITH ORAL SQUAMOUS CELL CARCINOMA	17
2.1. Genetics and epigenetics in oral cancer	17
2.2 Gene mutations in oral squamous cell carcinopma	22
2.3 Genomics and personalized medicine in oral squamous cell carcinoma	26
Chapter 3. ALTERNATIVE TECHNOLOGIES FOR SCREENING AND DIAGNOSIS APPROACHES IN ORAL SQUAMOUS CELL CARCINOMA	27
3.1. Lesions development and evolution of oral squamous cell carcinoma	27
3.2. Diagnosis and tumor staging	30
3.2.1. Biopsy in oral squamous cell carcinoma – clinical considerations	31
3.2.2. Tumor staging and evaluation	32
3.3. Oral cancer screening	33

3.3.1. Tissue fluorescence imaging as an adjunctive tool.....	36
3.3.2. Biomarkers and precision medicine in oral squamous cell carcinoma	38
SPECIAL PART	
Chapter 4. A RETROSPECTIVE ANALYSIS OF ORAL CANCER HISTOLOGICAL SUBTYPES AND RISK HABITS	40
4.1. Introduction and objectives	40
4.2. Materials and Methods.....	42
4.2.1. Study design	42
4.2.2. Study variables.....	43
4.2.3. Tumor sample histological investigation	44
4.2.4. Statistical analysis.....	46
4.3. Results	46
4.4. Discussions.....	59
4.5. Conclusions	61
Chapter 5. GENE COL9A1 GENOTYPING – A COMPARATIVE ANALYSIS IN THE DIAGNOSIS OF ORAL SQUAMOUS CELL CARCINOMA: A PILOT STUDY ...	63
5.1. Introduction and aim of the study	63
5.2. Materials and Methods.....	65
5.2.1. Study design	65
5.2.1.1. Study cohort.....	65
5.3. Biologic samples processing.....	66
5.3.1. DNA analysis.....	68
5.4. The Statistical Analysis	70
5.5. Results	71
5.5.1. Gender and Age Analyses	71
5.5.2. Tagged SNP of COL9A1 Gene Identification.....	74
5.6. Discussions.....	79
5.7. Conclusions	84
Chapter 6. EVALUATION OF FLUORESCENCE IMAGING IN THE ORAL MUCOSA SCREENING – A CLINICAL STUDY	86
6.1. Introduction and aim of the study	86
6.2. Materials and methods.....	89

6.2.1. Study design	89
6.2.2. Assessment of the variables	90
6.2.3. Data collection and clinical protocol	92
6.2.4. Data analysis.....	94
6.3. Results	94
6.4. Discussions	103
6.5. Conclusions	105
PERSONAL CONTRIBUTIONS.....	107
CONCLUSIONS	110
REFERENCES	112
ANNEX	I

ABSTRACT

The World Health Organization reports that the healthcare system faces an alarming increase of multiple pathologies, among which oral cancer. The continuous exposure to various carcinogens determines a change in the dynamic of carcinogenesis and influences the development of malignancies. As it was already described, cancer and especially oral cancer is a complex and multifactorial disease. The ascending incidence is concerning researchers and opens new discussions regarding the etiology of oral cancer, the diagnosis approaches and prognosis with an urge in the implementation of new methods for prevention, diagnosis, treatment and surveillance. Although there have been identified associated risk factors and habits, the incidence does not in all cases follow a predicted pattern.

The first part of the thesis is divided into three main chapters that are meant to introduce and discuss the current existing information related to the incidence, epidemiology and associated risk factors of oral cancer. Also, by providing a description of the genetic alterations that can be linked to entire carcinogenesis process of oral squamous cell carcinoma. The importance of the oral screening is another aspect, outlining its contributions in improving the conventional oral examination with the help of additional information provided by the potential use of alternative technologies.

The current preoccupation of the health system is, besides the improvement of the diagnosis and clinical steps, raising awareness among the population regarding this malignancy and its consequences. For many years, the etiology was linked to a specific socio-economic status, risk habits and factors, as well oral conditions. Currently, these factors that have been associated to the etiology have suffered a redistribution and organization, and although the malignant cells exhibit genetic alterations, the correct relationship between this event and the potential exposure to carcinogens is not completely understood in order to improve the preventive measures and the management of this malignancy.

Current epidemiological analyzes reveal important aspects related to oral cancer, highlighting the importance of the impact that the lifestyle habits have upon the individual risk. These factors actively contribute to the direction of implementing

the " personalized medicine " concept, focusing on each individual by evaluating the personal risk. Introducing the use of biomarkers could improve the early diagnosis and screening process and the assessment of the individual risk, by providing a non-invasive, simple and quick approach for the clinician and accepted by the patient.

Taking into consideration that the main risk factors of oral cancer are known and manageable, it can be assumed that it can become a preventable malignancy. Although, there are reports that evaluate the diagnosis of oral cancer in low-risk populations, their conclusions outlining the fact that a more complex initiation process, such as a genetic and epigenetic alterations are associated with oral cancer. Nevertheless, the primary preventive measures for oral cancer should consist in the education of the individuals, limiting the existing risk factors and encourage routine check-ups.

Oral cancer is represented by oral squamous cell carcinoma (OSCC) in the majority of the cases, followed by tumors of the salivary glands, melanomas, and lymphomas. OSCC includes all the malignant oral tumors that are arising directly from the epithelial layer, taking into consideration its permanent exposure to the direct action of various risk factors.

It is important to acknowledge the contribution of molecular biology, the action of the molecules within the cells and the interaction of the cells within the tissue in order to evaluate correctly the carcinogenesis process. The assessment of specific genes that can be directly linked to the malignant process of OSCC is the first step in a possible new era of the diagnosis, treatment and prognosis. The DNA micro-arrays investigations allow to quantify the gene expression profile in order to outline the potential changes that could be linked to OSCC. The main genetic changes related to oral cancer are mutations in the oncogenes, the tumor suppressor genes, genes involved in the DNA repair and other genes that are influenced by associated risk factors such as betel, alcohol, tobacco and viruses. The genomic analysis of oral cancer has to overcome several challenges due to the molecular complexity and gene heterogeneity of the oral tumors. The Cancer Genome Atlas (TCGA) with the contribution of the National Human Genome Research Institute (NHGRI) and National Cancer Institute (NCI) aimed to quantify the genetic and epigenetic changes in various malignancies. The database provided by TCGA outlined the existence of specific genetic mutations associated with OSCC, discovering new pathways for screening and diagnosis.

Oral squamous cell carcinoma is usually preceded by clinical lesions included in the category of the oral premalignant lesions (OPMD), being characterized by several neoplastic features that are known as oral dysplasia. The evolution of oral epithelial dysplasia and transformation into OSCC implies a progress of the carcinogenesis process towards developing specific malignant characteristics. The early detection and management of these lesions is crucial for the clinical outcome, although there have been discussions regarding the concept of the field cancerization and recurrence of these lesions based on the molecular alterations that affect at the same time the apparent normal oral mucosa, preceding the precancerous state.

The clinical evaluation of the oral mucosa and the assessment of the potential exposure to different carcinogens is a priority for the clinician. The oral mucosa is exposed to the effects of field cancerization and can develop lesions in different stages, fact that could imply more multiple biopsies. The early detection is an important factor for improving the survival rate and prognosis and a complete clinical examination of the oral cavity and the head and neck regions can identify in the majority of the cases the malignant lesions.

An identified oral mucosa lesion should be assessed as suggested by World Health Organization, mainly any oral mucosa lesion that has persisted more than 2 weeks although any known local factors have been out ruled, must undergo biopsy and further histopathological examination. An option in some cases could be the cytologic examination of the epithelial surface, but the sensitivity and specificity of this examination is low for the OSCC diagnosis. Recently, modern and less invasive approaches have been introduced in order to improve the clinical examination distinguishing the benign from potentially malignant lesions.

The screening of oral cancer and oral premalignant lesions should be a routine examination, especially in populations with a high-risk. The examination is simple, non-invasive and painless, based on a direct visual examination of the oral mucosa and loco-regional palpation. Usually, there are specific risk areas with a high incidence in developing lesions. Screening is defined as having the aim to identify an unrecognized disease in apparently healthy and asymptomatic individuals by the application of a test to distinguish the presence or absence of certain features. It is important to acknowledge the fact that the screening procedure is not a diagnostic

test and is intended to recognize specific changes that are associated with a high risk of developing oral cancer. Oral cancer screening is designed to focus on the presence of mucosa alterations, potentially malignant disorders and early cancer onset. Dentists can play a major role in the screening and early identification of oral potentially malignant disorders and malignant lesions fact that can positively influence the treatment onset and outcome. Unfortunately, reports suggest that there are unsatisfactory results related to the practice and knowledge of the dentists regarding these types of lesions.

The tissue fluorescence property became useful for the screening and potential early diagnosis in skin, lung and uterine cancer, and recently it was introduced for the oral mucosa evaluation. The principle that this imaging approach is based on is the property of the oral mucosa to become autofluorescence in certain circumstances. Changes in the oral mucosa cell structure (an increase of cellular or nuclear pleomorphism, hyperkeratosis or an hyperchromatin alteration), in the cells metabolism or changes localized in the subepithelial stroma will influence their reaction to a certain wavelength light. The alterations in the epithelium and stroma will influence the presence of the tissue components, known as fluorophores, and the way they emit after the interaction with an intense blue light, a process described as autofluorescence. The image resulted after the interaction of the blue light with the oral mucosa can be seen and evaluated by the clinician. The fluorescence technology can improve the visual conventional examination and provide further information in order to distinguish the normal areas of mucosa from areas exhibiting alterations. Over the past years, the property of autofluorescence has been widely investigated as an adjunctive tool for the diagnostic.

The special part of the thesis is focusing on three main independent studies that follow the pathway of the topics of the chapters discussed in the general part , revealing the personal contribution. The first study is five-year retrospective research that aimed to show the epidemiological trends of oral cancer, outlining the incidence of the histopathological subtypes, their differentiation grade and TNM (tumor, nodes, metastasis) classification, in relationship with age, gender and associated risk habits. By comparing the results with the ones existent in the literature, valid conclusions regarding the local epidemiological aspects and trends could be assessed.

The second research study is a pilot clinical study that gravitates around the existent genetic mutations that can be linked to oral squamous cell carcinoma patients, introducing the possibility of identifying COL9A1 gene mutation in fresh tumoral tissue and blood samples of oral squamous cell carcinoma patients. So far, this is the first study that included the genotyping of blood samples in order to identify the mutations of COL9A1 gene, and the results showed a positive correlation between the presence of the gene mutation in the two types of samples. The third study consisted of a comparative evaluation of the conventional oral examination performed during an oral screening process and the potential use of autofluorescence technology as an adjuvant in detection and improvement of the visual examination. The results point out the high sensibility of the autofluorescence technology while compared to the clinical findings, and the importance of performing an accurate oral screening of the mucosa in order to early identify the abnormal changes.

The results of the conducted studies highlight the complex etiology of this malignancy, revealing the epidemiological trends and the implication of the multiple risk habits, as well as the major role that the genetic alterations play in the carcinogenesis process. By introducing the possible use of blood samples and the presence of COL9A1(collagen type 9 alpha chain 1) gene mutations, the screening and early diagnosis process could be substantially improved in the future. The oral screening process has the major role in improving the early diagnosis, with the help of a step-by-step clinical examination performed by the clinician, an accurate anamnesis and risk factors assessment and nevertheless, by raising the awareness among patients, the survival rate can be substantially improved. With the additional information provided by the autofluorescence technology, the clinical examination can be improved, and the early cellular changes can be visualized.

Nevertheless, oral cancer is characterized by an insidious onset, no or little symptomatology, aggressive evolution and late diagnosis. This malignancy is described as a cumulative action of genetic alterations, local and general risk factors and habits that conduct the evolution of the disease, fact that should be reflected as well in the screening approaches and treatment plan.