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### RESEARCH ARTICLE

#### EVALUATION OF SOME HIGH RISK BACTERIAL SPECIES IN THE ORAL MICROBIOME OF HNSCC PATIENTS

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#### Abstract

In this study we evaluated the composition of seven high risk periodontitis species of microorganisms in the oral microbiome of patients with different forms of head and neck squamous cell carcinoma (HNSCC). The laboratory analyses were performed using the method of quantitative real-time amplification (qPCR) of 26 saliva and mouth swab samples collected from HNSCC patients. Laboratory data regarding the normal or pathological presence of studied species were integrated with patients clinical conditions and some lifestyle factors. The results show that the highest frequency of pathological concentration is in the case of *Fusobacterium nucleatum* and *Tannerella forsythia*, while the lowest frequency is observed for microorganisms *Aggregatibacter actinomycetemcomitans* and *Treponema denticola*.

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#### Introduction:-

During his biological evolution, *Homo sapiens* learned to coexist with a large number of microscopic organisms such as viruses, bacteria and fungi. These organisms are present in large quantities in the oral cavity, intestine, vagina, skin, etc. Through their diversity, density and composition they influence directly the immune activity and modulate the development of various pathologies. Studies regarding relationships between the human microbiome and malignant tumors have been of increasing interest in recent years and suggest that certain microbes may offer susceptibility of developing various forms of cancer. There are several mechanisms by which infections with certain microbial agents can cause the initiation of an oncogenic process. Bacterial metabolites and endotoxins can induce somatic mutations, signaling pathway disorders and various antiapoptotic cellular changes but most microorganism-induced tumors are preceded by a specific inflammatory status. Regarding the oral microbiota, there are various species which are linked with tumorigenesis of the head and neck squamous cell carcinoma (HNSCC). Previous studies suggest that such species as *Fusobacterium nucleatum*, *Tannerella forsythia* or *Porphyromonas gingivalis* possess high proinflammatory risk and may induce carcinogenesis [1,2,3,4]. They indicate that normal or pathological presence of these bacteria in oral microbiota influences the relationships between microorganisms and plays a major role in the formation of polymicrobial biofilm. Detection of some periodontitis agents in pre-cancer lesions may become an important element of cancer diagnostics and become a malignancy prognostic indicator [3]. In our study, which took place at the Institute of Oncology from Moldova between February and November 2021, we evaluated the concentration of seven high risk periodontitis species of microorganisms in the oral microbiome of patients with different forms of HNSCC (mucosal carcinoma). Laboratory data regarding the normal or pathological

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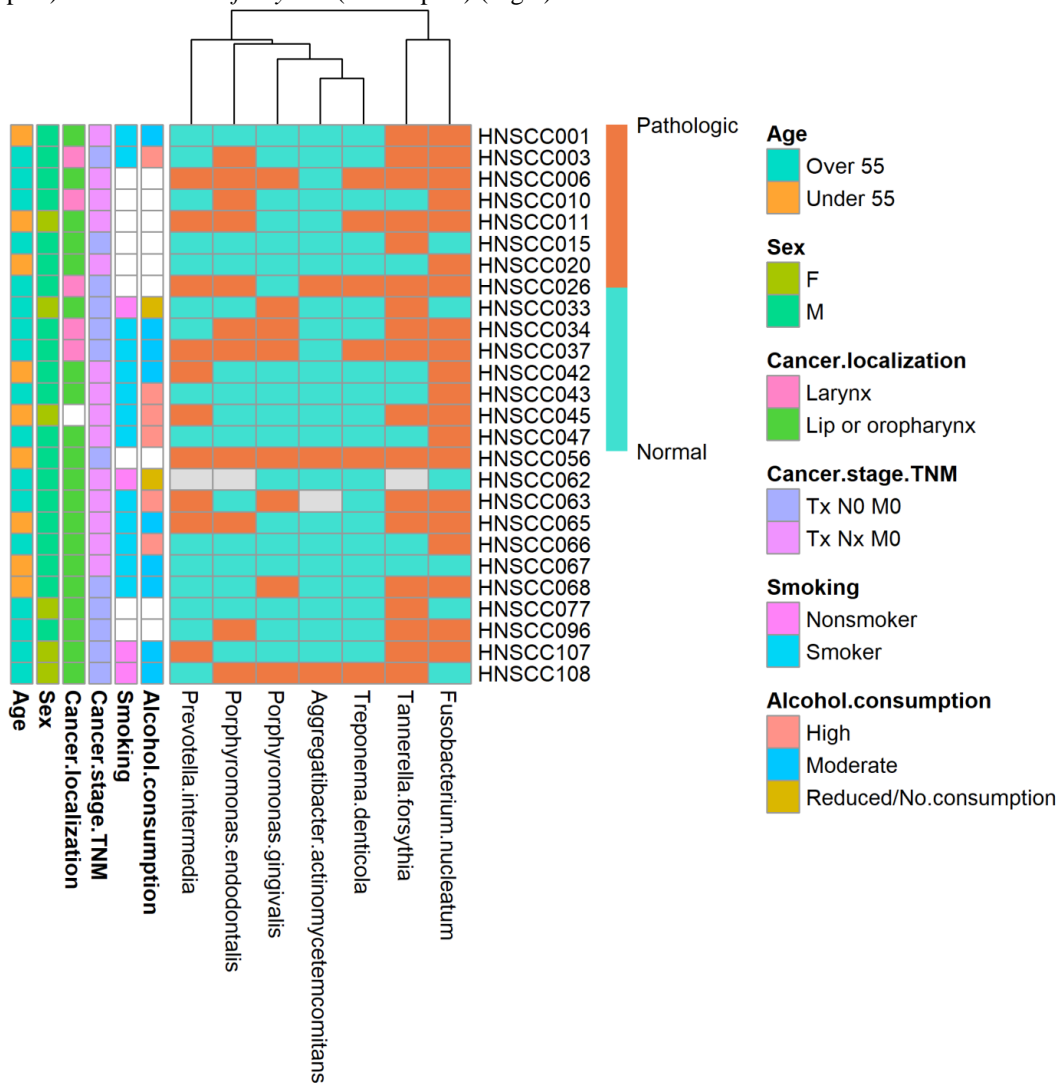
presence of these species in the oral microbiome were integrated with patients clinical conditions and some lifestyle factors.

**Materials and methods:-**

In order to carry out the laboratory analyses, it was used the method of *quantitative real-time amplification (qPCR)* of 26 saliva and mouth swab samples collected between February and November 2021 from 26 patients with lip, oropharynx and larynx squamous cell carcinoma. Immediately after harvesting and until molecular biology analyzes, the samples were stored in the biobank at -86°C. Bacterial evaluation was performed using the kit *Дентоскрин* (produced by Lytech Company Ltd.) [7] which allows DNA detection and quantification of 7 periodontal pathogens: *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Treponema denticola*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Tannerella forsythia*. Clinical data (cancer stage and localization, patient’s sex and age) such as lifestyle factors (smoking, alcohol consumption) were collected by clinicians with patient consent, during the routine hospital examination. The analysis of laboratory data, annotation with clinical data and plotting were performed in *R language* using the *heatmap* library [5,6].

**Results and discussions:-**

The results show that the highest frequency of pathological concentration is in the case of *Fusobacterium nucleatum* (20 samples) and *Tannerella forsythia* (18 samples) (Fig.1).



**Fig. 1:-** The concentrations of bacteria evaluated with *Дентоскрин* kit in the oral microbiome of HNSCC patients (annotation performed with data from clinical surveys)

*Fusobacterium nucleatum* was found in high concentrations in most smokers and only in one patient from the "Nonsmoker" category. Regarding the *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* microorganisms, they form a cluster that indicates a low number of samples with abnormal concentrations and suggests that these two microbes may have a relationship of "companion species" or "synergistic species". In general, samples with pathological values of *A. actinomycetemcomitans* show abnormal values for most species. Also, the integration with clinical data attests the pathological presence of *Porphyromonas endodontalis* in all patients with laryngeal carcinoma and in approx. 30% of patients with lip and oropharynx carcinoma. No clear differences were observed between sexes, age groups or tumor stages, however, given the small number of samples included in the study, these observations need to be verified in larger cohorts.

### Conclusions:-

1. The *Fusobacterium nucleatum* and *Tannerella forsythia* microorganisms show the highest frequency of pathological concentrations from all 7 studied species.
2. *Fusobacterium nucleatum* was found in high concentrations in most smokers and only in one patient from the "Nonsmoker" category.
3. The *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* microorganisms show the lowest frequency of pathological concentrations from all 7 studied species.
4. The samples with pathological values of *A. actinomycetemcomitans* show also high frequencies of abnormal values in other species.
5. All patients with laryngeal squamous carcinoma have pathological concentrations of *Porphyromonas endodontalis*.

### Acknowledgement:-

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