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Corellation between social status, salivary pH and oral microbiota

[1]Bianca Dumbrovca, [2]Carmen Defta, [3]Antarinia Craciun, [3]Marius Negucioiu, [3]Mirela Fluerasu,[3]Smaranda Buduru

[1] County Emergency Clinical Hospital Cluj-Napoca, Romania; [2] Department of Microbiology, Faculty of Stomatology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania; [3] Department of Prosthetic Dentistry and Dental Materials, Faculty of Dental Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

***All authors have equal contribution to this study**

Abstract. Objective. Lifestyle choices can significantly impact the oral health, such as patient's living environment, job stress, smoking habits and general health conditions that require ongoing treatment. These factors can influence the dental status, involving changes in pH value and type of oral microbiota, which will lead to the development of dental and periodontal issues. The aim of this study is to assess whether or not the oral microbiota is influenced by the social status (which implies factors such as age, smoking habits, environment, field of work and job-related stress, level of education and general health problems) and by the salivary pH value. Materials and methods. The study enrolled 20 patients and medical charts were filled highlighting general health status and both personal and professional lifestyle. Afterwards each of them underwent a clinical examination of teeth and gums, pH recording using special pH paper and dental plaque retrieval on glass slides. Consequently, the slides were fixed, stained and analyzed under a microscope in order to identify the microbiological aspects. Results. Gram-negative microbiota was statistically significant on slides in patients with a high level of education, whereas Gram-positive bacteria was found on patients with medium level of studies ($p<0.0001$). Also, Gram-negative bacteria was found on a greater number in subjects from urban areas, on the other hand Gram-positive bacteria being present mostly among subjects from rural environment ($p<0.0001$). Subjects with stressful jobs showed a dominance of Gram-negative oral biofilm ($p<0.0001$). Acidic pH level was correlated with presence of Gram-negative oral microbiota ($p=0.0102$). Conclusions. Gram-negative microbiota was correlated with higher level of education and stressful fields of work, alongside with urban origin environment. General health problems, smoking habit or age did not have an impact on salivary pH or oral microbiota.

Key Words: oral microbiota, salivary pH, social status

Corresponding Author: M. Fluerasu, email: mfluerasu@yahoo.com

Introduction

The mouth serves as the body's gateway to the exterior and it represents one of the most biologically intricate sites of the human body (Marsh & Martin 2016). This entire environment is influenced by modifications in salivary flow, nutrient availability, oxygen concentration, saliva, and gingival crevicular fluids, thus creating a dynamic and complex setting (Sedghi et al 2000). In the oral environment there is a high microbial diversity (up to approximately 1000 species of bacteria, viruses, fungi, protozoa and archaea (Radaic & Kapila 2021) linked with the oral health and oral homeostasis (De Filippis et al 2014), creating a "commensal, resident or indigenous" microflora (Samaranayake 2018). The resident microbiota is formed by both Gram-positive and Gram-negative bacteria, coexisting in the oral cavity during healthy or pathologic periods. Socranski described specific microbial groups which are present in the dental plaque: yellow complex of *Streptococcus spp*, green complex consisting of *Capnocytophaga spp*, purple complex containing *Veilonella parvula* and *Actynomices odontolyticus*. These three complexes are compatible with oral health, whereas both orange and red groups consists of periodontal pathologic Gram-negative bacteria, such as *Prevotella intermedia* for orange complex and *Porphyromonas gingivalis* for red complex. The interaction between these groups is very specific: the growth of yellow, green or purple complex precedes the multiplication of orange and red complex bacteria which are present simultaneously during periodontal disease development (Socransky & Haffajee 2000). The oral microbiota has been associated with various oral and systemic diseases, such as dental caries, periodontal disease, diseases of the oral mucosa, oral cancer, and peri-implantitis (Gao et al 2018; He et al 2015). It is also linked to systemic conditions like obesity (Claesson et al 2012), rheumatoid arthritis (Scher et al 2012), HIV infection (Gruffaz et al 2020), liver cirrhosis (Acharya et al 2017), inflammatory bowel disease (Zhu et al 2018), polycystic ovary syndrome (Akcali et al 2014), type 2 diabetes (Long et al 2017), atherosclerosis, cardiovascular disease (Olsen et al 2018), and more recently, Alzheimer's disease (Emery et al 2017). Furthermore, the oral

microbiota play a vital role in nitric oxide homeostasis, which affects blood pressure (Hezel&Weitzberg 2015).
Saliva is a complex fluid secreted by salivary glands, having immunologic and non-immunologic means for protecting mucosal surfaces (Mandel 1989). Normally, the average pH registered for saliva in 6.7, but this value can vary from 6.2 to 7.6 - interval valid for neutrality (Baliga et al 2013), which is maintained by several systems: the protein system, the phosphate system and the carbonic acid/bicarbonate system (Thavarajah 2006).
Metabolizing sugars from the daily diet results in the production of significant amounts of organic acids, including lactic acid. This process creates an acidic environment within the oral cavity, with higher levels of mutans group streptococci (Lamont & Jenkinson 2010) able to tolerate a lower pH which can get to 5.5, a condition often found in patients with severe dental caries (Lamont et al 2019). On the other hand, to initiate periodontal disease, the pH within the microbial plaque must exceed 7.6, as evidenced by the alkaline pH of saliva in patients with generalized chronic gingivitis (Baliga et al 2013).
Therefore, problems arise when there is an imbalance in the local homeostasis between the bacterial communities present in dental plaque, several factors being able to influence the oral environment (Patil et al 2013, Takahashi 2005). Factors such as age can have a significant influence on oral microbiota, adults with age between 20-40 years having the highest bacterial diversity, which declines during the 40-60 age period (Kazarina et al 2023). This high bacterial diversity may be linked with smoking habits, as one of the first effects of smoking that appear is a change in the oral PH as it lowers, by decreasing the buffer effect of saliva, increasing the risk of developing an acidic bacterial environment (Awan 2011).
Rural and urban environment might impact the oral microbiota when it comes to diversity and richness of flora, people in the urban area having more types of bacterial species present in the dental biofilm (Widyarman et al 2021). However, the association between oral microbiome and level of education is less clear, graduating university may be linked with a particular choice of diet which can influence the homeostasis of oral biofilm (Cheung 2022).
Stress-induced mediators contribute to the dysbiosis of the oral microbiome, as several studies showed a correlation between high levels of cortisol and presence of P. gingivalis, a periodontal pathogen of red complex, leading to the development of periodontal disease (Spector et al 2020). People working in fields such as medicine (Belkic&Nedic 2014) or police (Huang et al 2021) declared to have a higher level of job-related stress.
This study aims at assessing if there is a relation between oral types of microbiota (described in Gram-positive or Gram-negative dominant flora) and values of salivary pH and other social factors linked with personal and professional lifestyle: age, smoking habit, origin environment, highest level of education completed and job-related stress.
The first null hypothesis assumed that there is no significant correlation between the oral types of microbiota and value of salivary pH. The second null hypothesis assumed that there is no significant correlation between oral types of microbiota and the social factors above mentioned.

Materials and methods

The research was carried out on 20 patients with ages between 19 and 75 years old from Bucharest and Ilfov county, Romania. The interview and the clinical examination, together with collecting the specimens from the participants were taken in a private dental office in Bucharest between February 2023 and May 2023.
The patients were asked to give their written consent before the interview and the clinical examination. Patients were not promised any financial benefit from being part of the study and were not charged additionally for analyzing the slides or the salivary pH.
The exclusion criteria was: total or extensive edentulous patients, patients with immunosuppressive, radiotherapy or chemotherapy ongoing treatment, periodontal disease in active state or diabetes. Also, it was mandatory for inclusion criteria that patients had a complete arch (except in the third molar) with no prosthodontic restorations in order to collect the dental plaque from the same sites.
In order to gain information about patients' lifestyle, the clinician interviewed each patient prior to the clinical examination, performing a complete anamnesis focused on: age, the presence of general health problems and the treatment followed, origin environment, highest level of education completed, level of stress at the job correlated with the field of work and tabacco habits.
Clinical examination consisted of analysing the general dental and periodontal heath (presence of decays, gingivitis, pericoronaritis, periodontitis), alongside with measuring the salivary pH.
In order to obtain the salivary pH value, a pH kit (Ellementa, Romania) was used, which contained pH paper and a pH indicator scale- color chart.
Before collecting the saliva, each patient was asked to not eat, drink, smoke or perform oral hygiene one hour before the examination. For each patient, the saliva collection was performed during 8:00 am to 11:00 am, in order to avoid diurnal variations.
Each patient spit into a sterilized cup and a pH paper was immediately inserted into the collected saliva for 10 seconds, after that the color of the paper was examined. Acidic saliva (values less than 7) changed the paper color into a yellow-orange-red color and alkaline saliva turned the paper into intense green-blue color (values higher than 7).
The bacterial sampling was done by one clinician for all the patients, once for each patient. The sample originated from lingual, mesial and distal surfaces of inferior and upper central incisors and inferior second molars, using a sterile dental probe, after gargling with warm water to remove any residue.
The dental plaque collected was placed on a sterile glass slide on which, prior to applying the dental plaque a drop of saline solution was placed in the center of the slide. Circular movements were made in order to dissolve the dental plaque in the saline solution and the slide was left to air dry until a thin, non-adherent, white film formed on its surface, corresponding to the area where the pathological sample was spread.
The sample spread on the glass slide was fixed by passing it through the flame of a Bunsen burner 3-4 times. The slide was heated on the opposite side. Throughout this process, the temperature was monitored on the back of the hand to ensure it did not exceed 56 degrees Celsius, preventing alteration of the pathological sample at higher temperatures
Subsequently, the slides were stained using the Gram staining kit (POLYMED, Romania), which included Gentian Violet,

Lugol's Solution, alcohol-acetone mixture, Fuchsine (diluted 1:10), stand for placing slides and tap water.
Gram staining technique is widely used in microbiology for differentiating between Gram-negative and Gram-positive microorganisms, which appear differently colored following the staining protocol. Gentian violet stains Gram-positive bacteria in a shade of blue-violet. The next step involves staining Gram-negative bacteria in a shade of red-pink using fuchsine, with the color difference attributed to the thinner layer of peptidoglycan in the cell walls of Gram-negative bacteria. Between the application of the two types of stains, the alcohol-acetone mixture is applied. Finally, Lugol's solution is applied to fix the color (Coico 2023).
The final step involves examining the stained slides containing the pathological sample after they have dried. Prior to examination, a drop of cedar oil is applied to each slide to alter the refractive index during image magnification. The Zeiss optical microscope (Carl Zeiss GmbH, Austria) with a 100x immersion objective was used for this purpose. When used correctly, this objective magnifies the image 1000 times. At least 3 sites were examined on each slide.
The following variables were assessed: age, origin environment (urban or rural), highest lever of education completed (medium for high-school or higher education for university), job-related stress according to the field of work (assessed subjectively on a scale), smoking habits (present or absent), presence of general health problems.
Statistical analysis was performed using Microsoft Office Excel/Word 2021 (Microsoft, Redmond, DC, USA). Categorical variables were presented in absolute form and tested by using Repeated One-Way ANOVA test.

Results

A total of 20 patients were included in the study, with 60% female subjects and 40% male subjects. 50% patients included in the study were aged between 26-35 years old. 65% subjects had graduated university studies and 85% declared no general health problems. 70% declared to be stressed because of job field and job environment. The data from anamnesis and clinical exam, starting with gender, age, level of education, origin environment, smoking, field of work, stress level, general and oral health issues have been collected and analyzed (see Table 1).
Different bacterial cells have been identified in the sampled collected, based on their presence the slides being described as having a Gram-positive, Gram-negative, or equal dominant microbiota. Different types of cocci, diplococci, bacilli, filaments, fusobacteria have been found on slides with various prevalence (see Figure 1).
Value of salivary pH varied from 5 to 7, 70% of subjects having a pH level of 6. After describing the slides based on Gram-negative or Gram-negative dominant microbiota, 50% were found to have Gram-positive cells, 35% Gram-negative cells, and 15% having both types of cells (see Table 2).
Field of work, origin environment, highest level of education and salivary pH were discovered to have an impact on dominant oral microbiota, as differences between groups were discovered to be statistically significant according to the Repeated One-Way ANOVA test, which showed that:

Table 1. Data acquired from anamnesis and clinical exam

Variable		No	%
Gender	Male	8	40%
	Female	12	60%
Age group	18-25	7	35%
	26-35	10	50%
	36-45	1	5%
	46-55	0	0%
	56-65	0	0%
	>65	2	10%
Highest level of education completed	High school	7	35%
	University	13	65%
Origin environment	Rural	13	65%
	Urban	7	35%
Smoking	Non-smoker	12	60%
	Smoker	8	40%
Field of work	Administration	3	15%
	IT	3	15%
	Police	2	10%
	Medicine	2	10%
	Sales	2	10%
	Architecture	1	5%
	Constructions	1	5%
	Accountability	1	5%
	Engineering	1	5%
	Education	1	5%
	Hospitality	1	5%
Stress level	Stressed	14	70%
	Non-stressed	6	30%
	Retirement	2	10%
General health issues	Absent	17	85%
	Hipertension	1	5%
	Mitral regurgitation	1	5%
	Anxiety	1	5%
Oral health issues	Pericoronaritis	1	5%
	Periodontitis	3	15%
	Decay – deep lesion	14	70%
	Decay – medium lesion	7	35%
	Decay – incipient lesion	2	10%

Table 2. Registered values of pH and type of microbiota found on slides

Variable		No.	%
ph	5	3	15%
	6	14	70%
	6.5	2	10%
	7	1	5%
Oral microbiota	Gram-positive	10	50%
	Gram-negative	7	35%
	Equal	3	15%

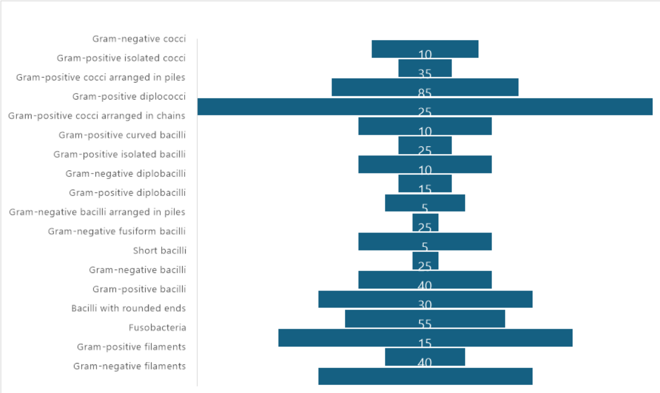

Figure 1. Identified bacterial cells and their prevalence in the collected slides.

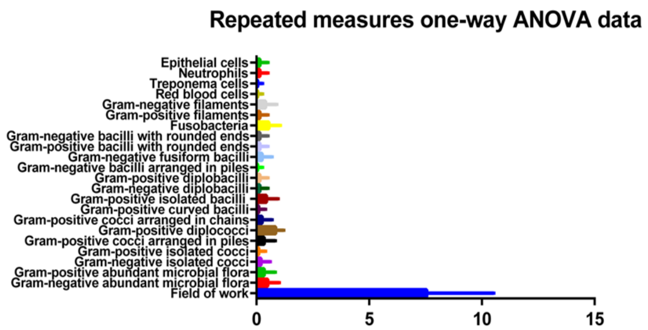

Figure 2. Correlation between oral microbiota and field of work

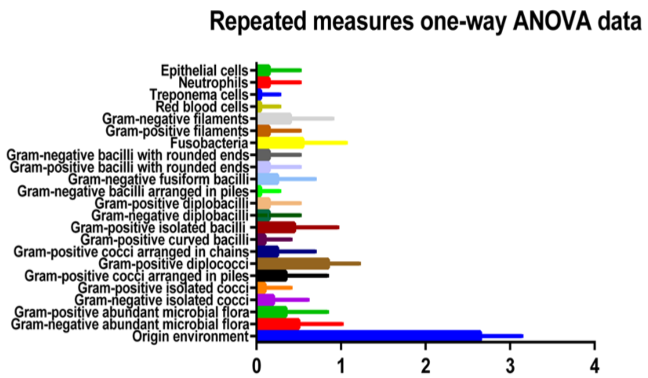

Figure 3. Correlation between oral microbiota and origin environment

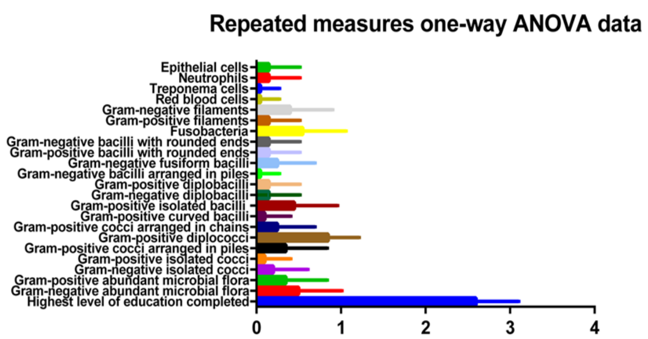

Figure 4. Correlation between oral microbiota and highest level of education completed

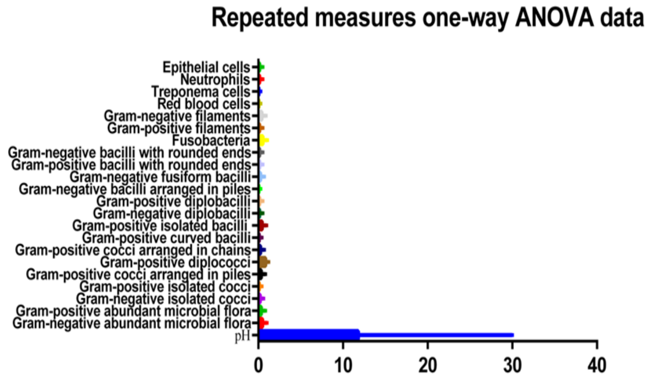

Figure 5. Correlation between oral microbiota and salivary pH

- Subjects with jobs in stressful fields of work (such as medicine, police) showed a dominance of Gram-negative oral microbiota ($p<0.0001$) (see Figure 2).

-Subjects from urban environment showed a dominance of Gram-negative microbial flora, as the ones from rural environment had a equality between Gram-positive and Gram-negative oral microbiota, or a tendency for Gram-positive microbiota dominance ($p<0.0001$) (see Figure 3).

- Subjects which graduated University studies showed a tendency to abundant Gram-negative microbiota, whereas subjects with only High Shool studies had a Gram-positive flora dominance on slides ($p<0.0001$) (see Figure 4).

- pH value of 5 was discovered amongs subjects with dominance of Gram-negative oral microbiota ($p=0.0102$) (see Figure 5).

Age, smoking habit or general health problems were not statistically significant, as there was no correlation between these social factors and type of oral microbiota found on slides.

Discussions

In this study the first null hypothesis assumed that there was of no correlation between the type of mycrobiota present and values of salivary pH. This hypothesis was not valid, as pH value was statistically significant on groups with different type of oral microbiota. The second null hypothesis assumed that there was no correlation between the type or oral mycrobiota and social factors. However, this study showed significant statistical difference between groups with Gram-positive abundant microflora and Gram-negative dominant microbiota correlated with origin environment, field of work, educational level and salivary pH value.

The oral microbiota can vary significantly between individuals from different geographic regions; people with similar origin environment, culture or ethnicity were found out to not have significant difference in oral microbiome, according to Ogbanga et al (2023). During our study, although subjects were from the same region (Bucharest and Ilfov county), different origin environment were addressed (rural and urban), significant difference being found on slides – as the ones from urban regions developed and abundant Gram-negative oral flora, Gram-positive abundant bacteria being found on the slides collected from subject with rural origins.

An acidic pH with the value of 5 was correlated with the presence of Gram-negative oral microbiota. Gram-negative oral microbiota was also dominant among patients working in stressful fields of work, thus some of these patients experienced an acidic salivary pH. According to a study made by Cohen and Khailaila (2014) , the pH levels in saliva were considerably lower in a group of students before and during the stressful exams periods comparative to non-exam period, when the levels of acidity were lower and the pH increased in value.

During our study, higher level of Gram-positive bacteria was found on slides collected from patients with a medium level of studies, whereas the ones which followed a higher level of education had a Gram-negative dominance on slides. According to Hallang et al (2021), a low educational level was associated with unfavourable microbiota composition which can lead to dental caries development. *Streptococci spp*. have an important role in evolution of dental caries, all these being Gram-positive bacteria (Deo & Deshmukh 2019). However, our study was limited in assessing the oral microbiota based on the Gram stain method and no specific bacteria species were identified.

In a study made by Jia et al (2021), Gram-negative species on oral microbiota were dominant on smokers subjects. During our study, the smoking habit did not seem to influence the dominance of Gram-positive or Gram-negative species, because both types of microflora were found on slides collected from smokers. During our study age did not showed an impact on oral mycrobiota, as no correlation was found, subjects from the same age interval having both Gram-positive and Gram-negative oral mycrobiota present. However, Faveri et al (2009) showed an increase level of pathologic periodontal Gram-negative species on elder subjects. Also, general health problems present among subjects from our study did not influence the level of Gram-positive or Gram-negative oral microbiota – according to Jia et al, (2018), several systemic diseases (cardiovascular, pulmonary, cancer) are linked with the presence of dysbiosis in the oral cavity.

This study offers an insight on how environmental factors can influence the oral microbiota and the salivary pH, everyday lifestyle being an important parameter to consider when it comes to evaluate the oral and general health of a patient. However, there are some limitations on our study, starting from the total number and social diversity of subjects and the method for describing the oral biofilm (as this study described oral microflora as Gram-positive or Gram-negative using Gram-stain method, and no specific microbiological device for species identification was used) . Also, idetifying the bacterial species instead of categorising them based on Gram stain may have conduct to more specific results and deeper insights into the subject. Thus, our study showed a correlation between oral microbiota, pH values and social factors such as living environment, education level and job-related stress, with no correlation of oral mycrobiota and smoking habits, age and general health conditions. A bigger sample of subjects may have lead to more significant statistic differences, along with a greater diversity in age and general heath conditions.

Conclusions

Gram-negative microbiota dominance was correlated with higher level of education and stressful fields of work, alongside with urban origins. General health problems, sex, smoking habits or age did not seem to describe a significant statistic difference. Clinicians should be aware when interviewing patients not only about general and oral heath history, but living environment, work field and job-related stress level, along with level of education should be taken into consideration. These factors can influence oral microbiota by creating proper conditions for specific pathogenic bacteria to spread, thus creating a dysbiosis which may lead to development of decays and periodontal disease.

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Authors addresses

•Bianca Dumbrovca: Department of Proshodontics, Clinical Emergency County Hospital Cluj-Napoca, 3-5 Clinicilor Street, 400006, Cluj-Napoca, Cluj, Romania, email bianca.dumbrovca@gmail.com;

•Carmen Defta: Department of Microbiology, Faculty of Stomatology, Carol Davila University of Medicine and Pharmacy, 37 Dionisie Lupu Street, 010458, Bucharest, Romania, email carmen.defta@umfcd.ro.

•Antarinia Craciun: Department of Prosthodontics, Faculty of Dental Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, 6 Pasteur Street, 400349, Cluj-Napoca, Cluj, Romania, email craciun.antarinia@umfcluj.ro,

•Marius Negucioiu: Department of Prosthodontics, Faculty of Dental Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, 6 Pasteur Street, 400349, Cluj-Napoca, Cluj, Romania, email marius.negucioiu@umfcluj.ro.

•Mirela Fluerasu: Department of Prosthodontics, Faculty of Dental Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, 6 Pasteur Street, 400349, Cluj-Napoca, Cluj, Romania, email fluerasu.mirela@umfcluj.ro.

•Smaranda Buduru: Department of Prosthodontics, Faculty of Dental Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, 6 Pasteur Street, 400349, Cluj-Napoca, Cluj, Romania, email dana.buduru@umfcluj.ro.

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