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A PILOT STUDY ON THE MICROBIOLOGICAL DIVERSITY IN ORAL LICHEN PLANUS (OLP)

Ayşe Gül ÖNER TALMAÇ, Gaye KESER, Hakan YÜLEK, Şükrü ÖNALAN, Filiz NAMDAR PEKİNER

APPROACH AND METHODS TO BE APPLIED:

Seven individuals who presented to the M.U. Faculty of Dentistry, Department of Oral and Maxillofacial Radiology Clinic and were diagnosed with "OLP" clinically and histopathologically (mucosa with OLP lesions and healthy mucosal tissue from the same patient) will constitute the patient group. Six healthy individuals who agreed to participate in the study and who presented to our clinic due to any complaints and who do not have any autoimmune symptoms will constitute the control group.

Clinical Examination:

1. Systemic and dental histories will be obtained for all patients included in the study.

2. Oral mucosal and dental examinations will be performed for all patients included in the study. The location and type of lichen planus lesions, the type and condition of the dentures (if any), and the type and distribution of dental materials will be assessed and recorded on the "Patient History and Evaluation Form."

Sample Collection

1. Three samples will be collected from patients with OLP: Two from oral lichen planus lesions (OLP) located on the buccal mucosa and one from unaffected oral mucosa (vestibular sulcus or labial commissure) (H-OLP).

2. Two samples will be collected from healthy patients: Two from bilateral healthy buccal mucosa (H).

Participants will be advised to brush their teeth and avoid food intake for at least one hour before sampling.

A 50 mg tissue sample will be collected from the patients using a cytobrash and transferred to 2.0 ml centrifuge tubes. The tubes will be stored at -80°C for DNA extraction.

DNA Isolation

DNA extraction will be performed using the QIAmp DNA Mini Kit (Qiagen) using the QIACube Lt automated isolation device, according to the manufacturer's protocol. A 50 mg tissue scraping sample will be homogenized in 200 µL of 0.9% NaCl before adding proteinase K and lysis buffer, following the Purelink protocol. Isolated DNA will be measured at A260 absorbance using a Nanospectrophotometer (QIAxpert). If DNA amounts exceed 30 ng/µl, samples will be submitted for microbiota analysis.

Microbiota analysis

Targeted amplification from total genomic DNA will be performed using metabarcoding library construction using specific rDNA primers, equimolar pooling, MiSeq high-throughput sequencing (Illumina, San Diego, CA, USA), and multiplexing of paired-end reads (2 × 300 bp).

Paired-end sequences will be clustered using the swarm clustering method, with a maximum of 3 allowed differences between amplicons (d = 3). All clusters will be filtered for chimeras using VSEARCH. Operational Taxonomic Units (OTUs) with an abundance of less than 0.005% will be discarded because they are more likely to be chimeras from rare clusters. Finally, the affiliation of all bacterial OTUs will be verified using sequences from type material in the EzBioCloud database and the 16S rRNA and ITS databases for BlastN at NCBI. Remaining identified chimeras will be manually filtered, and these improved OTU tables will be used to calculate biostatistics using the PhyloSeq R package v1.28.0 implemented in FROGS. Alpha-diversity metrics (Chao1 and inverse Simpson indices) will be calculated after rarefaction. The abundance-based dissimilarity (beta-diversity) index, the Bray-Curtis index, will also be calculated after rarefaction. Microbiota analysis will be performed as a service outsourced.

ANTICIPATED WORK DURATION: 1 year

Start date: June 15, 2023

Data evaluation period: 9 months

End date: March 15, 2024

Patient Consent Form

Lichen Planus (LP) is a skin disease that can also occur inside the mouth. It can appear anywhere within the mouth, usually symmetrically on the inside of the cheeks, under and around the tongue, on the gums, and on the lips. It is called "Oral Lichen Planus" (OLP). It usually doesn't cause any symptoms, but sometimes a rough surface or burning sensation may be experienced. Although various factors are thought to be contributing factors, the exact cause is unknown. Therefore, various studies and studies are being conducted to diagnose and treat the

disease. The study, titled "METAGENOMIC ANALYSIS OF ORAL LICHEN PLANUS AND RELATED ORAL PATHOGENS," will determine and evaluate the relationship between these factors and the disease. For this purpose, a 50 mg tissue sample will be collected from you using Cytobrash and transferred to 2.0 ml centrifuge tubes.

In this study, samples will be collected from 12 individuals with oral lichen planus and 12 healthy individuals and stored for analysis in a molecular analysis laboratory. This study aims to identify the relationship between oral lichen planus and oral pathogens, develop treatment options, and contribute to similar studies.

Therefore, your CV, contact number, disease-related findings, and a swab sample will be collected from you. These will be used solely for this study, and the information obtained will be kept confidential. You are not responsible for any fees for this study. If you cease to be eligible for the study or voluntarily withdraw, there will be no interruption in your treatment at our clinic.

I have been informed of these matters verbally or in writing. I have provided this form with the information provided. I have had the time and opportunity to ask relevant questions, and all my questions have been answered. I have read this form in its entirety. Under these conditions, I agree to participate in the clinical trial titled "METAGENOMIC ANALYSIS OF ORAL LICHEN PLANUS AND RELATED ORAL PATHOGENS" voluntarily, without any pressure or coercion. I also confirm the accuracy of all information I have provided about myself, including my medical history.

Volunteer's

Name-Surname Signature

Address: Tel:

Fax:

Date:

_____

Institutional official who witnessed the consent process from the beginning

Name-Surname Signature

Position: Tel:

Fax:

Date:

Researcher making the statement

Name-Surname: Signature

Date:

Dear Prof. Dr. Filiz NAMDAR PEKİNER informed me that a medical study would be conducted at the Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Marmara University, and provided me with the above information regarding this study. Following this information, I was invited to participate in this research as a "participant."

I believe that if I participate in this research, the confidentiality of my information, which must remain between me and the doctor, will be treated with the utmost care and respect during this research. I have been given sufficient confidence that my personal information will be meticulously protected during the use of the research results for educational and scientific purposes.

I may withdraw from the research without giving any reason during the project's implementation. However, I am aware that it would be appropriate to notify the researchers in advance of my withdrawal to avoid inconvenience. I also understand that I may be withdrawn from the research by the researcher to prevent any harm to my medical condition. I assume no financial responsibility for research expenses. I will not be reimbursed. I have been given the necessary assurance that any health problems I may experience, whether directly or indirectly, arising from the research will be treated with all necessary medical attention. I know that I will not be financially burdened with these medical interventions.

I know that if I encounter a health problem during the research, I can contact Prof. Dr. Filiz NAMDAR PEKİNER at any time. I am not obligated to participate in this research and may choose not to participate. I have not been subjected to any coercive behavior to force me to participate. I also know that if I decline to participate, this will not compromise my medical care or my relationship with the physician.

I will detail all information provided to me.