Salivary and serum leptin levels in oral lichen planus patients : A Case-Control Study.

مستوى اللبتين في لعاب ومصل الدم في مرضى الحزاز المسطح الفموي
دراسة الحالات والشواهد.

Protocol submitted to faculty of dentistry, Cairo University for partial fulfilment of the requirements for the Master's Degree in oral medicine

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Date: 18-7-2023

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Evidence based committe (Reviewers)

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2.		

Research plan committe

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I. Administrive information:

1. Title:

Salivary and serum leptin levels in oral lichen planus patients : A Case-Control study.

-Research question :

Do levels of salivary and serum leptin differ between oral lichen planus patients and healthy control subjects ?

(Primary outcome is serum leptin levels)

2. Protocol registration:

The study protocol; will be registered on www. clinicaltrials.gov upon approval by the ethics committee of Faculty of Dentistry, Cairo University.

3. Protocol version:

18 July 2023, protocol number: 1

4. Funding:

Self-funding

5. Roles and responsibilities:

Investigator: Bahaa Mahmoud Fawzy El Nomrosy, B.D.S 2015 Misr university for science and technology.

The principal investigator, responsible for funding and undergoing the clinical and theoretical work of the trial, collecting data, clarification and conclusions.

Prof Weam Rashwan, Professor of Oral medicine and Periodontology Department, Faculty of Dentistry, Cairo University.

Role: Main supervisor
Responsibilities: initiated the study design. She will afford guidance through the clinical work, revising the theoretical part and help with the conclusions and completion of the data.

Professor Olfat Gamil Shaker, Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University.

Role: co-supervisor

Responsibilities: Assessment of leptin expression profiles in saliva and serum and interpretation of the data.

II. Introduction:

6a. Scientific background

One of the most prevalent muco-cutaneous chronic autoimmune illnesses is oral lichen planus. It has an unknown cause, however factors like systemic illness, psychological illness, dental work, and medication can increase your risk.

Leptin is a hormone that helps with weight loss and also has a role in the body's response to fasting. Leptin's effects on the immunological system, the neuroendocrine system, and development are all worth noting. Infections, endotoxins, and cytokines including tumour necrosis factor (TNF) and interleukin-1 (IL-1) all play a role in triggering this response.

6b.

One of the most prevalent muco-cutaneous chronic illnesses is oral lichen planus. There is no doubt that this is an autoimmune disorder. Despite an unknown cause, several elements, such as systemic conditions, psychogenic disorders, dental restorations, and certain medicines, should be taken into account.

Oral lesions are typically found on both sides of the mouth, most frequently in the inner buccal mucosa. It manifests in three distinct clinical presentations: reticular, atrophic, and bullous erosive.

Because of the high risk of malignant progression, the condition is classified as premalignant. Cytoid bodies and T-lymphocyte infiltration into the epithelial basal cell layer are histopathological hallmarks of the disease.

Leptin is a hormone secreted by adipocytes that stimulates autoimmune reactions. LP is linked to dyslipidemia. therefore, this research was carried out to evaluate the lipid profile and serum leptin levels in LP patients. White adipose tissue secretes the polypeptide hormone leptin.

Multiple studies have shown that elevated leptin levels correlate with increased body fat and increased body mass index. Additionally, it induces autoimmunity and participates in cellular immune response.

Leptin is thought to play a role in the pathogenesis of psoriasis due to its ability to stimulate the production of cytokines and regulate helper T cells. Beyond psoriasis, the evidence on the role of leptin in dermatological disorders is scant. It is possible that it contributes to the pathophysiology of LP.

However, there is scant information available on how much leptin is present in LP. LP is also linked to dyslipidemia. Many studies have found that LP patients have considerably different lipid profile levels compared to healthy control groups, leading researchers to believe that chronic inflammation and dyslipidemia contribute to an elevated risk of cardiovascular disease.

whether or not leptin has an effect on LP progression. The purpose of this research was to examine the relationship between blood leptin levels and LP severity in newly diagnosed individuals. We also compared leptin levels, lipid profile, and sickness duration to draw conclusions.

6c.

Assessment of salivary and serum leptin levels in oral lichen patients

III. Methods:

A) Study design and settings

7. Study design

Case-control study
The patients are divided into two groups

Case group: diseased oral lichen planus 39 patients

Control group: healthy patients 39 healthy controls

PCO:

Population: Oral Lichen Planus patients.

Control: Healthy patients.

Outcomes: Salivary and serum leptin levels.

8. Settings

All patients will be selected from the "out-patient clinic" of Oral Medicine – Oral Diagnosis and Periodontology Department; Faculty of Dentistry, Cairo University.
All controls will be selected from the "out-patient clinic" of any clinical department other than Oral Medicine; Faculty of Dentistry, Cairo University.

B) Participants

9. Eligibility criteria and selection methods

Inclusion criteria:

- Ages for both sexes fall between 30 and 70.
- Symptomatic OLP has been diagnosed clinically and verified histologically.
- Participants who sign a written consent form after being fully informed about the study

Exclusion citeria:

- Treatment with a systemic or locally administered systemic medication within the previous three months before the commencement of the research.

- Patients now taking or who have just stopped taking an NSAID (both steroidal and non-steroidal) for pain or inflammation.
- Patients who have been diagnosed with a malignant tumor or tumors.
- Women who are expecting or nursing.
- Inmates, the mentally ill, the elderly, etc., all fall into this category.

rationale for choosing cases and controls for case control studies

All patients will undergo a thorough history and physical examination, and an incisional biopsy of the oral lesion will be taken for histological analysis to confirm a diagnosis of Oral Lichen Planus.

In order to make a fair comparison of salivary and serum leptin levels between cases and controls, we will select individuals who do not have any systemic or oral lesions.

10. Matching criteria and allocation ratio

Matching will be according to Age and Gender

The ratio between cases to controls is 1:1

C) Variables

11. Details about variables

Outcomes: Salivary and serum leptin levels.

Potential confounders: Age and Gender, Type of OLP

Diagnostic criteria: presence of Oral Lichen Planus form(s) (Reticular, Atrophic or Bullous-Erosive forms)

12. Data sources and management

Salivary and serum leptin levels Measurement.

-For patients in Group I, we will collect a saliva sample. Saliva and serum samples from participants in Group II (healthy controls) will be frozen at -80 degrees Celsius until ELISA testing can be performed.

-Saliva will be collected from each individual after a brief water rinsing and placed on ice in a Falcon tube of the 50 ml capacity. It will take about 5-10 minutes to gather 400 l of unstimulated entire saliva.

- Centrifugation removes insoluble components (bacteria, cellular debris, and glycoprotein aggregates) from a saliva specimen that was snap frozen and packaged in ice or liquid nitrogen immediately after collection.

-Phlebotomists should collect blood samples to ensure participants are comfortable and the sample is of sufficient quality and quantity. Clear spoken and/or written instructions, including information regarding fasting and medication avoidance as appropriate for the intended analyses, should be provided to the study participants. The gold standard for blood donations.

- Under strict hygienic conditions, a venous blood sample of 6 mL is taken from the patient after a fast of 12 hours in a plain red-capped evacuated blood collection tube. Within an hour of sample collection, they will be analysed. After the blood has coagulate, it will be centrifuged at 2000 rpm for 10 minutes to separate the serum. On the same day, regular tests will be performed, and the remaining serum will be frozen at -200 degrees Celsius for serum leptin quantification at a later date.

-The Leptin concentration in the blood will be measured using a commercially available enzyme immunoassay (ELISA) kit from DRG.

-To facilitate data collection and sample identification, we will assign a unique identifier (ID) number to each participant. The main investigator will record all participant information on a standard Excel spreadsheet. The superiors will conduct a secondary inspection.

13. Addressing potential sources of bias

Random selection of cases and controls, all personal and clinical data will be confidential, exposed only on request

D) Study size:

A power analysis was created to have enough strength to use a two-sided statistical test of the null hypothesis that there is no difference between tested groups regarding salivary and serum leptin levels. By adopting an alpha (α) level of 0.05 (5%), a beta (β) level of 0.2 (i.e. power=80%), and an effect size (d) of (0.736) calculated based on the results of a previous study.[1]; the predicted total sample size (n) was found to be (62) cases (i.e. 31 cases per groups). Sample size was increased by (25%) to compensate for possible drop-out during different follow up intervals to be a total of (78) cases (i.e. 39 cases per group). Sample size calculation was performed using G*Power version 3.1.9.7[2]

14. Study size

Cases: 39… patients

Controls: 39…. Subjects,

E) Quantitative variables

15. Handling of quantitative variables in the analysis

Variables: Oral Lichen Planus disease, types and duration

Potential confounders: Age and Gender, personal habits

Patients will be grouped according to the disease and Outcomes. Potential confounders will be taken into consideration.

F) Statistical methods

16. Statistical methods

Categorical data will be analysed using the chi square test and given as frequency (n) and percentage (%). By examining the distribution of the data, computing the mean and median values, and applying the Kolmogorov-Smirnov and Shapiro-Wilk tests, numerical data will be examined for normality. If the data were found to be normal, the mean and standard deviation values would be reported, and an independent t-test would be used to compare the groups. The data will be given as median and range values if the assumption of normality is broken, and the Mann-Whitney U test and Freidman's test will be used to compare the groups. For all tests, the significance threshold will be set at P 0.05. With IBM® SPSS® Statistics Version 25 for Windows, statistical analysis will be carried out.

IV. Ethics and dissemination:

17a. Research ethics approval

The protocol will be proposed to the ethics committee of faculty of dentistry, Cairo University

17b. Protocol amendments

No amendments are planned; however, if any amendment was formed, it will be published as amendments to the pre-published protocol

17c. Informed consent

All participants in the study will be asked to provide an informed signed consent after full understanding of the study procedure and before allocation in the study.

17d. Confidentiality

To facilitate data collection and sample labelling, we will assign a unique identifier to each participant. Only the ethics committee will be able to see your personal information.

17e. Declaration of interest

No conflicts of interests to be declared

17f. Access to data

Study supervisors and principal investigators will have access to all data of the study. The data will be made available to the ethics committee when asked.

17g. Dissemenation policy

When the study is finished and analysis performed, the study details will be published in full report.

V. Appendices

18. Informed consent

A model of the informed consent will be available in the protocol appendix.

VI- Statement of originality:

Oral lichen planus patients' serum leptin levels have already been evaluated, but to the best of our knowledge, no experiments have been conducted on their salivary leptin levels. To learn more about leptin and other hormones involved in Oral Lichen Planus, we conducted the first study to measure leptin levels in the saliva of patients with oral lichen.

VII- References:

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3. **Nosratzehi, T.** Oral Lichen Planus: Overview of Potential Risk Factors, Biomarkers and Treatments. **Asian Pac J Cancer Prev**, 19, n. 5, p. 1161-1167, May 26 2018.

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