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Assessment of Salivary Sialic Acid in Patients with Oral Lichen Planus and Healthy Individuals

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Abstract: ***Background: Oral Lichen planus (OLP) is a T-cell mediated chronic inflammatory oral mucosal disease of unknown etiology. Sialic acid is a diverse family of sugar units with a ninecarbon backbone that are typically found attached to the outermost ends of these chains. In inflammatory skin diseases, the infiltration of skin by lymphocytes is mediated via recognition of sialic –acid containing molecules that act as ligands for the selectins the subsequent recruitment of innate immune cells into the area of inflammation is also mediated by the selectins. Purpose: To study salivary sialic acid as anti-inflammatory in OLP patients and healthy subjects. Methods: Twenty five patients with OLP were enrolled in this study. Age, gender, occupation, smoking status (smokers or non-smokers), lesion types, duration, location and size were recorded for each patient. After an oral examination, salivary samples were collected and flow rates (ml/min) were recorded. The collected samples were centrifuged at 3000 rpm for 10 minutes; the clear supernatants were separated and stored frozen at (-20 c) until analysis. Then salivary sialic acid was analyzed using thiobarbituric acid reagent(Warren method). Results: Twenty -five patients were with OLP (14 were females and 11 were males).The mean age was 48.3 years with an age range of 30-60 years. The control group consisted of 35 healthy subjects (20 were females and 15 were males) with an age range of 38-60 years and a mean of 48.2 years. Regarding salivary sialic acid, the present study showed a significant difference between control and OLP patients, OLP patients showed higher salivary sialic acid levels(85.34 mg/dl compared to control group(60.36 mg/dl) p value‹0.01..***

Keywords: OLP, salivary sialic acid

1. Introduction

Sialic acid is a diverse family of sugar units with a nine carbon backbone that are typically found attached to the outermost ends of these chains. Given their location and distribution, sialic acids can mediate or modulate a wide variety of physiological and pathological processes. Changes in sialic acid expression are seen in many physiological and pathological states. These changes can be detected in histological sections by using plant lectins or antibodies to detect specific sialylated glycans (1;2).

In inflammatory skin diseases, the infiltration of skin by lymphocytes is mediated via recognition of sialic –acid containing molecules that act as a ligands for the selectins The subsequent recruitment of innate immune cells into the area of inflammation is also mediated by the selectins (3).

2. Subjects, materials and methods

Materials and methods

Participants

Sixty subjects were participated in this study; twenty-five oral lichen planus patients who were diagnosed clinically and histopathologic ally as an oral lichen planus, and thirty-five healthy looking volunteers as a control group who were age matched with OLP patients. The study was approved by the Ethics Committee of Oral Diagnosis Department in the College of Dentistry –University of Baghdad.

Method of saliva collection:

Salivary samples collection and histopatholgical study was done in Imam Hussain Medical City/ Kerbala Dermatology Department during the period from January to May 2017.

Saliva collection was started after the clinical examination. Saliva was collected from all participants under the same circumstances (20). Saliva was allowed to accumulate in the mouth and to expectorate all saliva formed over five minutes period into a sterile graduated test tube. The saliva samples of all the participants were identified by a code number during the period of s collection and processing. After the disappearance of the salivary froth, the salivary flow rate was measured in millilitres per minutes. Samples were stored at - 20°C, until analyzed.

Samples collection was limited to the hours between 8:00 and 11:00 AM to minimize the effect of diurnal variations.
Sialic acid was measured using thiobarbituric acid reagent(Warren method). A description about the purpose and aim of the study was performed for participants.

The study was carried out using a structured case sheet; the first part was related to the demography regarding name, age, gender, occupation and marital statutes. The second part involved clinical oral examination that carried out under natural light using disposable plane mouth mirrors.

Oral examination was performed by the same examiner.

Inclusion criteria of the current study include patients who were clinically and histologically diagnosed as an OLP

Exclusion criteria were patients having any systemic treatment suppressing the immune system such as systemic

steroids or other immunosuppressive agents, as well as NSAIDs, antimalarial, diuretics, antihypertensive, antibiotics, and antifungals for the last 4 weeks and topical medications for last 3 weeks prior to sample collection. Also, patients with a history of trauma or any surgery 4 weeks prior to sampling, and those who were suffering from any systemic or dermatological disease affecting the immune system or any malignancy.

Furthermore, smoker patients were also excluded from this study.

Statistical analysis
Statistical analysis was performed with SPSS version 19.0. Descriptive statistical analysis, student T-test, analysis of variance (ANOVA) and linear and multiple linear correlation were used. A p-value of less than 0.01 was considered to indicate statistical significance

3. Results

Age and Gender:
Twenty -five patients were with OLP (14 were females and 11 were males).The mean age was 48.3 years with an age range of 30-60 years.

The control group consisted of 35 healthy subjects (20 were females and 15 were males) with an age range of 38-60 years and a mean of 48.2 years.

Occupation:
The majority of OLP patients were workers (18; 72%) followed by house wives (4; 16%) and officers 3(12%). Similarly, in control group subjects, 26(74%) were workers, 6(17%) were house wives, and 3(8%) were officers.

Oral lichen planus findings:
Oral lichen planus patients were divided into two subgroups according to the clinical presentation of the lesions at first presentation, 14 patients were with reticular form (56%) and 11 patients were with erosive form of OLP (44%).

Location:
The present study showed that buccal mucosa was the most common affected site (88%), followed by tongue (8%) then gingiva (4%).

In reticular form, buccal mucosa represented 86 % of the affected site followed by tongue 14%. While in erosive form of OLP, buccal mucosa represented 91% of the affected sites followed by gingiva 9%.

Size
In this study, the size of the OLP lesion was divided into three categories: 1.5, 2.5 and 3.3 cm. The majority of the reticular type of OLP was 1.5 cm in diameter, followed by 2.5 cm. In relation to the erosive type, the majority of OLP lesions were 1.5 cm followed by 2.5 cm in diameter.

Salivary flow rate (F/R):
Regarding salivary F/R, the present study showed a significant difference between the control and OLP patients ($p<0.001$); salivary F/R is significantly lower in OLP patients (0.04±0.06) compared to the control (0.05±0.02) ($p<0.001$)

In relation to the subgroups, there is no significant difference between reticular and erosive type of OLP in salivary flow rate.

Oral health status
Oral health status was divided into three scores: fair, moderate and good. The majority of patients with reticular form of OLP were seen with fair oral hygiene 6(43%) followed by 4(28.5%) moderate with similar number with good hygiene status. In relation to erosive form of OLP, 6 (43%) were observed with a fair oral hygiene, 3 (27 %) with moderate, and 2 (18 %) were seen with a good oral hygiene status.

Salivary sialic acid
Regarding salivary sialic acid, the present study showed a significant difference between control and OLP patients, OLP patients showed higher salivary sialic acid levels(85.34 mg/dl compared to control group(60.36 mg/dl) p value ‹0.01 Table 1 and figure 1.

Table 1: Mean and salivary sialic acid of OLP patients and control subjects

Study groups	Number	Mean mg/dL	Std.Deviation	Std.Error	p value
Control	35	60.36	0.83	0.14	0.0001
Patients	25	85.33	1.41	0.28	

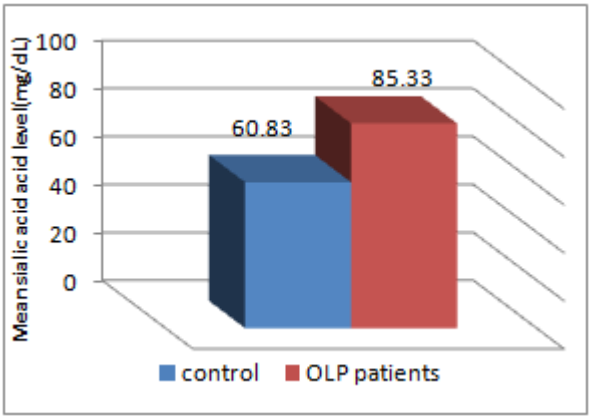

Figure 1: Mean salivary sialic acid level of control group and OLP patients

Sialic acid and age:
When we asses correlation between sialic acid and age, we found that it was non-significant, p=0.386.

Sialic acid and gender:
Regarding gender, no difference was found between male (60.48) and female (60.20) in salivary sialic acid p=0.968.

4. Discussion

Sialic acids(Sias) are unusual sugars with a shared nine-carbon backbone that are widely expressed on the surfaces of all cells in all animals of the deuterostome lineage (vertebrates and so-called "higher" intervertebrate), and also

in certain pathogenic or symbiotic bacteria that associate with them(4-10).

Since Blix isolated Sialic acid from bovine submaxillary gland mucin(BSM) in 1936(11), a great deal of effort has been directed toward elucidating the biological functions of these sugars, especially N-acetylneumaric acid(NANA). NANA occupies the terminal position of many naturally occurring glyconjugates including glycoproteins and glycolipids. Most studies on NANA have focused primerly on cell protection, fertilization, cell differentiation, cell adhesion, immunology, inflammation and tumors (12;13,14;15).

Sialic acid level in saliva is found to be significantly increased in OLP patients compared with healthy subjects, which agreed with previous studies(16).

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