



Complements C3 and C4 in serum and stimulated saliva of patients suffer oral erosive lichen planus

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ABSTRACT

Introduction: The objective of this study was to compare the level of complements C3 and C4 in serum, and stimulated saliva between oral lichen planus (OLP) and healthy individuals.

Methods: A case-control study was performed on 31 healthy and 31 who suffer the erosive type of OLP. Serum and saliva level of C3 and C4 were measured by immunoturbidimetry method.

Results: C3 and C4 were expressed at a lower level in serum and saliva of OLP patients compared to control groups. Serum C3 and C4 levels did not correlate with their saliva levels. The receiver operating characteristic analysis showed significantly diagnostic abilities for serum and saliva C3 and C4 to discrimination of OLP patients from controls (cutoff [mg/dl] for C3 were 83 in serum and 3.45 in saliva and for C4 were 9.5 in serum and 0.9 in saliva).

Conclusion: Serum and salivary levels of total C3 and C4 were lower in patients with OLP than in healthy controls. Therefore, they may able to discriminate OLP from healthy.

Keywords:

Complement C3
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Oral lichen planus
Saliva
Serum

Introduction

Oral lichen planus (OLP) is a chronic, inflammatory mucosal disease (Mittal et al., 2012). Its incidence is about 0.1% to 4% of the adult people (Li et al., 2020). There are several types of OLP and second most common of it, is the erosive form (Mollaoglu, 2000). OLP can be caused or triggered by a genetic malfunction or environmental factors. Although the cause remains unknown, the immunological system plays a substantial role. Cellular immune system dysfunctions play a key role in the onset and perpetuation of the OLP. The inflammatory infiltrate in OLP is principally made up of lymphocytes and a smaller number of macrophages. T lymphocytes predominate over B lymphocytes, and T helper

cells are more frequent than T cytotoxic/suppressor cells (Rodríguez-Núñez et al., 2001). Complement proteins are kinds of blood immune system proteins that are complicated in inflammation and the host defense system. These proteins have been shown to perform an important function in activating mast cells and macrophages. The complement system comprises of nine major proteins, the most important of them is C3, the most abundant complement in the plasma (Janssen et al., 2005). It plays an important role in the course of opsonization. C4 is another component in the system that activates C3, resulting in its lysis by pore formation on the target cell membrane (Afshar-Kharghan, 2017).

Studies have shown that deficiencies in complement

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lead to various autoimmune diseases. For example, there is a significant association between C4 deficiency and systemic lupus erythematosus. Also, C3 was involved in the basement membrane of lichen planus (Abbas et al., 2007). Most studies have shown that C3 and C4 levels are lower than normal in the serum of patients with OLP (Sun et al., 1986; Luo et al., 2015; Huang et al., 2016). Often, the clinical appearance of OLP mimics other kinds of vesiculobullous diseases; therefore, a biopsy of the lesion is required for definitive diagnosis (Cheng et al., 2016). Recently, it was revealed that the deposition of C3 in granular and linear patterns is one of the most common manifestations of OLP biopsies. Thus, the presence of C3 and C4 may be used for diagnosis of OLP (Buajeeb et al., 2015).

Saliva, like other body fluids (such as serum), can be used to diagnose and evaluate disease progression. Saliva is a type of biological fluid that is readily available and noninvasively, without stress can be collected (Agha-Hosseini et al., 2011a; Agha-Hosseini et al., 2011b; Agha-Hosseini et al., 2012; Agha-Hosseini et al., 2015). Saliva can be considered in the rapid diagnosis of a wide range of diseases (Buajeeb et al., 2015; Mirzaii-Dizgah and Agha-Hosseini, 2011; Mirzaii-Dizgah and Riahi, 2013; Mominzadeh et al., 2014; Kaczor-Urbanowicz et al., 2017). Therefore, the investigation of salivary concentrations has been the focus of researchers that can help in the diagnosis or evaluation of disease progression (Lawrence, 2002; Mirzaii et al., 2020). The objective of this study was to compare the level of complements C3 and C4 in serum and stimulated saliva between OLP and healthy individuals.

Material and methods

Participants

This study was performed on 31 individuals with erosive OLP (aged: 46.8 ± 10.5 ; male/female: 11/20) and 31 healthy individuals (aged: 45.3 ± 7.54 male/female: 8/23) referring to the Department of Oral & Maxillofacial Medicine. All patients provided written informed consent and the study protocol was approved by the Ethical Committees of Aja University of Medical Sciences (approval No. IR.AJAUMS.REC.1398.254) and was conducted according to the principles expressed in the Declaration

of Helsinki.

Inclusion criteria

The diagnosis of erosive OLP was confirmed by clinical and histopathological examination according to the modified World Health Organization diagnostic criteria for OLP (van der Meij and van der Waal, 2003). All patients had to be symptomatic and suffering from some degree of pain. Biopsies were performed in all cases of OLP.

Exclusion criteria

In both the case and the control groups, the exclusion criteria were lichenoid reactions to medications, pregnancy, nursing and any local or systemic disease like diabetes, hypertension, cardiovascular disease, kidney disease and so on. Also, people who had similar lesions adjacent to amalgam restorations, cigarette smokers, alcohol consumers, individuals with a history of radiotherapy and patients display dysplastic changes in biopsy were excluded.

Serum and saliva sampling

All subjects were on a regular diet and received no treatment prior to admission and serum as well as whole saliva samples were collected at their initial visit. Saliva and serum samples were taken between 10-12am. To collect stimulated saliva, patients were asked to chew equal pieces of natural gum for 1 minute and then spit into a vial. After saliva collection, 2ml of venous blood was collected from all participants and poured into the gel clot tube. The samples were centrifuged at 3000rpm for 10min. The supernatant of saliva and serum were stored at -70°C .

C3 and C4 assay

C3 and C4 levels were measured by immunoturbidimetry (ParsAzmoon, Karaj, Iran) according to the manufacturer's instructions.

Statistical analysis

Assessment of differences amongst groups was performed with an unpaired two-tailed student's t-test or Mann Whitney test. The Spearman correlation test was used to confirm the association between the parameters. Receiver operating characteristic (ROC) analysis was used to detect cut-off point for salivary C3 and C4

TABLE 1: Complements C3 and C4 levels in serum and saliva of patients suffer from oral lichen planus (OLP)

	Healthy individuals (n=31)	OLP patients (n=31)	P-value
Serum C3 (mg/dl)	115.5.4±7.543.0 100.8±)	72.9 ± 7.5 (64.1±74.4)	0.001 (0.002)
Stimulated saliva C3 (mg/dl)	4.1±0.4 (3.3±3.9)	2.6±0.2 (2.7±1.7)	0.009 (0.032)
Serum C4 (mg/dl)	12.0±1.3 (12.1 ±14.7)	6.5 ±0.8 (5.4 ± 6.4)	0.002 (0.007)
Stimulated saliva C4 (mg/dl)	2.3 ± 0.4 (1.2 ± 2.2)	1.2±0.3 (0.6 ±2.2)	0.004 (0.001)
Stimulated saliva flow rate (ml/min)	0.89±0.08 (0.83 ± 0.69)	0.55±0.06 (0.50 ±0.36)	0.006 (0.01)

Data are expressed as mean±SE (median±IQR) and analysed by unpaired student's t-test (Mann Whitney). *P*-value<0.05 considered as significant.

TABLE 2: Receiver operating characteristic curve analysis of C3 and C4.

	Cutoff (mg/dl)	Sensitivity%	Specificity%	Area	P-value
Serum C3	83.0	81	60	0.750	0.002
Stimulated saliva C3	3.45	69	80	0.717	0.008
Serum C4	9.5	70	77	0.778	0.001
Stimulated saliva C4	0.90	81	57	0.729	0.006

between OLP and healthy groups. Analysis was done using SPSS software version 16.

Results

This case-control study assessed 31 OLP as case group and 31 healthy as control. There was no significant difference in age and sex between the groups. The areas that were affected in OLP patients were buccal mucosa and vestibule (n=18), tongue (n=7) and gingiva (n=6). Serum and saliva levels of C3 and C4 did not have a normal distribution. The median serum C3 and C4 concentrations were lower in OLP than that of the healthy group (Table 1). Also, stimulated salivary concentrations of C3 and C4 were lower in OLP than the control group (Table 1). Stimulated salivary flow rate had a normal distribution and it was lower in OLP than the control group (Table 1).

The serum level of C3 was not significantly correlated with stimulated saliva C3 levels ($r=0.067$; $P=0.756$). Also, the serum level of C4 was not significantly correlated with stimulated saliva C4 levels ($r=0.270$; $P=0.080$). The ROC analysis results revealed that the evaluation of C3 in stimulated saliva (AUC [area under curve]= 0.717, $P=0.008$) and serum (AUC= 0.750, $P=0.001$) samples and also C4 in stimulated saliva (AU = 0.729, $P=0.006$) and serum (AUC= 0.778, $P=0.001$) could be used for determining OLP patients from control (Table 2).

Discussion

The complement system is an important part of the innate immune system and involves in various autoimmune diseases, which helps antibodies and phagocytic cells in eliminating pathogens from the body. Complement C3 is the most abundant complement protein in serum (Janssen et al., 2005). The specific embellishment of C3 deposition at the basement membrane is a prominent feature in the detection of OLP (Buajeeb et al., 2015). Earlier studies do not offer a definitive and convincing description of innate immunity in the course and pathogenesis of OLP (Popovska et al., 2015). Any other way, studies of serum complement have led to disagreements and conflict (Van der Waal, 2009). Due to these inconsistencies, it is considered that there is no adequate information on whether OLP is influenced by changes in the innate immune response. We examined the serum and saliva levels of C3 and C4 in erosive OLP and healthy subjects.

Our results showed that the serum level of C3 is lower in OLP patients than in healthy subjects. It is in agreement with Luo et al. (2015) report. However, it is in opposing one study (Popovska et al., 2015) which indicated that serum C3 value increases in OLP patients. Also, some studies implicated that serum C3 is in normal ranges in OLP (Rodríguez-Núñez et al., 2001; Talungchit et al., 2018). Other studies showed that the serum level of C3 does not differ between OLP and normal individuals (Sklavounou et al., 1983; Sklavounou et al., 1983; Sun et al., 1986; Gandolfo et al., 1994).

Serum C4 level was significantly low in OLP. It is not supported by studies that have been implicated serum C4 is not differ between OLP and healthy groups (Sklavounou et al., 1983; Gandolfo et al., 1994; Rodríguez-Núñez et al., 2001; Talungchit et al., 2018). However, some studies supported our finding (Sun et al., 1986; Luo et al., 2015; Huang et al., 2016). Stimulated saliva C3 and C4 concentrations were low in OLP patients than healthy individuals. The best of our knowledge, there was no report about stimulated saliva C3 and C4 in OLP. It has been shown that C3 and C4 present in the basement membrane and colloidal bodies in OLP (Gupta and Jawanda, 2015). It has also been pointed out C3 and C4 are consumed in or around the colloid bodies of lichen planus (de la Faille-Kuyper and de la Faille, 1974). Complements C3 and C4 are low in serum of autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis because of consumption (Lintner et al., 2016; Trouw et al., 2017). Therefore, it seems that the lower concentration of C3 and C4 in serum and saliva of patients in this study can be explained by increased consumption of C3 and C4.

Hepatocytes are the main source of most serum complements. However, complements such as C3 and C4 have been shown to be produced in many tissues, especially salivary gland epithelial cells and participate in local immune responses. The complement system has been shown to be active in saliva, indicating that complement activation participates in local immune and inflammatory responses in the oral cavity (Andoh et al., 1997). These may explain the lack of correlation between serum and saliva of C3 and C4. ROC analysis showed that the area under the C3 and C4 curves were 0.75 and 0.778 in serum and 0.717 and 0.729 in saliva, respectively. The best clinical cut-off level of C3 were 83.0mg/dl in serum and 3.45mg/dl in saliva. The best clinical cut-off level of C4 were 9.5mg/dl in serum and 0.9mg/dl in saliva. Thus, low complement levels have diagnostic value for OLP. It assumes that C3 and C4 may involve in the pathogenesis of erosive OLP. Our findings also suggest that salivary and serum C3 and C4 may be a useful marker for OLP.

OLP more commonly affected buccal mucosa in this study which is in agreement with previous studies (Chiang et al., 2018). The saliva flow rate was lower in erosive OLP patients than healthy individuals which is in accordance with other studies (Agha-Hosseini

et al., 2017; Agha-Hosseini et al., 2018). It indicates that OLP patients may suffer from dry mouth feeling. Since the erosive form of OLP shows usually dysplastic transformation and needs medication, so, these patients refer to the clinicians more than other forms of OLP. Therefore, we studied only the erosive form of OLP due to the time limitation. It is one of the limitations of this study that other forms of OLP were not studied. The second limitation of our study was the low number of patients and healthy individuals to calculate the valuable cut-off point for detecting patients from healthy individuals.

Conclusion

Serum and salivary levels of total C3 and C4 were lower in patients with OLP than in healthy controls. Therefore, they may able to discriminate OLP from healthy.

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Conflict of interest

The authors declare that they have no conflict of interests.

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