REVIEW PAPER

Relationship of oral candidiasis with salivary lysozyme and lactoferrin in HIV-positive patients: a systematic review

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Abstract

Oral candidiasis is one of the main consequences of human immunodeficiency virus (HIV) infection. Saliva contains proteins that play a key role in the health of oral cavity. The aim of this review study was to investigate the relationship of oral candidiasis with salivary lysozyme and lactoferrin in patients with HIV infection. In this systematic review study, all articles with English abstract were searched with the keywords of "Oral Candidiasis" or "Candida" and "Saliva" and "HIV" or "AIDS" and "Lysozyme" and "Lactoferrin" from the Google Scholar, PubMed, Web of Science, Cochrane, and Scopus databases from 1990 until April 2020. Amongst the 16 articles obtained after reviewing the abstracts, 13 appropriate articles were included in this study. In 11 studies, the relationship between salivary lactoferrin and oral candidiasis was investigated, 54% of which showed an increase in salivary lactoferrin in HIV-positive patients with candidiasis. Eight studies examined the relationship between salivary lysozyme and oral candidiasis, 63% of which showed no association. Understanding numerous factors and conditions involved in candida cloning would be broadly related to increasing our understanding of fungal pathogenesis and host defense factors. The results of our study could be useful in diagnosing and designing new strategies for the prevention and treatment of fungal infections in HIV-positive patients. In addition to anti-fungal properties, non-toxicity to human cells could lead to lactoferrin being used in the future as a drug to prevent and treat fungal infections in patients with immunity difficulties.

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Introduction

Oral candidiasis is one of the most common fungal infections, which affects the oral mucosa [1]. Ninety percent of patients with acquired immune deficiency syndrome (AIDS) suffer from oropharyngeal candidiasis in many stages of their disease. *Candida albicans* is the major cause of oral candidiasis in patients with human immunodeficiency virus (HIV) and AIDS. With the development of HIV infection, despite the use of antifungal drugs prophylaxis, oral candidiasis becomes a permanent resident of the oral cavity. Extensive lesions of this infection can be painful and cause eating disorders and malnutrition, which is frequent in patients with AIDS [2-4].

Saliva plays an important role in maintaining oral health and prevents mouth candidiasis with two mechanisms. First, saliva is mixed with bacteria and debris, and by swallowing, these organisms are cleared from the oral cavity. Second, saliva contains anti-microbial proteins as lysozyme (murami-

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dase) and lactoferrin, which are considered to regulate candida population in the oral cavity [5, 6]. It should be noted that lysozyme is an enzymatic protein that some of researchers have shown to be anti-fungal in the oral cavity [7, 8]. Lactoferrin is an iron-binding protein, which has proven *in-vitro* anti-fungal properties [9].

Changes in the host's salivary flow rate and composition during HIV infection may alter the host's defense mechanism that affects the candida's adhesion and cloning. Therefore, the increase of oral candidiasis in HIV-positive patients may be related to defects in salivary defense proteins [10].

Numerous studies have examined the association of salivary proteins with oral candidiasis. In one study, Muller *et al.* showed that a decrease in salivary lactoferrin and IgA was associated with an increase in recurrent oral fungal infections in HIV-positive patients [11]. Even though some researchers have reported no significant association, other studies have shown a direct relation between these proteins and candidiasis [12-15]. Giving such inconsistent conclusions in this regard, the aim of this review study was to investigate the oral candidiasis relationship with salivary lysozyme and lactoferrin in patients with HIV infection. Knowing this connection can be useful in diagnosing and designing new treatment strategies for the prevention and treatment of fungal infections in these patients.

Material and methods

Search strategy

This systematic review was conducted based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement for reporting systematic reviews [16].

A focused question was produced according to the participants, intervention, control, and outcomes (PICO) principles [17]. The focused question for this review was "Is there an association between oral candidiasis and salivary lysozyme and lactoferrin in HIV-positive patients?" In this review study, all published articles with English abstract with the medical subject heading (MESH) terms and keywords of "Oral Candidiasis" or "Candida" and "Saliva" and "HIV" or "AIDS" and "Lysozyme" and "Lactoferrin" from Google Scholar, PubMed, Web of Science, Cochrane, and Scopus databases were searched from 1990 until April 2020. In the initial phase, the titles and abstracts of articles were reviewed by two independent individuals, based on inclusion and exclusion criteria. Disagreements were resolved with the third author's discussion. Next, the full text of selected articles was reviewed. The quality of chosen studies was evaluated by the Newcastle-Ottawa scale method [18]. The data of selected articles were extracted using data extraction form. This form included the author's name, year of publication, sample size, and the results of study.

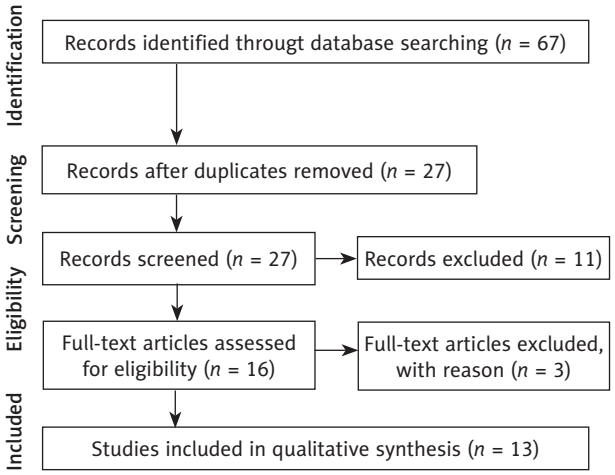

Figure 1. Flowchart of searching strategy based on PRISMA guidelines

Inclusion criteria

Inclusion criteria were the studies, in which levels of salivary lactoferrin or lysozyme in HIV-positive patients were assessed, and its association with oral candidiasis was evaluated.

Exclusion criteria

Reviews and case reports articles as well as in-vitro studies were excluded.

Results

During the initial search, 67 articles were obtained. Amongst 16 articles, after reviewing the abstracts, 13 appropriate articles were included in this study, based on the entry and exit criteria shown in Figure 1.

In 11 studies, the relationship between salivary lactoferrin and oral candidiasis was investigated, 54% of which showed an increase in salivary lactoferrin in HIV-positive patients with candidiasis. In 37% of studies, no association between oral candidiasis and salivary lactoferrin in patients with HIV infection was indicated, and other studies revealed that oral candidiasis was associated with low levels of salivary lactoferrin in HIV-positive patients. Eight studies examined the relationship between salivary lysozyme and oral candidiasis, in which 63% showed no association, and 37% demonstrated an increase in salivary lysozyme in HIV-positive patients with candidiasis (Table 1).

Discussion

The results of this systematic review demonstrated that HIV-positive patients with higher candida counts had higher salivary lysozyme and lactoferrin, but the secretion of these salivary anti-microbial proteins was suppressed in HIV-positive patients, whose CD4+ cell counts were significantly lower.

In patients with HIV infection, oral candidiasis is still known to be one of the most common opportunistic infec-

Table 1. Summary of data extracted from the studies included in this review

Authors [ref]	Year	Study design	Sample size [*n*]	Results
Chandrasekar Lakshmi *et al.* [14]	2016	Case-control study	90 HIV patients, 30 controls	The levels of salivary lysozyme and candida count were high in HIV-positive patients compared to the control group, but there was no significant relationship between them
Ferreira *et al.* [15]	2015	Case-control study	28 HIV patients, 10 controls	Lactoferrin levels were directly related to oral candidiasis
Alves *et al.* [19]	2014	Cross-sectional study	70 HIV patients, 50 controls	HIV-positive patients had higher salivary lactoferrin levels associated with candida cloning
Lourenco *et al.* [12]	2013	Case-control study	69 HIV patients, 40 controls	There was no association between oral candidiasis and salivary lactoferrin in HIV-positive patients
Laibe *et al.* [20]	2005	Case-control study	19 HIV patients, 11 controls	Salivary lactoferrin and lysozyme levels were high in HIV-positive patients with oral candidiasis
Namikoshi *et al.* [21]	2004	Case-control study	30 HIV patients, 50 controls	The secretion of salivary anti-microbial proteins against candidiasis increased in healthy individuals and HIV-positive patients, but was suppressed in HIV-positive patients whose CD4+ counts were significantly lower
Bard *et al.* [22]	2002	Case-control study	19 HIV patients, 11 controls	HIV-positive patients with candidiasis infection had higher salivary lactoferrin levels
Lin *et al.* [23]	2001	Cohort study	18 HIV patients, 34 controls	There was no association between anti-candidiasis activity and salivary lactoferrin and lysozyme
Van Der Strate *et al.* [24]	1999	Case-control study	95 HIV patients, 26 controls	The presence of candidiasis was associated with low levels of salivary lactoferrin
Tsang *et al.* [25]	1999	Cohort study	32 HIV patients, 32 controls	Salivary lysozyme levels were not associated with oral candidiasis
Mandel *et al.* [26]	1992	Case-control study	78 HIV patients, 27 controls	Oral candidiasis was not associated with salivary lactoferrin and lysozyme in patients with HIV infection
Muller *et al.* [11]	1992	Case-control study	44 HIV patients, 19 controls	An increase in candidiasis was associated with a decrease in lactoferrin in HIV-positive patients, but was not associated with salivary lysozyme levels
Atkinson *et al.* [13]	1990	Case-control study	37 HIV patients, 15 controls	Patients with HIV infection with higher candida counts had higher salivary lysozyme and salivary lactoferrin

tions [2, 3]. There are a variety of defense systems against *Candida albicans* in the host body, including specific immune system and non-specific immune system. The innate immune system creates a defense barrier against infections [27]. Lactoferrin and salivary lysozyme play an important role in innate immunity. These salivary proteins are produced by the salivary glands, gingival crevicular fluid, and phagocytic cells [9]. When patients with HIV are divided into three groups based on CD4+ cell count, the lowest levels of these anti-microbial proteins are seen in the third stage; when CD4+ cells are less than 200, this indicate that the innate immunity is suppressed at this stage. 54% and 37% of the studies showed a direct relationship between lactoferrin and salivary lysozyme with oral candidiasis in patients with HIV infection, respectively, and the rest of the studies revealed no association or reverse relationship. Such an inconsistency of the results could be due to the collection of saliva at different stages of the disease, and different methods of its analyzing. According to studies, in the early stages of HIV infection, with a decrease in number of CD4+ cells, the innate immunity of saliva to compensation increases [28]. The accumulation of lymphocytes and granulocytes in response to candidiasis infections in the tissue produces cytokines and stimulates the production of salivary lysozyme and lactoferrin [13, 19]. On the other hand, the release of lactoferrin and lysozyme from cytoplasmic granules of macrophage and neutrophils also occurs [29]. However, increasing lactoferrin sometimes does not prevent candidiasis, which is probably due to the emergence of candidate species that have developed a resistance to lactoferrin and lysozyme, as HIV progresses. With a decrease in number of CD4+ cells below 200 μg/ml, the host immune system is unable to produce enough lactoferrin and lysozyme [21]. Significant reductions in lactoferrin and salivary lysozyme in the AIDS stage are likely due to reduced production of neutrophils and leukocytes. HIV infection reduces the number of leukocytes and decreases the movement of leukocytes into the oral mucosa affected by candidiasis infection. Decreasing CD4+

cells also weakens granulocytes activity, and reduces the release of granules containing immune components [28, 29].

The numerous anti-fungal mechanisms of lactoferrin cause this protein to be used in combination with anti-fungal agents. The use of lactoferrin in combination with fluconazole may be the most effective combination against *Candida* [30-32].

Our study has some limitations. Firstly, the sample size was limited for some groups, and secondly, our search was limited to English articles, which may consider language bias.

Conclusions

Understanding of various factors and conditions involved in candida cloning would be broadly related to increasing our understanding of fungal pathogenesis and host defense factors. The results of our study could be useful in diagnosing and designing new strategies for the prevention and treatment of fungal infections in HIV-positive patients. In addition to anti-fungal properties, non-toxicity to human cells could lead to lactoferrin being used in the future as a drug to prevent and treat fungal infections in patients with immunity difficulties.

Conflict of interest

The authors declare no conflict of interest with respect to the research, authorship, and/or publication of this article.

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