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Potential gingival crevicular fluid and serum biomarkers by stage of HIV infection

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ABSTRACT

Objective: This study evaluates the potential of gingival crevicular fluid and serum cytokines as HIV stage biomarkers.
Methods: Gingival crevicular fluid (GCF) and serum samples from 78 HIV-positive adult male subjects (cases) and 39 HIV-negative male subjects (controls) from Mexico were examined for 17 cytokines using multiplex ELISA. Participants were divided into five subgroups by HIV stage of infection on age-specific CD4+ T-lymphocyte count and antiretroviral therapy (ART), and further correlated to the cytokine levels.
Results: GCF concentrations of IL-6, IL-7, IL-10, IL-12, G-CSF and MCP-1, as well as serum concentrations of IL-1β, IL-2 and IL-6 showed a statistically significant difference among subgroups. We found a significant effect size correlation on cytokines expression levels. Subjects who were not in ART showed significantly higher levels of some of the analyzed cytokines compared to the rest. We found that GCF IL-8 was a significant predictor for the Non-ART HIV status ($p < 0.05$). We observed the same result for GCF G-CSF in the ART Short-term group and serum GM-CSF in the ART Long-term subgroup.
Conclusion: Results indicate a high variability of GCF and serum cytokines concentrations and low frequency of their detection in different HIV/ART stages. However, within the limits of the present study, some GCF and serum cytokine concentrations correlate positively. Oral and periodontal innate immunity is affected by HIV viremia and ART. GCF IL-8, G-CSF, as well as serum IL-8, MCP-1 and GM-CSF may be useful biomarkers for the detection of disease presence and/or its severity due to HIV infection and ART use.

1. Introduction

Viruses and other periodontopathogens are risk factors in many oral and systemic diseases and human immunodeficiency virus (HIV) is not the exception. Co-pathogenesis is characterized by numerous complex interactions between the co-infecting pathogens and their host, inducing disruption of physical barriers, dysregulation of immune responses and arrears to achieve immune homeostasis. HIV targets and infects CD4+ T-cells by binding to cell surface glycoprotein CD4 and then to chemokine coreceptors CCR5 or CXCR4. Infected HIV cells releases newly formed virions in a semi-synchronous wave pattern and are also thought to spread them through direct contact with their neighbors via virological synapses and by pools of free virions [1]. The oral cavity seems to be a potential reservoir of HIV, as its RNA and DNA can be detected and quantified in the subgingival biofilm, gingival crevicular fluid (GCF) and saliva of HIV-infected individuals [2–4]. There-

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fore, the oral epithelial cells are susceptible to either cell-free or cell-associated HIV infection.

The biological and clinical basis of the probable relationship among oral chronic inflammatory disorders, such as periodontal disease and the exacerbation of HIV viremia have received limited attention [5]. Nevertheless, controversial data regarding the association among immunosuppression and prevalence/severity of oral and periodontal diseases in HIV-infected individuals had been reported [6,7].

The cytokines, chemokines, and their receptors have been receiving notable consideration afterward the discoveries that some of them could specifically block HIV infection and that some specific chemokine receptors were the long-sought co-receptors which, along with CD4+ T-cell, are required for the productive entry of HIV [8]. The intricate and complex balance of factors that regulate the HIV-1 pathogenic process is highlighted by both the inhibition and enhancement that cytokines, chemokines and their receptor signaling events elicit on the HIV entry and replication processes.

Extending our prior work, where we examined whether the levels of interleukin (IL)-2, IL-4, IL-6, IL-8, IL-10, granulocyte–macrophage-colony-stimulating factor (GM-CSF), interferon-gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) in GCF were altered in HIV-infected individuals with non-periodontal disease, and whether CD4+ T-cell count, HIV viral load and antiretroviral therapy (ART), affected those levels [9]. We decided to explore the possible association between GCF and serum levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, GM-CSF, IFN-γ, granulocyte colony-stimulating factor (G-CSF), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1β (MIP-1β), tumor necrosis factor alpha (TNF-α), with CD4+ T-cell count, HIV viral load and ART, as well as their association with HIV/ART status, in an effort to identify and quantify periodontal and systemic risk biomarkers by objective methods and measures.

2. Material and methods

2.1. Recruitment and participants

Participants were recruited from HIV/AIDS healthcare providers, non-governmental and community-based organizations in Monterrey, Mexico and adopted a non-probability and snowball sampling [10]. The research involved 202 consenting adult men who have sex with men (MSM). Eligible participants in the cross-sectional study were those 18 years or older and who had engaged in sex - anal or oral - with another male.

HIV-infected subjects were diagnosed in advance of the study as HIV-positive by enzyme-linked immunosorbent assay (ELISA) and confirmed by Western Blot [11]. All self-declared non-HIV subjects were provided with an opportunity to undergo voluntary counseling and testing for HIV (at screening and if enrolled, 3 months after all study samples were taken), detected by rapid HIV-1/2 test (OraSure Technologies, Inc., Bethlehem, PA).

2.2. Ethics

The study protocol was reviewed and approved by the ethics, research and biosafety committees at Tecnologico de Monterrey and Universitat Internacional de Catalunya. The study procedures were undertaken with the written consent of each subject and according to the Declaration of Helsinki [12].

2.3. Clinical examination

2.3.1. Anamnesis

All participating patients were offered a general health survey and baseline electronic medical/dental records were collected. Exclusion criteria included history or current manifestation of any systemic, autoimmune, and infectious diseases, or other than HIV/AIDS. Likewise, diabetes, concurrent psychiatric or psychological treatment, illicit drug use, current alcohol abuse, chronic or intermittent usage of anti-inflammatory or antidepressant drugs, antibiotics, chlorhexidine digluconate, previous vaccination (⩽4 weeks), previous periodontal treatment (⩽6 months) and current use of prosthetic or orthodontic appliances (fixed or removable), that could affect periodontal or systemic cytokine expression. Smoking habit was recorded and not excluded due to its prevalence among MSM with that among other men [13,14]. In like manner, obesity could not be avoided and body mass index (BMI) was taken into account due to obesity prevalence among Mexican adult population [15].

2.3.2. Oral examination

All participants underwent a standardized baseline oral examination. Gingival recession (REC), probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP) were measured using Florida Probe® (Florida Probe Corporation, Gainesville, FL). Six sites were measured in all teeth considering a fixed reference point on the occlusal surface of teeth and cementoenamel junction, except third molars. A total number of natural teeth was recorded. BOP was calculated as the percentage of positive sites per subject. BOP was considered positive if bleeding was elicited within 30 s following periodontal probing. Plaque index was recorded (PI) [16]. No radiographs were taken. Periodontitis was defined by the presence of one site with PPD ⩾ 4 mm and CAL ⩾ 3 mm on at least 4 different teeth with or without BOP. HIV-related oral lesions were recorded according to classified criteria EC-Clearinghouse [17], and OHARA case definitions [18].

2.4. Samples and laboratory tests

Prior sampling all subjects were rapid tested for the presence of anti-HBc, HBsAg and anti-HCV (Intec, Xiamen, China). In addition, non-fasted blood glucose levels (BGL) were determined (LifeScan, Inc., Milpitas, CA). Furthermore, oral fluid multiple drug screen test was used to determine drug abuse (Branan Medical Corp., Irvine, CA). All rapid tests were performed with appropriate counseling and referral.

2.4.1. GCF collection

Maxillary teeth including first molars, second premolars, and canines or central incisors (three teeth per subject) were selected. Sampled teeth were free of gingival inflammation, caries, prosthetic reconstruction and root canal therapy. After supragingival plaque was removed, paper strips (PerioPaper Strips, OraFlow, PlainView, NY) were gently inserted into the crevice for 30 s. GCF volume was measured and converted to microliters using Periotron 8000 and MCCONVRT V2.52 (Oraflow Inc., Amityville, NY). Paper strips with traces of blood were discarded and sampling was replicated from another non-sampled site of the tooth. Six paper strips of each patient were placed in 500 μl of phosphate-buffered saline (PBS). Following 10 s vortexing and 20 min shaking, the strips were removed and the eluates were centrifuged for 5 min at 5800 x *g* to remove plaque and cellular elements. The supernatant was harvested and divided into aliquots and stored at −80 °C until assayed.