REVIEW ARTICLE

Saliva as a Biomarker in Detection of Human Immunodeficiency Virus - A Clinical Perspective

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ABSTRACT

Human immunodeficiency virus (HIV) is one of the most infectious diseases which continues to increase worldwide. Saliva like many things in life has various roles within the oral cavity and its importance is usually not recognized until it is absent. Saliva an emerging biofluid is the most practical and a non-invasive diagnostic tool but favored to be more accurate than the available alternatives. The sensitivities and specificities of using saliva as a diagnostic tool in detection of HIV in developing countries reported to be in a range of 95–100% and 98–100%, respectively.

Key words: CD4 helper T-cells, collection device, human immunodeficiency virus, saliva

INTRODUCTION

Human immunodeficiency virus (HIV) infection is a condition caused by the HIV, which eventually destroys the immune system, making the body harder to fight against any other infections.[1] Saliva contains a variety of enzymes, hormones, antibodies, antimicrobial constituents, and growth factors and has been identified as mirror of body's health.[2] The current development of diagnostic biomarkers along with technological developments in salivary diagnostics will make saliva as a diagnostic tools for dentists in making clinical decisions and predicting treatment outcomes.[3] The benefit of using saliva as a diagnostic tool in detection of HIV is that the risk of needlesticks injury, and cuts from broken glass tubes are eliminated; hence, the collection of sample becomes easier than that of venous blood. A single collection device like an absorbent material can be used to collect oral fluid and can be discarded safely.[4] The collection of blood from the patients is difficult because sufficient amounts of venous blood are difficult to be obtained due to collapsed veins or due to cultural and religious reasons; hence, the compliance of using saliva becomes greater. The collection of saliva requires minimum training, hence, cost saving.[5] In addition, the risk infection due to reuse of unsterilized needles is reduced due to the use of saliva. The drawback of using saliva as a diagnostic aid is that there may be denaturation of proteins including the immunoglobulin by proteolytic enzymes if whole saliva is been used without any stabilizer. The amount of saliva collection in a large volume may be difficult for quality control panels. The risk of spread of the infectious agents like *Mycobacterium tuberculosis* may be greater with oral samples.[4] The purpose of this article is to review the literature on the diagnostic applications of saliva for the detection of HIV infection.

Saliva collection methods

Direct collection

Whole saliva can be collected in a container by dribbling, drooling, spitting, or with the use of substances that stimulate the salivary glands such as candies or citric acid. Following collection, ELISA buffer or a non-ionic polyoxyethylene surfactant, for example, Triton X-100, that is most frequently used as a component of cell lysis buffers or other solutions intended to extract and solubilize proteins.

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Collection devices are absorbent pad on a plastic stem consist of a buffer used as a transport media contains antimicrobial agents and antiproteolytic stabilizers. Following salivary collection, the absorbent pad is transferred to the buffer and then transported to the laboratory where the fluid is used for testing. Alternatively, the patient can use as a cedar to chew cotton roll for collecting saliva where the patient is asked to chew, during centrifugation the fluid will subsequently with the being eluted. At room temperature, the collected saliva remains stable for around 3 weeks and remains for a longer period when refrigerated.[4]

Salivary pH and buffering capacity and its association with dental caries in patients with HIV infection

The HIV affects the major salivary gland, especially the parotid gland which causes infection and enlargement of the gland, significantly leads to reduced stimulated parotid flow rates resulting in xerostomia and increased risk of caries.[6]

A study done by Hedge *et al*., in 2013, to evaluate the buffering capacity and salivary pH and to compare with HIV-positive and negative individuals. Both stimulated and unstimulated saliva of 200 HIV-positive subjects of age group between 20 and 40 years were collected. The pH and buffering capacity were analyzed using the saliva check kit and the results showed that there was a decrease in pH and buffering capacity of saliva in HIV-infected patients which was one of the major factors responsible for increased caries.[6]

The HIV-infected subjects due to low salivary flow and low buffering capacity had a higher yeast count which leads to a higher incidence of fungal infection compared to that of normal individuals.[6]

Total antioxidant status in HIV patients

Antioxidants are known to play a key role in the immune system, by excessive production of reactive oxygen species (ROS) and by reducing oxidative stress. In an HIV-infected individual, immunodeficiency occurs due to the development of oxidative stress which may be defined as the modification and accumulation of biological molecules altered by various kinds of ROS and reactive nitrogen species (RNS).[7]

Immune suppression and decrease in CD4 helper T-cells in HIV disease are a continuous process throughout the progression (Douek *et al*., 2009).[7] During various phases of the disease abundance production of oxygen-derived species mark's the inefficiency of antioxidant system (Aquaroetal., 2008; Aukrust *et al*., 2003). The various natural antioxidant systems including enzymes glutathione reductase, superoxide dismutase, glutathione transferase, methyltransferase, and catalase neutralize the damaging oxidative stress. Small non-protein cellular antioxidants, non-enzymatic Vitamin C and E, carotenoids, flavonoids, and uric acids provide protection against free radicals.[8]

A study was conducted by Hedge *et al*., 2013, to estimate the total antioxidant levels in saliva of healthy and HIV-infected individuals and to correlate the same with CD4 count. Unstimulated saliva was collected and total antioxidant activity was estimated by phosphomolybdenum method and concluded that the total antioxidant level in saliva decreases with decrease in CD4 and has a correlation with disease progression in HIV-infected individuals.[7]

Nitric oxide concentration (NO_2 NO_3) in saliva in HIV individuals

An active biomolecule nitric oxide plays a major role in defense against bacteria, protozoa, and tumor cells. L-arginine gets degraded by the enzyme nitric oxide synthase and reacts with oxygen *in vivo* and forms two stable end product, nitrite and nitrate. The production of these substances may be deleterious and may contribute to AIDS pathogenesis. There was an increase in nitrate and nitrite level, the two stable end product in the mononuclear cells, polymorphonuclear neutrophils cells, and serum of patients with AIDS, in individuals with neurological disorders and pulmonary disease caused by intracellular opportunistic pathogens.[9]

A study was conducted by Hegde *et al*., in 2014, to evaluate the correlation between nitric oxide levels and CD4 count in saliva of HIV-infected individuals. Unstimulated saliva was collected and nitric oxide was estimated by Griess method and the result of the study showed that salivary nitric oxide can serve as an indicator of HIV replication.[9]

Oral lesions in HIV patients

Oral health is an important component of the overall health status in HIV infection. A variety of oral lesions can occur throughout the course of HIV infection; hence, proper awareness and coordination between the physician, dentist, and the health-care professional is required for the overall improvement of the patients health. Hence, the mortality rate of the population can be reduced by early diagnosis and an appropriate treatment of the disease.[10]

Xerostomia HIV-infected individuals are more prone to dental caries due to decreased salivary flow which acts as a contributing factor. Approximately 30–40% of HIV-infected individuals experience moderate-to-severe xerostomia due to proliferation of CD8+ cells in the major salivary gland and the effects of medications (e.g., didanosine).

Candidiasis

The three common presentations of oral candidiasis are angular cheilitis, erythematous candidiasis, and pseudomembranous candidiasis.

Oral hairy leukoplakia

Epstein–Barr virus causes oral hairy leukoplakia which is commonly present in the lateral border of the tongue. It appears as a white corrugated lesion which cannot be wiped away.

Linear gingival erythema

Linear gingival erythema, or "red band gingivitis," presents as a red band along the gingival margin and may or may not be accompanied by occasional bleeding and discomfort.

Kaposi's sarcoma

Kaposi's sarcoma is still the most frequent HIV-associated oral malignancy, Kaposi's sarcoma-associated herpesvirus has been identified as the etiologic agent.[10]

A study was conducted by Hedge *et al*., in 2012, on the prevalence of oral lesions in HIV-positive patients and to investigate the association between the level of immunosuppression and its relationship with oral lesions. The CD4 count as well as any therapy being instituted was recorded and correlated with the oral manifestations. The result of the showed that the prevalence of oral lesions among HIV-infected individuals was 71% with periodontitis - 52% and erythematous candidiasis - 48% being the most prevalent oral lesions.[11]

Bodhade *et al*., 2011, conducted a study to evaluate the association between the CD4 count and the oral manifestations in HIV-infected individuals. 399 individuals were used in the study, of which the CD4 count was determined in 369 patients and correlated with oral manifestations. Oral candidiasis was found to be significantly correlated to a reduced CD4 cell count <200 cells/mm^3 with good sensitivity, best specificity, and positive predictive value.[12]

Salivary genomics, transcriptomics, and proteomics: The emerging concept and their use in diagnosis of HIV infection

The popular area of research in the recent years was to study the genomics, transcriptomics, and proteomics of saliva and the oral cavity due to its non-invasive, safe, and cheap source of complex genetic information. Saliva contains a variety of proteins that are found to have a wide range of biological functions, as well as mRNA and microRNA transcripts, and metabolites. Any changes in the salivary concentrations of these molecules aid in the detection of oral and systemic diseases.[13]

Genome (Human DNA)

The genetic material of an organism is known as a genome which included the coding and non-coding genes and the genetic material of mitochondria and chloroplast. The total DNA content in whole saliva was 1.8–128.4 μg/mL with a mean value of 21.6 μg/Ml.[14]

Transcriptome (human mRNA)

The total amount of mRNA presents in the gene of the organism. mRNAs get rapidly degraded within the cells which happen within a few minutes unlike rRNSs and tRNAs which are stable within the cells. This may be due to the reason that mRNAs are present in a small proportion of the total RNA compared to that of rRNA and tRNA fractions which are present abundantly.[14]

Salivary proteome

The entire amount of proteins which can be expressed by a genome, cell, tissue, or organism at a certain time period is called proteome. Whole saliva has a protein ranging between 0.5 and 3 mg/ml with the proteome consisting of 1000 distinct protein sequences, of which 300 sequences are of human origin.[14]

Antibodies against viruses and viral antigens can be detected by the identification of viral DNAs and RNAs, in the saliva of infected patients one of the most sensitive and specific methods available. In case of HIV type 1 detection of salivary proviral DNA, that is, the host cell integrated viral sequence may also be used with moderate sensitivity value of 40%. Detection of *viral RNA* in saliva may also be used with moderate to high sensitivity in detection of HIV-1.[14]

CONCLUSION

Saliva is gaining importance in recent years and is considered a diagnostic tool for various reasons. Saliva is identified to be functionally equivalent to the serum of the body reflecting the physiological state of the body including hormonal, emotional, nutritional, and metabolic variations. In epidemiologic surveys, salivary test can be considered as a good alternative to blood samples aiming to estimate the prevalence of HIV in general populations and in high-risk groups. Overall, the majority of studies have demonstrated high sensitivity and specificity of oral fluid-based rapid HIV test in comparison with routinely utilized methods.[15]

Clinical perspective of the article

V arious studies have been conducted in the field of saliva in HIV patients, such as detection of salivary pH, buffer capacity, nitric oxide, antioxidants , and early detection of oral lesions. hence the future scope of the research is to analyze the CD4 levels in the saliva that can help in the early diagnosis of the immunodeficiency syndrome. Counseling the patients to maintain proper oral hygiene will help to improve their lifestyle and social well-being.

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