Indian Journal of Dental Sciences.
October 2013 Supplementary Issue
Issue:4, Vol.:5

www.ijds.in

Original Article

Indian Journal
of Dental Sciences

E ISSN NO. 2231-2293 P ISSN NO. 0976-4003

Evaluation And Diagnostic Usefulness Of Saliva For Detection Of HIV Antibody

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Submission : 18th July 2012
Accepted : 17th August 2013

Quick Response Code

Abstract
Background and Objectives: use of saliva as specimen for detection of antibodies to infectious agents has generated particular interest in AIDS research community since 1980's. HIV specific antibodies of immunoglobulin isotypes IgA, IgG and IgM are readily found in salivary secretions, in the present study HIV specific antibodies were detected in saliva and serum samples by ELISA in confirmed HIV seropositive and healthy individuals.
Methods: The 100 saliva and serum samples were collected from age and sex matched confirmed HIV seropositive subjects and 100 Healthy Controls. HIV antibodies were determined by Enzyme Linked Immunosorbant Assay (ELISA).
Results: The results were found to be 99% sensitive and 100% specific for saliva samples while it was 100% sensitive and specific for serum samples.
Interpretation and Conclusion: Saliva can be used as alternative to blood for detection of HIV antibodies as saliva collection is painless, non-invasive, inexpensive, simple and rapid. Salivary antibody testing may provide better access to epidemic outbreaks, children, large populations, hard-to-reach risk groups and may thus play a major role in the surveillance and control of infectious diseases.

Key Words
HIV, Saliva, Serum ,Antibodies

Introduction

HIV infection is a major global health problem. As per recent reports by United Nation AIDS Control Society (UNAIDS) number of people living with HIV till 2010 were 33.2 Million (30.6–36.1 Million) and people who died of AIDS were 2.1 Million (1.9–2.4 Million). It is estimated by National AIDS Control Organization (NACO) that the number of people living with HIV infection in India till 2010 were 2.5 million (2-3.1 million).[1] Prevalence is high in the 15-49 age group (88.7 percent of all infections), indicating that AIDS still threatens those in the prime of their working life. World Health Organization (WHO) and National AIDS control organization (NACO) in 1997, enumerated the different modes of transmission of HIV. These are Sexual intercourse (anal / vaginal / oral) with an infected partner (man to woman, woman to man and man to man), transmission with infected blood, blood products, organs, tissue transplantation and artificial insemination, contaminated syringes and needles, and from an infected mother to child, i.e. perinatal or vertical transmission.Worldwide, HIV is most commonly transmitted by sexual activity. HIV is found in blood and other body fluids including semen, vaginal fluid and saliva. The immense majority of HIV infections are produced during unprotected sexual intercourse via the vaginal mucosa and especially the anal mucosa.[2],[3] The risk of HIV transmission via oral secretion is an issue of growing interest to dental health professionals, above all with the upsurge in the number of infected individuals.

Although HIV RNA, proviral DNA and infected cells are readily detectable in salivary secretions and Gingival Crevicular Fluid (GCF) of infected individuals, the transmission of HIV by oral route is very low or virtually non-existent. The mechanism of this oral immunity is poorly understood. Reports of antiviral activity in the saliva of both healthy individuals and HIV infected individuals suggest the presence of a factor or factors in saliva that can inhibit HIV infection. Furthermore, it is well established that human saliva inhibits HIV infectivity in vitro.[4],[5],[6],[7] The anti-HIV inhibitory factors in saliva may make a major contribution to the extremely low or negligible rates of oral transmission of the virus reported by epidemiological studies.[1],[4],[5]

Evaluation and diagnostic usefulness of saliva for detection of HIV antibody has been studied since 1986 assaliva is a body fluid containing antibodies of diagnostic significance. Unlike venipuncture, saliva collection is painless, non-invasive, inexpensive, simple and rapid. By using sensitive immunoassays in salivary specimens it is possible to diagnose immunoglobulin's against a wide range of infectious diseases e.g. hepatitis A, B and C, measles, mumps, rubella, human immunodeficiency virus, Epstein Barr virus, parvovirus B 19, human herpes virus 6 and Helicobacter pylori infections. Salivary antibody testing may provide better access to epidemic outbreaks, children, large populations, hard-to-reach risk groups and may thus play a major role in the surveillance and control of infectious diseases. Evaluation and diagnostic usefulness of saliva for detection of HIV antibody has been done by Enzyme linked immune assay (ELISA) which has been modified by increasing the specimen volume, altering

the incubation periods, reagent concentrations, and reducing the assay cutoff values[6],[8],[9] These modifications have resulted in improved ELISA sensitivity and specificity compared with those of matched serum test.

Material And Method

The present study was carried out in Dept.of Oral Medicine and Radiology, K.M Shah Dental College, Piparia, Vadodara, Gujrat. The study was approved by Ethical Committee of Sumandeep Vidyapeeth, Vadodara. The total of 200 subjects – 100 HIV confirmed seropositive as study group and 100 age and sex matched healthy individuals who had undergone a checkup by qualified medical physician as control group were randomly selected for the study from the OPD of Dhiraj General Hospital & K M Shah Dental College and Hospital Piperia,Vadodara and Non Governmental organizations working for HIV positive persons in Vadodara. Written consent was obtained from each participant. The aims and objectives of the study were to detect HIV antibodies in saliva and serum of newly diagnosed confirmed HIV seropositive patients by ELISA and to evaluate the sensitivity and specificity of ELISA test in serum and saliva samples of HIV positive and healthy individuals.Newly diagnosed confirmed seropositive patients before starting anti-retro viral therapy (ART) were selected. Three separate positive ELISA tests were considered confirmatory. Participants were excluded if they were on anti-retro viral therapy (ART). Patients with history of autoimmune disorder eg: Systemic Lupus Erythmatosis(SLE) or Discoid Lupus Erythmatosis(DLE), Rheumatoid arthritis who are likely to give false positive ELISA test were excluded from the study. Saliva collection and blood collection apparatus was used which included whole saliva Collector (50ml), 10 ml vial (transparent), lemon juice, dropper, tourniquet, spirit, cotton, bi ended needles, connector, vaccutainer tubes – 4 ml, 10 ml vial were used .

Results And Observations

The age range for study group was from 6years to 65 years with mean age of 34.14 ± 11.51 years whereas age range for control group was from 11 years to 62 years with mean age of 31.02 ± 7.15 years. The general socio-demographic data of the population revealed that most of HIV positive males were laborers (33.3%) and truck drivers(21%) by occupation whereas most of HIV positive females were housewives(46.5%). Most common mode of HIV transmission in the study group was unprotected sexual practices (70%) followed by blood transfusion (18%), vertical transmission (9%) and intra-venous drug users(3%).Out of total 25 married females of study group 21(84%) had given history of single partner and 4(16%) had multiple partners whereas 3(27.2%) out of 11 widows also gave history of multiple partners. Out of total 28 cases of sexual transmission of HIV infection only 7(25%) females gave history of multiple partners.

Thus the results indicated that total 95% married males and 16% married females of study group had unprotected sexual activities with multiple partners which indicates 84% females acquired HIV infection from there HIV positive spouses.

Out of total 100 subjects in study group, 99(99%) were tested positive for HIV antibodies in saliva samples with one false negative result and all the subjects were detected positive for HIV antibodies in serum samples whereas all the subjects of control group were tested negative for HIV antibodies in serum and saliva samples.

Thus, the ELISA test was found to be 99% sensitive and 100% specific for detection of HIV antibodies in saliva samples of study group whereas it was found 100% sensitive and specific for detection of HIV antibodies in serum samples of study group.

Discussion

Generation of specific antibody response is a critical component of the host defense against pathogenic microorganisms and HIV is no exception. The presence of virus-specific antibodies in mucosal secretions including saliva has been well documented. HIV specific antibodies of immunoglobulin isotopes IgA, IgG and IgM are readily found in salivary secretions of infected people but at levels considerably lower than those in blood[10]. Detection of HIV-specific antibodies in oral fluid transudate has been exploited recently as a highly sensitive and specific alternative to blood for diagnosis and population surveillance.

Spencer Hedge et al in 1998 explained the diagnostic significance of antibodies in oral secretions. Immunoglobulin's (IgG) were identified in human saliva nearly 50 years ago and shortly thereafter in 1963, the prevalence of IgA in saliva was demonstrated.[11]

Parry J. V. et al 1987, performed a sensitive assays for viral antibodies in saliva. They described methods for detecting antibodies to HIV as well as antibodies to other viruses and proposed saliva as an alternative specimen for epidemiological investigations.[12]

ELISA has been modified by increasing the specimen volume, altering the incubation periods, reagent concentrations, and reducing the assay cutoff values for detection of HIV antibody in saliva.[6],[7],[13],[14] These modifications have resulted in improved ELISA sensitivity and specificity in saliva compared with those of matched serum test as reported by Timothy C Granade etal in year 1995 and 1998.12,13 [13],[14]

In the present study we have evaluated diagnostic usefulness of saliva for detection of HIV antibodies. Unlike venipuncture, saliva collection is painless, non-invasive, inexpensive, simple and rapid.

In our study saliva and serum samples of 100 confirmed seropositive patients and 100 healthy individuals were tested by ELISA kit. The result was found to be 99% sensitive and 100% specific for saliva samples while it was 100% sensitive and specific for serum samples. The results were congruent with studies done by Soto-Ramirez et al[15] in 1992, Chamanput etal[16] in 1993,Ishiwaket al in 1995 [17], Schramm et al in 1999 and recently by Nitika et al in 2007[18]. Diagnostic sensitivity and specificity of saliva for detection of HIV antibodies is reported by various authors is given in the following **Table 1**.

Thus, in the various studies, diagnostic sensitivity of saliva, analyzed by ELISA, is ranged from 95% to 100% and diagnostic specificity of under 90% has been reported. 3,5,7,8,14,15,16,17,18, [4],[5],[6],[7],[8],[9],[10],[11],[12],[13],[14],[15],[16],[17],[18],[19]

In conclusion, Saliva can be used as alternative to serum and plasma for

Table 1: Sensitivity And Specificity Of Saliva For Detection Of HIV Antibodies By Various Authors

Authors	Year	HIV Positive Subjects	HIV Negative Subjects	Senstivity	Specificity
Parry Et Al	1987	43	10	100	100
Arichbald Etal	1991	21	---	95.2	---
Chamanput Etal	1993	100	100	99	100
Luo N Etal	1995	50	57	96	100
Ishiwak Etal	1995	63	76	100	100
Pasquier Etal	1997	530	---	100	99.8
Prudenico Etal	1998	187	115	95.2	97.4
Schramm Etal	1999	684	652	100	99.1
Wesolowski Etal	2006	26066	---	90	99.8
Nitika Etal	2007	146	304	100	100

detection of HIV antibodies as a highly sensitive and specific alternative to blood for diagnosis and population surveillance.

Still much more work is required in this field so that saliva can be used as alternative to blood for detection of HIV antibodies.

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Source of Support : Nill, Conflict of Interest : None declared