

Characterization of Bacterial and Fungal Colonizers of the Oral Cavity among the Human Immunodeficiency Virus (HIV/AIDS) Infected Individuals before and after Initiation of Highly Active Antiretroviral Therapy (HAART)

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Abstract It is a well known fact that human immunodeficiency virus (HIV) infection is a world wide pandemic. HIV and acquired immunodeficiency syndrome (AIDS) with oral infections are among world's most severe public health threat. HIV infection is associated with risk of developing opportunistic infections and especially contribute to oral infections which are further complicated as most of the infections are due to normal flora/microorganisms of endogenous origin. Therefore, rapid and early detection, isolation and characterization of these oral infections related to oral micro biome enhances knowledge of physicians involved in HIV patient care to control and prevent HIV infected individuals from future complications which could be responsible for severe morbidity and mortality. Asymptomatic carrier detection of potential pathogens is important to control infections and also act as a treatment guide for clinical and epidemiological purposes for both clinicians treating HIV patients and clinical microbiologists. Oral infections in HIV infected patients have become an increasing cause of clinical concern for several reasons: firstly they are associated with adverse clinical outcomes and secondly most of them are asymptomatic carriers. This study is an attempt made by us to determine the prevalence of oral micro biota and their association with opportunistic infections in HIV infected patients before and after initiation of highly active antiretroviral therapy (HAART).

Keywords: HIV infections, Oral micro biota, highly active antiretroviral therapy (HAART), opportunistic infections in HIV infection, normal bacterial and fungal colonizers of the oral cavity

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1. Introduction

HIV/AIDS patients suffer from frequent opportunistic infections in which oral infections/lesions take first place. They mainly include fungal, viral and bacterial infections ranging from mild, in apparent to life threatening illnesses. Most of the microorganisms responsible for these oral manifestations are believed to be a part of normal flora [1]. Anatomically oral cavity extends from lips to oropharyngeal isthmus. The microbial population of oral cavity is referred to as oral flora/oral micro biota/oral micro biome as coined by Joshua Lederberg which signifies community of commensals both symbiotic and potentially pathogenic organisms. The pathogenic threat of these colonizing microorganisms increases due to

immunosuppression and prolonged antibiotic therapy, in turn altering the microbial population of oral cavity.

The Dynamic change in the oral micro biome is usually influenced by age of an individual, immune status and long-term antibiotic therapy. The complex nature of oral flora associated with oral manifestations play a crucial role in changing the course of HIV disease. Hence it is important to know microorganisms colonizing in the oral cavity and their changing pattern and other risk factors which may be related to cause of infections in HIV/AIDS before and after initiation of highly active antiretroviral therapy [HAART]. These observations can profoundly uncover some of the commonest oral inhabitants responsible for frequent to less frequent infections in HIV infected individuals. Occurrence of oral manifestations might be different in HIV infected population who are

HAART naive and those who are undergoing antiretroviral therapy.

The Present study aims to determine and characterize cultivable oral colonizers in HIV/AIDS patients and their relation with oral lesions and degree of immunosuppression before and after initiation of HAART. To the best of our knowledge this study is first of its kind as there is no data available in literature on the changing oral flora in HIV infection before and after initiation of antiretroviral therapy. The degree of immunosuppression was interpreted based on available data of CD4+ T cell counts.

The oral flora is affected by altering temperature, pH, nutrients, host defenses, immunosuppression and prolonged usage of antimicrobial agents [2,3,4,5,6]. In HIV/AIDS most of these factors contribute to oral flora taking over the niche and assisting in flourishing and establishing of secondary infections which are commonly encountered in immunocompromised individuals [7]. Hence oral manifestations are considered as the direct indicators of immunosuppression, confirming/indicating the progression of HIV disease. Therefore appearance of oral lesions plays a pivotal role in the management of HIV disease and assessing the progression during antiretroviral therapy. It becomes necessary for the HIV care physicians and clinical microbiologists to understand the potential role of oral colonizers in causing opportunistic infections and occurrence of multidrug resistant microorganisms which may further complicate the treatment of oral infections.

2. Materials and Methods

Present study is a descriptive longitudinal study including 240 HIV sero-positive individuals conducted over a period of 2 years between July 2012 and Sep 2014. Each patient was followed at an interval of every 6 months after initiation of HAART at the ART center RMC Loni, India. The patients were grouped into 2 categories that include one group of individuals with oral lesions and those without oral lesions, the other group contained patients before and after initiation of HAART.

2.1. Limitations

Uncultivable and Unnamed human oral isolates and phylotypes were not described in the study.

2.2. Methods

HIV patients were asked to rinse their mouth with 20ml sterile normal saline for 20 seconds. The rinsed saline was collected and serially diluted [1 in 2 with saline] and inoculated on Blood Agar, Mackonkey's Agar and Sabourauds dextrose agar (SDA) media plates. In patients presenting with oral lesions two swabs were collected from the infection site out of which one was processed for direct KOH mount (demonstration of fungi) and grams stain, and the other was used for culture. The cultivable aerobic isolates were identified up to species level using conventional laboratory methods that included growth characters, biochemical tests and special biochemical characters. Antibiotic susceptibility testing was also performed by standard clinical laboratory methods. For *Candida* isolates identification was based on germ tube test, morphology on corn-meal agar, sugar fermentation and assimilation patterns [8,9].

3. Results

A total of 240 HIV patients registered at ART Center, RMC, Loni, India were included in the study who were followed up every 6 months for a period of 2 years. Most of the HIV sero-positive patients presented 1 or more oral lesions before initiation of HAART and during the course of treatment. In the present study out of 240 patients 181(75.41%) patients showed oral infections majorly by *Candida* spp, oral manifestations due to viral infections and other inflammatory conditions. 169(70.4%) presented symptoms which were clinically suggestive of oral candidiasis, among them 102(60.3%) were newly registered for HAART and 69(40.8%) were on treatment for at least six months.

The list of organisms isolated from the patient's before starting HAART included *Streptococcus* spp, predominantly *S.mutans*, *Staphylococcus* spp including coagulase negative *Staphylococci* (CONS), *Candida* spp, Gram negative rods, Gram negative cocci, *Actinomyces* spp and anaerobic bacteria Table 1. All these organisms were observed to be the normal colonizers. *Candida* is an asymptomatic colonizer and was noticed in 198(82.5%) patients where as 169 were having symptomatic infection. The common species responsible for candidiasis was *C.albicans* followed by non albicans group such as *C.tropicalis*, *C.parapsilosis*, *C.kefyr*, *C.krusi* and only one isolate was *C.dublinensis* Table 2. Due to lack of resources oral lesions associated with the viral infection were not included in this study.

Table 1. The oral microbial flora in HIV seropositive patients before and after HAART

Organisms isolated	Number of isolates(240)	Before HAART	After HAART
<i>Streptococcus</i> spp	387	179	208
<i>Candida</i> spp	198	102 symptomatic	96 symptomatic
<i>Staphylococcus</i> spp	93	79	14
CONS	61	39	22
<i>E.coli</i>	48	31	17
<i>Klebsiella</i> spp	37	29	8
<i>Pseudomonas</i> spp	11	9	2
<i>Acinetobacter</i> spp	5	5	NIL
<i>Enterococcus</i> spp	2	2	NIL
<i>Actinomyces</i> spp	4	3	1
Anaerobes mostly <i>Bacteriodes</i> spp	47	33	14

Table 2. The characterization of *Candida* spp isolated from HIV seropositive patients before and after HAART

Organisms isolated	Number of Isolates(198)	Before HAART	After HAART
<i>C.albicans</i>	102	69	33
<i>C.tropicalis</i>	43	27	16
<i>C.paropsilis</i>	24	19	5
<i>C.kefyr</i>	17	11	6
<i>C.krusi</i>	11	8	3
<i>C.dublinensis</i>	1	NIL	1

4. Discussion

The oral micro biota of HIV infected patients before starting antiretroviral therapy contrasts with the oral colonizers of HIV patients after initiation of HAART. In the present study it was observed that among the patient's who were not on HAART, most of organisms isolated from oral lesions were either normal flora or from endogenous origin as observed in previous studies [10,11]. The major isolates were *Streptococcus* spp and *Candida* spp in both groups where as yeast carriage rate was noted to be higher in HIV individuals before initiation of antiretroviral therapy. The present study has observed that there was a major change in the oral flora of the patients undergoing the treatment as indicated by gradual decrease in the oral micro biome. In this study overall prevalence of oral manifestations was increased when the patients had low CD4+ T cell counts with the maximum count recorded being 1468 cells/mm³ and a minimum of 11cells/mm³. The study has also noted that oral manifestations were also observed in patients with CD4+ T cell counts more than 350 cells/mm³ and 500cells/mm³. In current study overall prevalence of *Streptococcus* spp (86.6%) was higher than *Candida* spp in patients undergoing HAART. *S.mutans* was the most frequent isolate which was associated with dental caries followed by *Candida* spp, *Staphylococcus* spp and few isolates of Gram negative rods including *E.coli* and *Klebsiella* spp. Previous literature has noted that asymptomatic or symptomatic presence of *Candida* is usually associated with oral manifestations, which increases the risk of opportunistic infections in HIV/AIDS patients [12].

Before HAART the incidence of *Candida* was high and the colonization showed a gradual decrease after initiation of HAART. This can be attributed to improved immune response, raised CD4+ T cell counts after initiation of HAART [13]. On the other hand prevalence of *Streptococcus* spp both before and after antiretroviral therapy may be due to other host related factors that may include diet, life style, consumption of tobacco and its products in this particular region and prolonged antibiotic therapy which may have adverse effect on oral health status [14].

Majority of patients had periodontal diseases like dental caries, necrotizing and ulcerating lesions hence most isolates were *S.mutans*. In our study which included 240 patients 208 presented clinical features of dental caries, gingivitis and edema. The percentage of candidemia was very high especially in patients with low CD4+T cell counts. Studies previously have also noted that infection with *Candida* spp should not be considered as a secondary invasive opportunistic infection which occurs at low CD4+T cell counts and that oral candidiasis was a primary indicator of immunosuppression. Among the various

species of *Candida* isolated *C.albicans* was the most frequent isolate in acceptance with other previous reports [15,16]. The decreasing levels of CD4+T counts are a pre-disposing factor for the development of opportunistic infections [17]. Though the yeast infection rate was higher, our study revealed *Streptococci* as another major colonizer which was isolated in almost every case associated with dental problems and other oral infections including sore throat. These observations were similar to a study from India by Mukherjee et.al which was performed among HIV positive individuals and compared with HIV negative individuals [18].

5. Conclusion

The results of the present study reveal that the HIV patient's oral cavity is colonized by *Streptococcus* spp both before and after initiation of HAART. *Candida* spp was the most common isolate from oral cavity before antiretroviral therapy and that there is a decrease in the colonization rates of *Candida* spp after initiation of HAART. The present study couldn't delineate as to which of the two reasons i.e. whether it is the effect of HAART or improved oral hygiene are the causes of changing/improving oral health status. However our results suggest that oral manifestations/infections are mostly acquired endogenously during the course of HIV disease and therapy. Therefore, by understanding the oral microbial colonizers, efforts can be made to prevent the active development of disease. This study highlights the importance of having a good knowledge of human oral micro biome in HIV seropositive patients which can lead to a better understanding of microbe-man interaction in terms of HIV infection in future. With respect to HIV disease and the impact it is making on the human health and medical fraternity, we should understand that we continue to win the battle against HIV but the war is still on because there remains much to be researched and understood.

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