

## IDENTIFICATION OF BACTERIA CAUSING DIARRHOEA IN HIV/AIDS PATIENTS AND ITS CORRELATION WITH CD4 COUNT

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### ABSTRACT

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#### BACKGROUND

The number of HIV-positive patients is increasing in India. Data on the prevalence of diarrhoea and the spectrum of bacteria responsible for diarrhoea in HIV-positive patients is lacking in our area. The identification of enteric pathogens in patients with HIV/AIDS is important because an increasing array of therapeutic regimens is becoming available to treat many of these infections. Thus, an attempt is done to elucidate the associations between causative bacteria of acute and chronic diarrhoea and CD4 count.

#### METHODS

Stool specimens were obtained over a period of eighteen months from HIV infected adults with diarrhoea presenting to Shri B M Patil Medical College Hospital and Research Centre, Vijayapura. In all patients with diarrhoea, stool specimens were examined by microscopy and cultures to identify bacterial pathogens and blood sample was analysed for CD4 count.

#### RESULTS

A total of 80 individuals were enrolled in this study. Cases included 46 males and 34 females. Among the cases, maximum subjects were found to be in the age group of 30-40 years in which 23 (62.2%) were males and 14 (37.8%) were females. 56 had acute and 24 had chronic diarrhoea. The percentages of bacteria isolated were 5 (8.9%) in acute and 16 (66.7%) in chronic diarrhoea respectively. The most common bacteria isolated was E. Coli (17.5%) followed by Klebsiella (5%) and Shigella Sps (3.75%). Patients with chronic diarrhoea had lower CD4 cell counts. The maximum bacterial isolation was in the patients whose CD4 cell counts were below 200 cells/mm<sup>3</sup>.

#### CONCLUSION

Bacterial isolation was most strongly associated with low CD4 counts and chronic diarrhoea. E. coli was isolated maximum among all the bacteria in the HIV patients. Over two-thirds of diarrhoeal episodes were undiagnosed, suggesting that unidentified agents or primary HIV enteropathy are important causes of diarrhoea in this population. There is a strong negative association between duration of diarrhoea and CD4 levels.

#### KEYWORDS

HIV, Diarrhoea, Bacteria, CD4.

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**HOW TO CITE THIS ARTICLE:** Ambali AP, Murthy S, Mulimani MS. Identification of bacteria causing diarrhoea in HIV/AIDS patients and its correlation with CD4 count. J. Evid. Based Med. Healthc. 2016; 3(44), 2165-2168.

DOI: 10.18410/jebmh/2016/481

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**INTRODUCTION:** Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) is a major problem in India. HIV patients are prone to develop numerous opportunistic diseases during their lifetime. Among them diarrhoea is a significant cause of morbidity observed in majority of studies. In fact diarrhoea is the second leading cause of hospitalisation in developing nations and makes its place in top 10 worldwide. The information on the cause of diarrhoea and the possibility of isolation of

pathogens has largely come from various cross-sectional studies.

Expectedly, infectious aetiology leads the list in developing nations in contrast to noninfectious aetiology in developed nations. There are many reports regarding frequency of various pathogens causing diarrhoea from different parts of India. Some studies also demonstrated regional variability of pathogen, as well as changing trends of aetiology in the same population.<sup>1</sup>

Diarrhoeal diseases in AIDS are most often due to infectious pathogens and include various parasite, bacteria and virus. The degree of immune-suppression, as defined by the CD4+ T-cell count, determines to a large extent when individuals with HIV infection develop opportunistic infections. The incidence and outcome of many of these

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*Financial or Other, Competing Interest: None.*

*Submission 02-05-2016, Peer Review 16-05-2016,*

*Acceptance 25-05-2016, Published 31-05-2016.*

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*DOI: 10.18410/jebmh/2016/481*

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complications; however, can be altered by preventive measures like primary and secondary prophylaxis.<sup>2</sup>

Infectious diarrhoea is a major cause of morbidity and mortality in patients with HIV infection and AIDS. Most patients with AIDS who develop diarrhoea have some degree of malabsorption. Patients with AIDS and diarrhoea also have lower numbers of CD4+ cells and a higher incidence of extra-intestinal opportunistic infections than those without diarrhoea suggesting that patients with AIDS who develop diarrhoea have a higher degree of immunosuppression.<sup>3</sup>

Patients with chronic diarrhoea have lower CD4 counts than those who have acute diarrhoea. Bacteria that are more common in HIV-infected patients include *Salmonella* spp., *Shigella*, *Campylobacter jejuni*, *Escherichia coli* (enterotoxigenic, enteroadherent, and enteroaggregative), and *Listeria monocytogenes*. *Clostridium difficile* toxin-associated diarrhoea may also be more common among individuals with HIV disease, particularly among hospitalised patients and those who have recently received antibiotic therapy. Other, less common, bacterial causes of enterocolitis include *Aeromonas*, *Plesiomonas*, *Yersinia*, and *Vibrio* spp. Mycobacterial infections of the small bowel are usually associated with late stage HIV disease and disseminated *M. Avium* complex, although enteritis caused by *M. tuberculosis* has been reported.<sup>4</sup>

The number of HIV-positive patients is increasing in India. Data on the prevalence of diarrhoea and the spectrum of bacteria responsible for diarrhoea in HIV-positive patients is lacking in our area. The identification of enteric pathogens in patients with HIV/AIDS is important because an increasing array of therapeutic regimen is becoming available to treat many of these infections. Thus, an attempt is done to elucidate the associations between causative bacteria of acute and chronic diarrhoea and CD4 count.

## MATERIALS AND METHODS:

### Source of Data:

**Type of Study:** A descriptive cross-sectional study design is used.

**Study Population:** A total of 80 individuals were included in the study after the approval of Institutional Ethical Committee and with the consent of the subjects.

**Cases:** Eighty prospective randomly selected patients seropositive for HIV1 and/or 2 with diarrhoea and all patients presenting with diarrhoea and diagnosed HIV1 and/or 2 seropositive on admission for the first time, in Shri. B.M. Patil Medical College Hospital and Research Centre, Vijayapura as inpatients or outpatients for period of two years.

**Inclusion Criteria:** Patients admitted to hospital within the study period, irrespective of age and sex with seropositive for HIV 1 or HIV 2 or both with diarrhoea and all patients presenting with diarrhoea and diagnosed HIV1 and/or 2 seropositive on admission for the first time. Also patients

seropositive for HIV1 or HIV2 or both with recurrent diarrhoea were also included.

**Exclusion Criteria:** HIV-positive patients who had received antibacterial medications for diarrhoea prior to admission were excluded.

All the included patients were subjected to the investigations like HIV1 and HIV2 by ELISA, CD4 Count, Stool microscopy, Stool Culture and Sensitivity.

**RESULTS:** A total of 80 individuals were enrolled in this study. It included all patients seropositive for HIV1 and/or 2 with diarrhoea and presenting with diarrhoea and also those who were diagnosed HIV1 and/or 2 seropositive for first time. Fresh stool samples were collected from all the subjects and were examined for bacteria by microscopy and stool culture. Cases included 46 males and 34 females. Among the cases, 37 (29.6 %) subjects were found to be in the age group of 30-40 years which constituted major group in which 23 (50%) were males and 14 (41.18%) were females.

*E. coli* was the most common bacteria isolated which constituted about 14 (17.5%). *Klebsiella* was the next most common isolated which constituted about 4 (5%) and the last was *Shigella* Sps. which constituted 3 (3.75%). In 59 (73.75%), enteropathogenic bacteria was not isolated on culture.

Bacteria are found more in chronic diarrhoea as compared to acute diarrhoea. Among 80 patients, about 56 patients had acute diarrhoea in which bacteria was isolated in 05 (23.81%) patients and 24 patients had chronic diarrhoea in which bacteria was isolated in 16 (76.19%) patients with statistical significance.

Among 80 patients, 28 patients had CD4 count <100 cells/mm<sup>3</sup>, 23 patients with CD4 between 100-200 cells/mm<sup>3</sup> and 29 patients had CD4 >200 cells/mm<sup>3</sup>. Bacteria isolation in stool sample of patients with CD4 count between 100 - 200 cells/mm<sup>3</sup> was higher i.e. 52.38% than patient with CD4 count less than 100 cells/mm<sup>3</sup> i.e. (47.62%) with statistical significance.

Among 56 patients of acute diarrhoea, about 28 (50%) had CD4 >200 cells/mm<sup>3</sup> and 16 (28.57%) had CD4 count <100 cells/mm<sup>3</sup>. Among 24 patients of chronic diarrhoea, 12 (50%) patients had CD4 <100 cells/mm<sup>3</sup> and 1 (4.17%) patient had CD4 count >200 cells/mm<sup>3</sup> with p-value of <0.0001 which is statistically significant.

**DISCUSSION:** HIV/AIDS (Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome) is a major problem in India with more than 23.9 lakh recorded cases by the end of 2009.<sup>5</sup> Diarrhoea is a common complication in HIV-positive patients and occurs in 90% of AIDS patients in developing countries. Identification of the aetiological agent of diarrhoea in a patient with AIDS is very important as it can help in the institution of appropriate therapy and the reduction of morbidity and mortality in these patients. This study was therefore conducted to ascertain the scope and frequency of potential enteric bacterial pathogens isolated from stool samples of HIV-positive individuals with

diarrhoea. The aetiology for such diarrhoea could be parasitic, bacterial, fungal, enteric viruses, or HIV itself may contribute to diarrhoea. In addition to microbes, other factors such as medication, immune deregulation, autonomic dysfunction, and nutritional supplementation play a substantial role in diarrhoea of patients with HIV/AIDS.<sup>6</sup>

In India, enteric infections remain common in the general population and for those people infected with HIV, with geographic differences in the reported prevalence of individual pathogens reflecting differences in pathogen prevalence, standards of hygiene, and diagnostic methods used. There are many reports regarding the frequency of various pathogens causing diarrhoea from different parts of India.<sup>7</sup> Some studies also demonstrated regional variability of pathogens, as well as changing trends of aetiology in the same population.<sup>8</sup>

**1. Distribution of Patients in the Study Group:** In the present study, there were 80 HIV-positive individuals, of which there were 46 males and 34 females. Those having diarrhoea for less than 2 weeks were considered to have acute diarrhoea and diarrhoea lasting more than 2 weeks was considered to be chronic.<sup>9</sup>

Among HIV infected with diarrhoea, 56 patients had acute form of it and the remaining 24 patients were suffering from chronic diarrhoea; acute diarrhoea subjects outnumbered chronic diarrhoea. Our rate represents a minimum burden of diarrhoea, as episodes were only counted if a patient came to the clinic. Diarrhoea which was self-limiting or which responded to over-the-counter medication would not have come to our attention.

**2. Distribution of Bacteria in the Study Group:** The overall infection rate in this study was 21 (26.25%). In the present study, bacteria were found in higher numbers in males (15%) than in females (11.25%). Male preponderance in infection rates among HIV infected has been shown in studies by Dwivedi K et al.<sup>9</sup>

The higher bacterial infection rate was seen in 30-40 years age group in HIV positive subjects, with 62.2 % males and 37.8 % females being in that age group. In the study by Mukhopadhyaya et al,<sup>10</sup> mean age of infected individuals was found to be in 30-40 years age group. The infection rates observed in the present study were comparable to the studies of Kumar SS et al<sup>11</sup> which has reported higher infection rates in HIV-positive patients.

**3. Spectrum of Bacteria Identified in the Study Population:** In the present study, *E. Coli* was the most common bacteria isolated which constituted about 14 (17.5%). *Klebsiella* was the next most common isolated which constituted about 4 (5%) and the last was *Shigella* Sps. which constituted 3 (3.75%) as compared to the study of Beena U et al<sup>6</sup>

in which *Escherichia coli* in 24% cases, *Clostridium difficile* in 12.5% cases, *Salmonella* species in 5% cases, *Vibrio cholerae* in 5% cases, and *Shigella* species in 2.5% cases were isolated. Figures from various studies demonstrate striking geographic variations in the prevalence of individual pathogens in HIV patients. These variations may relate both to the prevalence of pathogens within the community, and to drugs used prophylactically in patients with HIV infection. Moreover, the quantitative differences in bacteria infecting HIV patients with diarrhoea may be due to differences in genotypic/phenotypic characters.

**4. Diarrhoea and Intestinal Bacterial Infection:** In our study, higher bacterial infection was found in those with chronic diarrhoea (66.70%) than those with acute diarrhoea (8.90%). Similar observations were made in studies conducted by Beena et al,<sup>6</sup> Christine et al<sup>12</sup> and Samie et al.<sup>13</sup>

**5. Diarrhoea and Immune Status of the HIV-positive patients:** Among cases, mean CD4 counts were found to be 319.93 cells/mm<sup>3</sup> study. Those with chronic diarrhoea (179.1 cells/mm<sup>3</sup>) had significantly lower CD4 counts compared to acute diarrhoea (239.26 cells/mm<sup>3</sup>). There was a strong negative association between the duration of diarrhoea and CD4 levels and this was comparable to a study done by Attili et al.<sup>1</sup> Mean CD4 counts were observed to be 141 cells/mm<sup>3</sup> diarrhoea (265 cells/mm<sup>3</sup> in acute and 123 cells/mm<sup>3</sup> in chronic) in the study of Dwivedi KK et al.<sup>9</sup>

The theories for chronic diarrhoea at low CD4 is not clear but one of the reasons could be regional immunosuppression as suggested by Schneider et al.<sup>14</sup> They found, loss of CD4 cells in intestinal mucosa of the patients with diarrhoea, which were more pronounced than peripheral CD4 levels and their relation is quite variable. The mucosal immunity, an important factor to prevent diarrhoea is therefore variable even in patients with good immunity (i.e. peripheral CD4 > 200 cells/mm<sup>3</sup>). We did not perform the mucosal CD4 levels, thus any comments on the mucosal immune status based on blood CD4 levels would be inappropriate. But we can presume that probably the low mucosal immunity could be a cause of diarrhoea in patients with high CD4 levels.

**6. Bacterial Isolation and Immune Status of Cases:** Maximum number of bacteria was seen in those with CD4 counts less than 200 cells/mm<sup>3</sup>. The prevalence of bacteria was lesser in CD4 counts of >200 cells/mm<sup>3</sup>. Of 80 cases, *E. coli* were identified in eight patients with CD4 <200 cells/mm<sup>3</sup> and four patients with CD4 >200 cells/mm<sup>3</sup>. *Shigella* sps and *Klebsiella* were isolated with CD4 <200 cells/mm<sup>3</sup>. Studies by Attili et al<sup>1</sup> also showed highest prevalence

of bacterial infection in patients with a low immune level CD4 < 200/mm<sup>3</sup>.

The diagnostic yield of stool analysis is low in patients with higher CD4 cell counts. The probable reasons may be<sup>1</sup> effective anti-retroviral therapy helps eradicating bacterial infection, and associated with the influx of CD4 positive cells into the lamina propria and the opportunistic infections causing diarrhoea in AIDS become less common. Other gastrointestinal diseases, which are common in young age group, like inflammatory bowel disease and coeliac disease, irritable bowel syndrome and idiopathic steatorrhoea are presently leading the list of aetiological agents.

**CONCLUSION:** Individuals with HIV/AIDS, because of their compromised immune status are at a higher risk of enteric bacterial infection with grave prognosis.

As bacteria cause prolonged, life-threatening diarrhoea in patient with AIDS, identification of these bacteria at the earliest will enable the clinician to give effective treatment.

Diarrhoea due to *Escherichia coli* bacteria was found to be more common followed by *Shigella* species and *Klebsiella* organisms.

HIV-infected individuals with lower immunity, as indicated by CD4 counts, suffered more from chronic diarrhoea. Bacteria were common in lower immune status. Highest rate of infection was seen with CD4 counts 100-200 cells/mm<sup>3</sup>. Identification of bacteria causing diarrhoea with HIV or AIDS especially at lower CD4 count should be performed and treated accordingly.

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