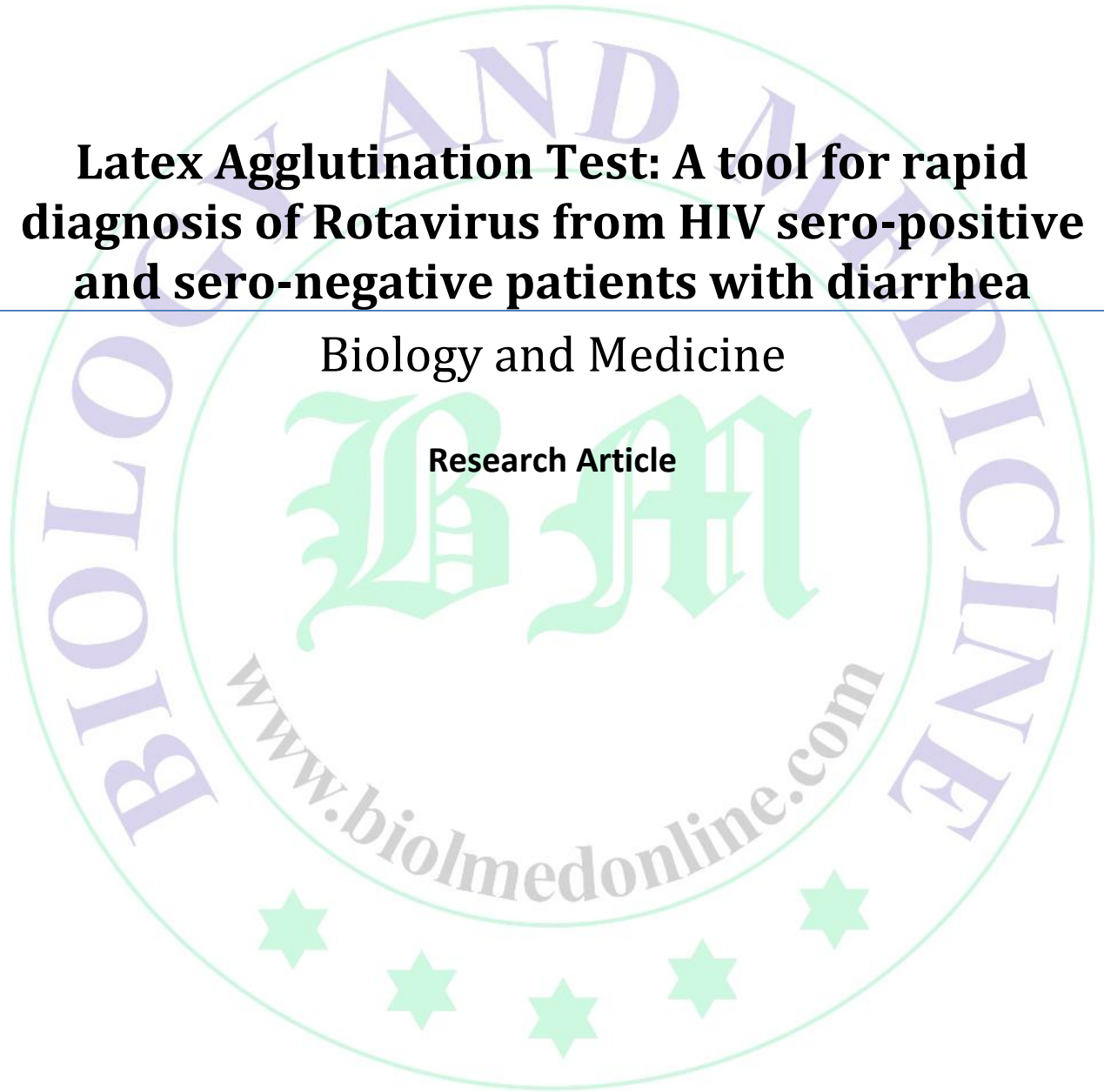# Latex Agglutination Test: A tool for rapid diagnosis of Rotavirus from HIV sero-positive and sero-negative patients with diarrhea

Biology and Medicine

Research Article

Latex Agglutination Test: A tool for rapid diagnosis of Rotavirus from HIV sero-positive and sero-negative patients with diarrhea

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Abstract

The aim of this study was to determine the incidence of Rotavirus in HIV sero-positive and sero-negative patients with diarrhea using a rapid Latex agglutination test and to correlate it with the clinical symptoms. A total of 126 patients (74 HIV sero-positive and 52 HIV sero-negative) with diarrhea were enrolled for this study. Of the total study population, 100 (79.37%) were adults and 26 (20.63%) were children. The incidence of acute diarrhea was 54 (42.86%) and chronic diarrhea was 72 (57.14%). Statistically, the difference between them was found to be significant ($p = 3.33E-06$). Chronic diarrhea was seen in 55 (74.32%) HIV sero-positive patients and acute diarrhea was seen in 35 (67.31%) HIV sero-negative patients. Rotavirus was detected in 9 (12.16%) HIV sero-positive patients and in 3 (5.56%) HIV sero-negative patients. Rotavirus positivity was higher in HIV sero-positive patients than in HIV sero-negative patients. Latex agglutination test was found to be a good tool for an easy and rapid detection of Rotavirus in stool specimen, making it an ideal bedside procedure.

Keywords: Rotavirus; rapid diagnosis; Latex agglutination test; HIV; diarrhea.

Introduction

Rotavirus is the leading cause of viral diarrhea in infants, young children and adults worldwide. An estimated 2.3 million hospitalizations and 527,000 deaths in children aged <5 years are caused by Rotavirus in south Asia and sub-Saharan Africa annually (WHO, 2011). Adults are also found to be at risk of rotavirus infection (Anderson and Weber, 2004) which is usually milder or can be asymptomatic, except in the elderly diarrheal illness (Peter *et al.,* 1983). In the immune compromised patient, Rotavirus may cause fatal chronic diarrhea (Cunliffe *et al.,* 2001; Raboni *et al.,* 2002). Transmission of Rotavirus occurs through the feco–oral route. As the virus is stable in the environment, transmission can occur through ingestion of food or water (CDC, 2011). The main symptoms of the infection are fever, stomach cramps, vomiting and diarrhea.   Rotavirus is shed continuously in large numbers during the course of the disease and is usually detected up to a week after infection or for more than 30 days in immune compromised patients.

The AIDS epidemic has progressed with an estimated 34 million people living with HIV infection worldwide (UNAIDS, 2011). India has the third largest number of HIV/AIDS infection with 2.39 million people living with it (NACO, 2012). Gastrointestinal involvement is common in HIV/AIDS. Diarrhea is a common clinical manifestation of HIV infection, regardless of whether or not the patient has AIDS (Joshi *et al.,* 2002). It is more prevalent in HIV sero-positive patients then in HIV sero-negative patients (Mohammed *et al.,* 2003).

Early detection of Rotavirus would help in proper management and treatment of patients. Rapid, accurate and an early diagnosis is necessary to prevent severe complications due to Rotavirus infection. Various techniques are used for the detection of rotavirus like electron microscopy, enzyme immune assay (EIA), Immunofluorescence, Polyacrylamide gel electrophoresis (PAGE), radioimmunoassay (RIA), tissue culture etc. But these techniques are tedious and time consuming. Latex agglutination is a rapid, simple and sensitive test for the detection of rotavirus. In this study we

have used Pastorex® Rotavirus (Biorad, France) Latex agglutination test kit for the direct detection of Rotavirus antigen from stool samples in HIV sero-positive and HIV sero-negative patients with diarrhea.

Materials and Methods

The present study was carried out in the Department of Microbiology, Topiwala National Medical College and BYL Nair Charitable Hospital, Mumbai, India. The study was approved by the local Ethics Committee of BYL Nair Charitable Hospital. Informed consent was obtained from the patients before enrolling them for the study. A total of 126 patients (74 HIV sero-positive and 52 sero-negative) with diarrhea, admitted or attending the Outpatients department (OPD) were enrolled.

HIV status of the patients was confirmed by standard protocol as per WHO strategy III by testing antibodies against HIV by ELISA followed by two HIV rapid test kits at the Voluntary Counseling and Testing Centre (VCTC), BYL Nair Charitable Hospital, Mumbai, India.

(a) Selection of cases

Inclusion criteria: Patients with diarrhea for the first time or with recurrent attacks of loose motion along with passage of mucus and / or blood, vomiting, abdominal pain and weight loss were included.

Exclusion criteria: Patients without history of diarrhea and / or those who did not consent for the study.

(b) Collection of stool specimens

The patients were instructed to collect the specimen in a clean, dry, wide mouth sterile container with a tight lid which was provided to them. They were asked to take care that the specimen should not be contaminated with water or urine. Patients who brought formed stools were instructed to repeat the specimen. The samples were subjected to Latex agglutination test after direct microscopic examination and routine bacteriological analysis immediately. The samples were stored at 4ºC and processed within 24 hrs and aliquot of samples were stored at -80ºC.

(c) Latex agglutination test

Pastorex® Rotavirus code 52200 (Biorad, France) Latex agglutination test kit was used. All the reagents were brought to room temperature and mixed well before the test. The test was carried out as per the manufactures instructions. Results were subjected to appropriate statistical analysis wherever necessary.

Results

The present study was carried out on 126 patients (74 HIV sero-positive and 52 HIV sero-negative) suffering with diarrhea. As given in Table 1, out of 74 HIV sero-positive patients, 54 (42.86%) were male and 20 (15.87%) were females. Out of 52 HIV sero-negative patients, 31 (24.60%) were male and 21 (16.67%) were females. Table 2 shows the mode of transmission of the HIV virus among the HIV sero-positive patients. Most of the patients were in the age group 20 to 40 years (70.27%). 54 patients (72.97%) had history of heterosexual contact.

In Table 3, we see the comparison of the status of diarrhea with the HIV status of the patients. The incidence of chronic diarrhea was 72 (57.14%) and acute diarrhea was 54 (42.85%). As seen in Table 4, the symptom of weight loss was more (48.65%) in HIV sero-positive patients. Rotavirus positivity was more in HIV sero-positive patients (12.16%) than in HIV sero-negative patients (Table 5).

Discussion

Diarrhea is one of those things that no one likes to talk about but almost everyone has experienced. For most people, it is a short lived and highly unpleasant occurrence. But it can be dangerous for children, the elderly and the immune compromised. Diarrhea is divided into acute and chronic categories based on whether it is present for less than or greater than 4 weeks. Infectious diarrhea is the most common cause of diarrhea worldwide. Rotavirus infection is the leading cause of severe diarrhea in children aged less than 5 years.

Table 1: Categorization of the cases on the basis of HIV status, age and sex.

Age / Sex	HIV sero-positive patients (n = 74)		HIV sero-negative patients (n = 52)		Total
	Male	Female	Male	Female	
Less than 14	05 (6.76%)	06 (8.11%)	07 (13.46%)	08 (15.38%)	26 (20.63%)
15 – 19 years	02 (2.70%)	01 (1.35%)	02 (3.85%)	03 (5.77%)	08 (6.35%)
20 – 29 years	12 (16.22%)	02 (2.70%)	06 (11.54%)	02 (3.85%)	22 (17.46%)
30 – 39 years	27 (36.49%)	06 (8.11%)	09 (17.31%)	05 (9.62%)	47 (37.30%)
40 – 49 years	08 (10.81%)	04 (5.41%)	05 (9.62%)	01 (1.92%)	18 (14.29%)
50 +	00(0%)	01 (1.35%)	02 (3.85%)	02 (3.85%)	05 (3.97%)
Total	54 (72.97%)	20 (27.03%)	31 (59.62%)	21 (40.38%)	126 (100%)

Table 2: Correlation of age / sex with route of HIV transmission.

<20 years	20 to 40 years	>40 years	Mode of Transmission n = 74	Male	Female	Total
00 (0%)	04 (5.41%)	00 (0%)	Homosexual contact	4 (5.41%)	00 (0%)	4 (5.41%)
01 (1.35%)	45 (60.81%)	08 (10.81%)	Heterosexual contact	42 (56.76%)	12 (16.22%)	54 (72.97%)
02 (2.70%)	02 (2.70%)	00 (0%)	Blood Transfusion	02 (2.70%)	02 (2.70%)	04 (5.41%)
00 (0%)	01 (1.35%)	00 (0%)	Intravenous drug user	01 (1.35%)	00 (0%)	01 (1.35%)
11 (14.86%)	00 (0%)	00 (0%)	Mother to child	05 (6.76%)	06 (8.11%)	11 (14.86%)
14 (18.92%)	52 (70.27%)	08 (10.81%)	Total	54 (72.97%)	20 (27.03%)	74 (100%)

Table 3: Comparison of duration of diarrhea with HIV status.

Duration		HIV sero positive patients	HIV sero negative patients	Total
Acute diarrhea	No.	19	35	54
	%	25.68	67.31	42.85
Chronic diarrhea	No.	55	17	72
	%	74.32	32.69	57.14
Total	No.	74	52	126
	%	100.00	100.00	100.00
(p=3.33E−06)				

Table 4: Comparison of symptoms between HIV sero-positive and sero-negative patients with diarrhea.

Symptoms	HIV sero-positive patients n = 74	HIV sero-negative patients n = 52	Total n = 126
Weight loss	36 (48.65%)	16 (30.77%)	52 (41.27%)
Vomiting	26 (35.14%)	16 (30.77%)	42 (33.33%)
Nausea	8 (10.81%)	8 (15.38%)	16 (12.70%)
Fever	21 (28.38%)	23 (44.23%)	44 (34.92%)
Abdominal pain	25 (33.78%)	11 (21.15%)	36 (28.57%)
Malaise	1 (1.35%)	5 (9.62%)	6 (4.76%)

Table 5: Rotavirus detection by Latex agglutination test in HIV sero-positive and sero-negative patients with diarrhea.

Rotavirus	HIV sero-positive patients	HIV sero-negative patients	Total
Present	9 (12.16 %)	3 (5.76 %)	12 (9.52%)
Absent	65 (87.84 %)	49 (94.24%)	114 (90.48%)
Total	74 (100 %)	52 (100 %)	126 (100%)

Table 6: Comparison of clinical and Laboratory parameters of Rotavirus antigen positive in HIV sero-positive and HIV sero-negative patients with diarrhea.

Clinical & laboratory findings	Rotavirus positive	
	HIV sero-positive patients (n = 9)	**HIV sero-negative patients (n = 3)**
Symptoms		
1. Weight loss	5 (55.56%)	2 (66.67%)
2. Vomiting	7 (77.79%)	2 (66.67%)
3. Nausea	Not seen	Not seen
4. Fever	2 (22.22%)	3 (100%)
5. Abdominal pain	1 (11.11%)	2 (66.67%)
6. Malaise	Not seen	Not seen
Status of Diarrhea		
1. Acute diarrhea	1 (11.11%)	1 (33.33%)
2. Chronic diarrhea	8 (88.89%)	2 (66.67%)

Diarrhea in patients with AIDS can be caused by various factors like infection with conventional and/or opportunistic organisms or sometimes even by treatment with antibiotics. 30 to 90 % of patients with AIDS suffer from diarrhea at some point of their illness (Iyer *et al.,* 2001). The rapid worldwide spread of AIDS has placed an urgent need to halt the spread of the pandemic, care for those persons affected and manage the vast social and ethical impact of this disease.

In this study, we have compared the prevalence of Rotavirus infection in HIV sero-positive and HIV sero-negative patients with diarrhea. At the same time we have also studied the sign and symptoms observed in these patients.

A total of 126 (n) patients suffering with diarrhea and satisfying the inclusion criteria were studied. Out of these patients, 74 (58.73%) patients were HIV sero-positive and 52 (41.27%) patients were HIV sero-negative (Table 1). Out of the 74 HIV sero-positive patients 54 (72.97%) were male and 20 (27.03%) were female patients. The male to female ratio was 2.7. There were more male patients than female patients. The mean age in our study was 33 (1-65) years. 33 (44.59%) patients were in the age group of 30-39 years. 11(14.86%) patients were in pediatric group. Most of the patients were in the age group of 30 to 39 years (37.30%). Malebrache *et al.* (1983) found the mean age to be 32-54 years, which was similar to that observed in our study. 65.52 % patients were male and 27.03% were female patients which were lower than our study. Barbara *et al.* (1983) reported the mean age of participants was 35 years (21 – 29 years) and Phillip *et al.* (1988) reported the mean age of patients as 37 years

and all patients were males in their studies. These observations were not in accordance with our study. Prasad *et al.* (2000) reported 77 % were males and 23% were females while the mean age was 32 years. This result was in accordance with our study. Mohammed *et al.* (2003) in his study reported 110 (57.3%) male patients and 82 (42.7%) female patients. The male patients were lower in numbers and female patients were higher than our data.

In a comparative analysis of HIV sero-positive and sero-negative patients, we observed that there was a significant difference in the age and gender distribution. Most HIV sero-positive patients were in the age group of 30 to 39 years. In HIV positive patients 54 (72.97%) were male and 20 (27.03%) were female while sero-negative patients 31 (59.62%) were males and 21 (40.38%) were females. In our study, 54 (72.97%) patients had a history of heterosexual contact as the probable rout of transmission of HIV virus followed by mother to child transmission 11 (14.86%) (Table 2).

The incidence of chronic diarrhea was 55 (74.32%) in HIV sero-positive patients while 17 (32.69%) in HIV sero-negative patients. The incidence of acute diarrhea was 19 (25.68%) in HIV sero-positive patients while 35 (67.31) % in HIV sero-negative patients. The difference was statistically significant (p=3.33E–06) (Table 3). As compared, the incidence of chronic diarrhea was higher in HIV sero-positive patients and the incidence of acute diarrhea was higher in HIV sero-negative patients.

Rotavirus antigen was detected in 12 (9.52%) stool samples by latex agglutination technique. In HIV sero-positive patients, rotavirus was detected in 4 (5.41%) male patients and 5 (6.76%) female patients. In HIV

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negative patients, rotavirus was found in 1 (1.92%) male and 2 (3.85%) female patients. Peter *et al.* (1983) identified rotavirus as the only etiological agent in 5% of adults with diarrhea. The prevalence rate is lower than our study. The most common symptoms observed were nausea (86%), headache (79%), vomiting (61%) and fever (61%). The values did not coincide with those in our study. Barbara *et al.* (1988) observed rotavirus positivity of 3.45% in sero-positive patients with diarrhea. The prevalence rate was low as compared to that in our study. Gassama *et al.* (2001) observed rotavirus positivity in 8.2% patients. The prevalence rate was lower as compared to that in our study. Sharif *et al.* (2000) detected rotavirus in 38.7% of their samples. This value was higher than that observed in our study.

Phukan *et al.* (2003) detected rotavirus in 23.27 % stool samples. Vomiting (100%), fever (57.45%), dehydration (95.74%) was observed with rotavirus infection. The prevalence rate of rotavirus positivity was higher than our study and symptoms values did not coincide with those in our study.

Prevalence of weight loss was high (48.65%) in HIV sero-positive patients while it was found to be only 30.8% in HIV sero-negative patients. This difference was statistically significant (p=0.045). The prevalence of fever in HIV sero-negative patients was high (44.23%). Prevalence of malaise was higher in HIV sero-negative patients (9.62%) as compared to sero-positive patients (1.35%). The difference between them was statistically significant (p=0.032).

The Rotavirus positivity was higher in HIV sero-positive patients as compared with HIV sero-negative patients. Vomiting and weight loss were predominantly seen in both HIV sero-positive and sero-negative patients. Fever was seen as a most common symptom in HIV sero-negative patients. Rotavirus positivity was observed more commonly in chronic diarrhea in both the HIV sero-positive and HIV sero-negative study groups.

Latex slide agglutination technique was used for the detection of Rotavirus antigen in the present study. Mascarenhas *et al.* (1997) used electrophoretic profiles for the characterization of Rotavirus strains. Adeyi *et al.* (2010) used ELISA for identification of Rotavirus infection. Lee *et al.* (2007) compared Latex agglutination, Immunochromatography and Enzyme immunoassay and found Latex agglutination test to be rapid and easy to perform but showed low

sensitivity. They found Immunochromatography to be the test of choice. Paesi (2012) evaluated the Latex Agglutination Kit for Rotavirus detection and found it to be high in sensitivity and specificity and a valuable tool for diagnosing rotavirus infections.

Conclusion

Latex slide agglutination technique was used for the detection of rotavirus antigen in the present study. A rapid diagnosis of rotavirus by detection of rotavirus antigen provided the physicians with useful information. This technique has further simplified the diagnosis of rotavirus making it a bed side procedure. Rapid diagnosis may prevent inappropriate treatment and help prevent spread of infection.

Ethical Approval

This study was approved by the institutional ethics committee of TN Medical College and BYL Nair Charitable Hospital, Mumbai, India.

Conflict of Interests

The authors declare that they have no conflicting interests with regard to this study.

Authors' Contributions

All authors take full responsibility for the content of the paper and contributed equally to the work.

Acknowledgement

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