

Original Article

A Comparative Study of Rotaviral Antigen Detection by ELISA and ICT in Children below Five Years with Acute Diarrhoea in A Tertiary Care Hospital

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Abstract

Rotavirus is responsible for acute severe watery diarrhoea in young children. Early and rapid detection of rotavirus infection can help to reduce inappropriate administration of antibiotics and has future positive impact on prevention of drug resistance. This cross-sectional study was designed to determine the role of rotaviral antigen detection by ICT from stool sample of acute diarrhoeal children below five years admitted in Sylhet MAG Osmani Medical College Hospital, Sylhet and was carried out in the department of microbiology in collaboration with the department of paediatrics during the period from 1st January to 31st December, 2018. Total 184 children of under five years of age with acute watery diarrhoea were enrolled in this study. Rotaviral antigen was detected by ELISA (Enzyme Linked Immunosorbent Assay) and ICT (Immunochromatographic test) from stool samples. Out of 184 stool samples, rotaviral antigen was found positive in 84 and 86 cases by ICT and ELISA methods, respectively. ICT showed sensitivity of 90.70% and specificity of 93.88% when compared with ELISA. The rotavirus infection was found

highest in male children (61.90%) and in age group of 7 to 12 months (51.89%). Considering the importance of rotaviral diarrhoea, rapid detection of rotavirus infection by ICT is essentially needed and should be practiced routinely as it is relatively reliable, easy to perform and cost-effective. It is particularly important in Bangladesh, where diarrhoea is still contributing a significant proportion of mortality and morbidity in under five children.

Keywords: Rota virus, diarrhoea, ICT, ELISA, sensitivity, specificity

INTRODUCTION

Acute diarrhoeal disease is a major public health problem leading to high morbidity and significant mortality in both developed and developing countries¹. In Bangladesh, diarrhoea is the second commonest cause of death in children below five years after pneumonia. UNICEF estimated that diarrhoea is responsible for 6% of total under five deaths in the year 2013 in Bangladesh². Among hospitalized children, most of the deaths are due to diarrhoeal complications³. There are several bacterial, parasitic and viral agents responsible for acute gastroenteritis. Among viral agents, Rotavirus is the major cause of acute severe dehydrating diarrhoea in young children.^{1,4}

Rotavirus is transmitted from one person to another through the faeco-oral route having a very low infective dose.

Rotaviruses infect and multiply in the cytoplasm of enterocytes in the villi of small intestine. NSP4, one of virus encoded protein, is an enterotoxin which induces secretion by triggering a signaling transduction pathway. Damaged villus cells are replaced by non-absorbing immature crypt cells which leads to impaired glucose and sodium absorption. Consequently, there is loss of water and electrolyte which leads to dehydration, acidosis, shock and even death. Restoration of normal gut function requires 3 to 8 weeks.^{5,6}

Rotaviral infection is responsible for significant morbidity and mortality. Globally, this pathogen alone causes approximately 215,000 under five deaths. In Bangladesh, rotavirus causes 1000-2700 deaths each year in children below 5 years of age.⁷

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There are different methods used in rotavirus diagnosis like tissue culture, electron microscopy, enzyme linked immunosorbent assay (ELISA), latex agglutination techniques, immunochromatographic test (ICT) and reverse transcriptase polymerase chain reaction (RT-PCR).⁸

Direct detection of viral particles by electron microscopy is conclusive evidence of rotaviral infections but it is not available everywhere. Rotavirus can be isolated from stool sample by culture but it is a cumbersome process and needs equipped laboratories and skilled personnel⁹. RT-PCR although a very sensitive diagnostic tool, is available in only few reference and research centers and is particularly used for identification of outbreaks.⁸

Detection of antigen in stool sample is easy, rapid, relatively inexpensive and very sensitive method. It is a non-invasive procedure and there is no need of specialized laboratory facilities. So, the recent advancement of antigen detection methods based on immunological techniques like ELISA and ICT have gained the attention of researchers and clinicians.⁹

A study conducted by Agarwal and coresearchers in Delhi, India used both ELISA and ICT to detect stool antigen of rotavirus and showed that ICT had a sensitivity of 96.97% and specificity of 100% when compared to ELISA as a standard method.¹⁰

Rapid and accurate methods for the detection of rotavirus is important both for diagnosis and management of acute gastroenteritis. Rapid diagnosis also has a role in preventing spread of the disease. Due to high morbidity and significant mortality rate of rotavirus infection, there is a need for rapid and reliable methods like ELISA and ICT for Rotavirus detection in routine diagnostic laboratories.

ELISA is a sensitive method for detection of Rotaviral antigen and is ideal for screening of large number of fecal specimens in single sitting. Though it is a reliable method, it requires proper laboratory facilities and is time consuming. On the other hand, ICT is rapid, easy, single step procedure and can be done for a single specimen even at bed side. It can save time, labour and cost. It will help the clinician to take prompt decision regarding management.

So, this study was designed to compare between ICT and ELISA and also to evaluate the efficacy of ICT as a sensitive, rapid and easy diagnostic tool in diagnosis of Rotaviral diarrhoea among children less than 5 years.

MATERIALS AND METHOD

This cross-sectional study was carried out in the department of Microbiology in collaboration with the department of Paediatrics, Sylhet MAG Osmani Medical College Hospital from 1st January 2018 to 31st December 2018. All admitted children under 5 years of age with acute watery diarrhoea were included in this study. Children suffering from chronic diarrhea (diarrhoea for ≥ 14 days) and bloody diarrhea were excluded. After explaining the purpose of the study, informed written consent was taken from legal guardians of the patients. Prior to the beginning of this study, approval of the research protocol was obtained from the Ethical Review Committee of Sylhet MAG Osmani Medical College, Sylhet.

Specimen collection

Fresh stool samples were collected from the patients after admission in properly labeled clean, dry, wide mouthed, leak-proof plastic containers were used. Stool samples were carried within 1 to 2 hours to the virology laboratory of the department of microbiology.

Laboratory procedure

Macroscopic examination was done for colour, consistency, presence of mucus, blood or worms in stool samples. Then ICT was done in fresh samples. After performing ICT, samples were stored at -20°C until ELISA was done.

Antigen detection:

• Detection of rotavirus by ELISA:

Monoclonal antibodies against the product of the sixth viral protein (VP6) used in a sandwich type method. The assay was carried out according to the manufacturer's instructions.

• Detection of rotavirus by ICT:

ICT is a single-step, immuno-chromatographic lateral-flow test. Two bands (red and blue band) appear to indicate rotavirus positive. If only the blue band is visible, it is Rotavirus negative. It was carried out according to the manufacturer's instructions.

For data analysis, χ^2 test was done and $p < 0.05$ was considered significant.

RESULTS

Table-I Shows It was revealed from our study that, prevalence of Rotaviral diarrhoea (ICT positive) was highest (51.89%) among 7-12 months of age group children followed by 13-24 months of age group children (36.36%) .

Table I : Prevalence of Rotavirus diarrhoea (ICT +ve) among under 5 years old children according to age group (n=184)

Variable	ICT (+ve)		ICT (-ve)		P value
	N	(%)	N	(%)	
Age (months)					
0-6	8	30.77	18	69.23	0.004*
7-12	55	51.89	51	48.11	
13-24	16	36.36	28	63.64	
25-59	2	20.00	6	80.00	

Table-II Shows It was found from our study that, prevalence of ELISA positive Rotaviral diarrhoea was highest among 7-12 months age group (50.56%) followed by 13-24 months age group (48.28%) .

Table II: Prevalence of Rotavirus diarrhoea (ELISA +ve) among under 5 years children according to their age (n=184)

Variable	ELISA (+ve)		ELISA (-ve)		P value
	n	(%)	N	(%)	
Age (months)					
0-6	2	11.11	16	88.89	0.004*
7-12	50	50.56	40	49.44	
13-24	28	48.28	30	51.72	
25-59	6	33.33	12	66.67	

From our study, we found that prevalence of Rotaviral diarrhoea (both ELISA and ICT positive) was found higher among male children (61.90%) compared to female children (38.10%) (p= 0.247).

Table-III showed that stool antigen was found positive in 84 (45.65%) and negative in 100 (54.35%) patients by ICT and was positive in 86 (46.74%) and negative in 98 (53.26%) patients by ELISA.

Table III: Distribution of study population according to stool antigen test by ICT and ELISA

Diagnostic test	Positive	Negative	Total
ICT	84(45.65%)	100(54.35%)	184 (100.0)
ELISA	86(46.74%)	98(53.26%)	184 (100.0)

Table-IV showed that stool antigen was found positive in 78 cases by both ICT and ELISA and negative in 92 cases by both ICT and ELISA. However, 6 cases were false-positive as these 6 cases were positive by ICT but

found negative in ELISA. Again, 8 cases were false-negative as these 8 cases were negative by ICT but found positive in ELISA.

Table IV: Cross tabulation between total number of positive and negative cases observed by ICT and ELISA

Diagnostic Test ICT	ELISA		Total
	Positive	Negative	
Positive	78	6	84
Negative	8	92	100
Total	86	98	184

Considering ELISA as the gold standard diagnostic test, ICT revealed 90.70% sensitivity and 93.88% specificity. Positive predictive value (PPV), negative predictive value (NPV) and accuracy of ICT were found as 92.86%, 92.00% and 92.39% respectively.

DISCUSSION

In the present study, highest prevalence of Rotaviral diarrhoea was found in children of 7-12 months of age group (51.89%). This is in agreement with the results of a study done in Nigeria by Junaid et al. (2011) where most of the infected children (42%) were found between 7 to 12 months of age group¹¹. Ahmed (2009), Salwa (2014) and Dhiman (2015) also reported the same.^{12,13,14} It appeared that infants below 6 months of age are initially protected to some extent against rotavirus diarrhoea due to presence of maternal antibodies. After 6 months when maternal antibody decreases, rate of infection increases (Majumder et al., 2018).¹⁵ In this age group (7 – 12 months), children start crawling and develop tendency to put almost everything into mouth which can increase the chance of infection (Ahmed et al., 2009).¹² Another reason can be that the weaning is started at this age. So, there is chance of contamination of food during preparation if hand washing and food hygiene is not maintained properly. Frequency of rotaviral infection was less in higher age group due to acquisition of antibody by natural infection (Junaid et al., 2011).¹¹

In this study, highest prevalence of rotaviral diarrhoea was found among male (61.90%). This result is in agreement with previous Bangladeshi studies, where it was reported that around 58% (Roy et al., 2012)⁹ and 54% (Verkerke et al., 2016)¹⁶ children were male. Similar result was found from an Indian study done by Agarwal and co-workers where 62.7% male children were affected (Agarwal et al.,

2016)¹⁰. This male predominance is not clearly understood. It can be explained by social reason that the tendency of parents to prioritize their male children than female in seeking any kind of health care. This finding can also be explained by more resistance to infection in females due to XX chromosome (Dhiman et al., 2015).¹⁴

Present study found that ICT is 90.70% sensitive and 93.88% specific for diagnosis of Rotaviral diarrhoea compared to ELISA. This finding is in agreement with Momenzadeh¹⁷ and Salwa.¹³ Considering ELISA as gold standard, Momenzadeh et al. compared ICT with ELISA and found the sensitivity and specificity of ICT to be 87.7% and 98.6%, respectively and Salwa et al. found sensitivity and specificity of ICT 90.0% and 100%, respectively. Rougemont et al. tested ICT in comparison to ELISA and found sensitivity and specificity of ICT 83.0% and 99.9%, respectively. They suggested that ICT and ELISA were fairly comparable. They also suggested ICT as an effective and alternative diagnostic test for Rotaviral infection and it is also good for ambulatory practice.¹⁸ Kim and his co-workers compared ICT with ELISA, ELFA (enzyme linked fluorescent assay), real-time PCR, and mRT-PCR (multiplex reverse transcription PCR) and found that the ICT had no interference and an acceptable agreement rate with the ELISA, ELFA, real-time PCR and mRT-PCR. Therefore, ICT method can be useful in clinical practice for the rapid detection of Rotavirus infection.¹⁹ Considering ELISA as standard test, Agarwal et al. (2016) compared ICT with ELISA and found ICT was equivalent to ELISA regarding sensitivity and specificity. It is rapid, easy to perform, requires less handling of the sample and easy to interpret in the routine clinical laboratory and made the diagnosis simple, rapid, cost-effective and convenient. ICT has another important advantage that it can be kept in room temperature. On the other hand, ELISA is relatively expensive and equipments are not readily available in the health facilities of low resource countries.¹⁰

CONCLUSION

This study demonstrated that ICT is a useful method for the rapid screening of rotaviral antigen as it has the advantage of being quicker, convenient application, cost-effective, easy to perform, long shelf life, useful for testing single specimen, not requiring additional equipment and easy interpretation. As rotaviral infection causes severe dehydrating diarrhoea, rapid diagnosis by applying ICT may help in prompt treatment decision. It is particularly important in Bangladesh, where diarrhoea is still contributing a significant proportion of mortality and morbidity in under five children.

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