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A COMPARATIVE STUDY OF PARASITIC AND ROTAVIRUS A GASTROENTERITIS IN CHILDREN BELOW 5 YEARS AT EASTERN UTTAR PRADESH

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Abstract
Diarrheal diseases continue to be a major concern for developing countries. An estimated 248 million episodes of diarrhea occurs every year in children under 5 years. A diversity wide enough, these diarrheal agents include non-infectious (e.g., toxins) and infectious agents (bacterial, protozoal, and viral).
323 stool samples were collected which were tested for the presence of parasites , Rotavirus antigen through ELISA and Rotavirus A was confirmed through RT-PCR.
Among the 231 OPD patients, Rotavirus positive cases were found to be 95 (41.1 %), 31(13.42 %) were infected with parasites and other diarrhoeal cases were 105 (45.45 %).
Of the 92 IPD patients, children infected with Rotavirus A were 48 (52.2 %), 13 (14.13 %) were infected with parasites whereas other diarrhoeal cases were 31 (33.7 %)
Co-infection of Rotavirus-A with parasites were found in six patients (4 Giardia , 2 Entamoeba histolytica)

Keywords: Diarrhoea , Rotavirus A, prasites, co-infection

Introduction

Diarrheal diseases continue to be a major concern for developing countries. An estimated 248 million episodes of diarrhea occurs every year in children under 5 years [(1)]. In both developed and developing countries, viruses are the leading cause of acute diarrhea. Whereas, in low and middle-income countries, bacterial and protozoal pathogens also play an important role [(2)].
India has an estimated 457,000-884,000 hospitalizations and 2 million outpatients visits for diarrhea. Acute diarrheal disease accounts for 13% of the mortality among children under 5-year of age.
A diversity wide enough, these diarrheal agents include non-infectious (e.g., toxins) and infectious agents (bacterial, protozoal, and viral) [(2, 3)].
Globally, the commonest bacterial pathogens causing gastroenteritis are *Campylobacter* spp (especially *Campylobacter jejuni*), *Vibrio cholerae* (the cause for cholera), *Salmonella* spp,

including *Salmonella typhi* (typhoid fever), *Shigella* spp, *Yersinia enterocolitica*, and a variety of enteropathogenic *Escherichia coli* strains, including the enterotoxigenic *E. coli* (ETEC) strain [2].
The intestinal protozoan like *Giardia intestinalis*, *Cryptosporidium* and *Entamoeba histolytica* also known to cause diarrheal illness [2,4,5].
In high- income countries, viruses account for the majority of gastrointestinal infections, with about 70% of disease reported to be of viral origin [2,6]. Among the viral agents, rotaviruses and noroviruses, together with adenoviruses, astroviruses, and sapoviruses are the major etiological agents of viral gastroenteritis in children [7].
Acute diarrheal disease accounts for 13% of the mortality among children under 5-year of age.

Materials and Methods

It was tertiary hospital based cross-sectional study for children under 5 years with diarrhoea / acute gastroenteritis who came in as IPD/OPD patients in Dr KNS Memorial Institute of Medical Sciences ,Barabanki

1. Inclusion and Exclusion criteria

Inclusion criteria: Children under 5 years with acute gastroenteritis or diarrhoea alone were included in the study.
Exclusion criteria: Children under five years with dysentery or Parents/children not accepting to participate were excluded from the study
Institutional Ethical Committee approval was taken before initiating the study.

2. Sample collection

Parents were instructed to collect stool specimen of the child in a sterile wide mouthed screw capped plastic container (15-20 ml) provided to them and submit it in the laboratory within an hour of collection.

3. Sample processing

The sample was divided into two parts:

i. The first part was used for routine microscopic / macroscopic examination for detection of parasites
ii. The second part of the sample was stored at -20 degrees with buffer for further investigation of rotavirus antigen.

a. Routine Microscopy

Wet mount was prepared using Saline and Lugol's iodine and stool sample and observed under 10X and 40X objectives.
Screening of the slide was done for the presence of ova, cyst or trophozoite.

b. ELISA

Rotavirus antigen was detected in stool sample using EDI Fecal Rotavirus ELISA kit. The assay utilizes the microplate-based enzyme immunoassay technique by coating highly purified antibody onto the wall of microtiter well. Assay controls and fecal specimen, as well as HRP-conjugated monoclonal antibody that specifically recognize the inner capsid protein of the rotaviruses.

c. REAL TIME PCR DETECTION OF ROTAVIRUS A

Helini Rotavirus -A Realtime PCR kit was used for detection of Rotavirus A from stool sample.
Initial sample preparation was done using Helini Purefast stool processing buffer.
The stool was transferred in the stool processing buffer, vortexed and then centrifuged at 13000 RPM for 5 minutes. The supernatant was used for RNA purification.
HELINI Purefast viral nucleic acid mini spin prep kit was used for RNA purification as per the kit's instruction.

The detection of Rotavirus A was done with Helini Rotavirus-A Realtime PCR Kit.

Result

- A total of 323 stool samples from children with gastroenteritis were collected which comprises of both OPD and IPD cases. Among the children suffering from gastroenteritis 174 were boys and 149 were girls.
- Majority of the children suffering gastroenteritis were from the age group of 6-12 (123/323 i.e.38.1%) months followed by 13-24 (94/323 i.e. 29.1%) months and the least from 25-60 months (46/323 i.e. 14.2%). **(TABLE 1)**

TABLE 1. AGE AND GENDER WISE DISTRIBUTION OF GASTROENTERITIS

AGE	MALE	FEMALE	TOTAL
(In Months)	**Number (%)**	**Number (%)**	**Number (%)**
0-6 Months	32(53.3%)	28(46.7%)	60(100%)
6-12Months	71(57.7%)	52(42.3%)	123(100%)
13-24 Months	53(56.4%)	41(43.6%)	94(100%)
25-60 Months	18(39.1%)	28(60.9%)	46(100%)
Total	174(53.9%)	149(46.1%)	323(100%)

- Of the 323 cases of gastroenteritis, majority of the patients were from OPD i.e., 231 whereas IPD constituted of 92 patients.
- Among the 231 OPD patients, Rotavirus positive cases were found to be 95 (41.1 %), 31(13.42 %) were infected with parasites and other diarrhoeal cases were 105 (45.45 %).
- Of the 92 IPD patients, children infected with Rotavirus A were 48 (52.2 %), 13 (14.13 %) were infected with parasites whereas other diarrhoeal cases were 31 (33.7 %). **(Table 2)**
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TABLE 2. WARD WISE DISTRIBUTION OF ROTAVIRUS CASES

WARD	NUMBER OF CASES	NUMBER OF ROTAVIRUS POSITIVE CASES	NUMBER OF CASES WITH PARASITIC INFECTION	NUMBER OF OTHER DIARRHOEAL CASES
	Number (%)	**Number (%)**	**Number (%)**	**Number (%)**
OPD	231	95(41.1%)	31(13.42 %)	105 (45.45 %)
IPD	92	48(52.2%)	13 (14.13 %)	31 (33.7 %)
Total	323	143(44.3%)	44 (13.62 %)	136 (42.1%)

- Infection due to Rotavirus A showed a similar inclination as corresponding to the overall cases of gastroenteritis among children.
- Positive cases were high among the age group of 6-12 months followed by 13-24 months and lowest among 0-6 months.
- Parasitic infection was hight among 13-24 months followed by 25-60 months. No parasitic infection was found among children under the age group of 0-6 months of age.

Other cases for gastroenteritis was high among the age group of 6-12 months followed by 0-6 months and least in the age group of 25-60 months.

TABLE 3. AGE WISE DISTRIBUTION OF ROTAVIRUS A, PARASITES AND OTHER DIARRHOEAL CASES

AGE	NUMBER OF CASES	NUMBER OF ROTAVIRUS A CASES	NUMBER OF CASES WITH PARASITIC INFECTION	NUMBER OF OTHER DIARRHEAL CASES
(In Months)	**Number**	**Number (%)**		**Number (%)**
0-6 Months	60	19(31.7%)	0 (0.0%)	41(68.3%)
6-12Months	123	63(51.2%)	11 (8.9%)	49(39.8%)

13-24 Months	94	41(43.6%)	19(20.2%)	34(36.1%)
25-60 Months	46	20(43.5%)	14(30.4%)	12(26.0%)
Total	323	143(44.3%)	44(13.6%)	136(42.1%)

- Parasites were found be in 44 patients suffering from gastroenteris where the number of boys were 26 and the girls were 18 in number.
- The parasites detected were Giardia, Entamoeba histolytica, Taenia and Ascaris.
- Entamoeba hystolytica constituted of 21 patients followed by Giardia which was 17 in number. Taenia and Ascaris were 3 each .
- Co-infection of Rotavirus-A with parasites were found in six patients (4 Giardia , 2 Entamoeba histolytica)

Discussion

The study shows a prevalence of gastroenteritis higher among boys (53.86%) than in girls (46.13 %) in the age less than 5 years. A similar trend was noticed in a study by Khan et al where prevalence was around 61.4% among boys and 38.6 among girls [8] Study conducted by Kazi et al also had higher percentage among boys (57%) and significantly lower among girls (43%). [9]

In a contrasting study conducted by Sreekant P et al in the slums of southern India showed higher prevalence of gastroenteritis among females (52.1%) than in males (47.9%). [10]

This study showed higher prevalence in the age group of 6-12 months (38.1%), followed by 13-24 months (28.1%) and among 0-6 months (14.2%). A similar age distribution was unavailable for gastroenteritis.

Study by Saha J et al showed an overall prevalence of diarrhea of 14.4% in the age group of 0-11 months followed by 12-23 month (13.89%) and lowest being 48-59 months (4.75%). [11]

The study showed prevalence of Rotavirus at 44.3% which is close to study conducted by Arun, P at Chennai where the prevalence rate was 41.64%. [12]

In the present study, proportion of children having high rotavirus infection were from the age group of 6-12 months (44.05%) followed by 13-24 months (28.67%) and least among the age of 0-6 months (18.6%).

The age group of 6-12 months, where infection with RVA is high i.e., 44.05% is proportionate with Girish Kumar, C et al also had similar outcomes based on age which was 40.7% in the age group of 6-11 months. [13]

Parasitic infection was hight among 13-24 months followed by 25-60 months. No parasitic infection was found among children under the age group of 0-6 months of age.

A similar trend was found in the study done by Shariq et al in Behraich , Uttar Pradesh. [14]

Conclusion

These findings show a higher prevalence of Rotavirus A infection among the rural population. This call for renewed efforts for preventing rotavirus infection by increasing efforts to improve vaccination coverage, water and sanitation. The rise in parasitic infection suggests more stringent adherence to deworming process among children.

Limitations

Bacterial, fungal and viral agents of diarrhoea were not detected

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