The role of rotavirus associated with pediatric gastroenteritis in a general hospital in Lagos, Nigeria

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Abstract

Introduction Bacterial, viral and parasitic agents have been implicated and confirmed as causative agents of gastroenteritis in children with ages below 5 years old. The major role of rotavirus as causative agent is not widely recognized within the public health community, particularly in developing countries. This study examined the role of rotavirus as a causative agent of childhood gastroenteritis in infants and young children below 5 years of age in a General Hospital in Lagos, Nigeria.

Methods Parents and caregivers of children admitted to the hospital were interviewed using a structured questionnaire. Viral RNA was extracted from the stool samples collected and analyzed using RT-PCR for genotyping and agarose gel electrophoresis for identification of rotavirus electrophoretotypes.

Results Out of the 71 samples analyzed, 16 (22.5%) were positive for rotavirus. A total of 12 (75%) males and 4 (25%) females were positive for rotavirus gastroenteritis with most cases (7, 43.8%) distributed to the 13-24 months age group, followed closely by the 1-6 months age group, with 6 cases, 37.5%. Rotavirus G2 genotype was the most prevalent strain in the hospital (10 patients, 62.5%) followed by G1 (6 patients, 37.5%). These were the only rotavirus genotypes detected in the hospital.

Keywords Rotavirus, gastroenteritis, children.

Introduction

Bacterial, viral and parasitic agents have been implicated and confirmed as causative agents of gastroenteritis in children with ages below 5 years old. Rotavirus gastroenteritis is a major cause of hospitalization in children¹ with recent studies reporting a maximum incidence in the age group 13-24 months.² However, the major role of rotavirus as causative agent of gastroenteritis and dehydration is not widely recognized within the

public health community, particularly in developing countries. Despite the existence of approved, efficient vaccines, national immunization schedules often leave out rotavirus from the list of mandatory campaigns. Strain surveillance is important when it comes to rotavirus detection and characterization for the antigenic and molecular identification of rotavirus strains circulating in the locality. The incidence, clinical significance and major role of rotavirus will be widely recognized and possible risk factors determined in order to reduce child illness and death from diarrhea.

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The main objective of this study is to determine the role of rotavirus associated with pediatric gastroenteritis in a General Hospital in Lagos Nigeria in order to update policies and generate information for government intervention to reduce the morbidity and mortality due to rotavirus infection in children less than five years old in the study area.

Methods

Study site

This study was conducted in the Maternal and Childcare Center Clinic of the Randle General Hospital, Gbaja, Surulere, Lagos State of Nigeria in children who reported to the hospital with symptoms of acute diarrhea.

Study Population

The Maternal and Child Care Center of the Randle General Hospital, Surulere, Lagos is a General Public Hospital owned by the Lagos State Government of Nigeria and Managed by the Lagos State Health Management Board. It serves mainly the population of Surulere district of the city of Lagos with about 300,000 inhabitants.

A systematic stool sample of children below 5 years of age was obtained. The patients were admitted with acute diarrhea (defined as 3 or more watery or unformed offensive stools per day) with or without vomiting. After initial examination of the patients by a doctor (pediatrician), the mothers were interviewed and a detailed questionnaire regarding the management and course of the illness before hospital admission was completed.

Fecal Samples

A stool sample was collected from each child on admission and was transported on ice on the same day to the virology research laboratory of the Department of Medical Microbiology and Parasitology of the University of Lagos College of Medicine, Lagos University Teaching Hospital (LUTH), University of Lagos, Nigeria for examination. Bloody stools associated with dysentery were excluded from this study.

Fecal Sample Processing

Fecal samples were collected into clean, dry, leak-proof, universal containers by mothers or caregivers. The samples were frozen and stored

on ice at -20°C prior to use for rotavirus agarose gel electrophoresis and PCR genotyping. Ten percent suspensions of the sample in 1x phosphate buffered saline (PBS) were prepared.

Double-stranded ribonucleic acid (dsRNA) genome extraction

Each of the stool samples was subjected to the process of dsRNA genome extraction using the Qiagen RNA extraction Kit. Into each sterile Eppendorf vial containing lysis buffer (AVL), 140 µL of stool suspension were added and the vial was shaken vigorously on a vortex mixer at room temperature.

A loopful of diatomaceous sand (Qiagen, Germany) was added to each vial followed by 560 µL ethanol; the mixture was allowed to stand at room temperature for 20 minutes while being shaken intermittently every 5 minutes on a vortex mixer. The mixture was then spun at 13,000 rpm for 1 minute; supernatant was discarded. The pellet in each vial was then subjected to serial washings using two different kinds of washing buffer (AW1 and AW2; 500µL each) (Qiagen, Germany) and acetone 400 µL. Each washing step was concluded with spinning at 13,000 rpm for 1 minute with the resulting supernatant poured off. The pellet was then dried on the heat block for 15 minutes. This was followed by the addition of 100 µL RNase-free water, vigorous shaking on a vortex mixer and spinning down at 13,000 rpm for 2 minutes. The resultant supernatant, containing the dsRNA extracts was carefully removed, using a 200 µL pipette set at 60 µL, avoiding the sand and dispensed into new sterile Eppendorf vials.

Gel electrophoresis of rotavirus dsRNA

All the stool suspensions were subjected to a rapid and initial screening test using agarose gel electrophoresis. A 200 mL of 1.5% agarose with 1x Tris-acetate EDTA (TAE) buffer was prepared. The mixture was heated until boiling on a hot plate.

The clear colorless solution was then allowed to cool to about 45°-50°C before being poured gently, to avoid air bubbles, into a gel electrophoresis tray. A manufacturer's comb fitted into the gel container impacted the wells (into which the RNA extracts were loaded) and upon removal the gel was cast. The gel contained

1-23 sample wells. Each well was carefully loaded with 10 µL of sample (dsRNA extract) mixed with 2 µL loading dye inside the well plates. The electrophoresis tank was filled with TAE buffer as the running buffer. Electrophoresis was carried out at room temperature for 30-45 minutes at 80V. Finally, the separated dsRNAs in the gel were visualized, under ultraviolet (UV) light transilluminator for the photodocumentation of the distribution of the rotavirus electrophoretotypes obtained.

Reverse-transcription polymerase chain reaction (RT-PCR) of rotavirus dsRNA

5 µL of the extracted RNA were transferred into a PCR tube. The master mix was prepared and added into the extracted rotavirus RNA in the PCR tube for the amplification of the rotavirus dsRNA by RT-PCR.

Amplification of the rotavirus dsRNA by RT-PCR was carried out using the Ag path-ID (Applied Biosystems, Life Technologies Inc, USA) which contain core reagents for one-step real-time RT-PCR (qRT-PCR) detection and genotyping of rotavirus.

The RT-PCR master mix was prepared by adding 12.5 µL 2KX RT-PCR buffer, forward PCR primer con I and reverse PCR primers T-I, T-2, T-3, T-4, TaqMan probes, 1 µL 25 x RT-PCR enzyme mix, and 1 µL RNA sample, nuclease-free water to the PCR tube containing the RNA extract to make up a total volume of 25 µL reaction mixture each, according to the number of samples. RT-PCR master mix was prepared on ice to a volume of 25 µL. A quantity of 5-10% extra master mix was also prepared.

Negative controls, which included a duplicate no-template control using nuclease-free water instead of sample was also prepared. The RT-PCR master mix of 25 µL total volume per reaction mixture each was distributed into the PCR tubes and subsequently amplified by incubation in a thermocycler with ROX passive reference dye included in the RT-PCR buffer.

The reaction volume of 25 µL was amplified by incubation in a thermocycler using the following cycling conditions: 1. Reverse transcription (45°C, 10 min), 2. RT inactivation/initial denaturation (95°C, 10 min), 3. Amplification (95°C, 15 s).

In the amplification of the extracted and purified rotavirus RNA, the thermal cycle was run and RT-PCR data analyzed according to the manufacturer's instructions. The tubes containing the amplified RT-PCR products were arranged on a rack ready to be identified by electrophoresis in agarose gel, with a 100 base pair (bp) DNA ladder serving as a molecular marker in addition to the negative and positive control templates included in the reaction.

Identification of the RT-PCR products

The RT-PCR products were subjected to agarose gel electrophoresis for the detection and confirmation of the rotavirus electrophoretotypes.

1.5 g of agarose powder was weighed and mixed with 100 mL of 1x TAE buffer (Molecular Biology Grade - Genaxxon BioScience) in a conical flask. The combination was put in an oven to gel. The flask containing the gel was put in cold water trough to gel. After cooling, the gel was poured into the electrophoresis tank, mould or plastic tray with 5 mm comb that makes holes for the loading of reaction mixtures. 5 µL of each amplified dsRNA/RT-PCR reaction mixture were mixed with 1 µL of 6x loading buffer per hole. This was then loaded into the holes on the gel alongside the gene maker (fermenters) with a molecular weight of 100 bp or 1kb DNA ladder. The agarose gel underwent electrophoresis at 120V for 30 minutes.

RNA amplification was evaluated according to the indicators of the band sizes. Standard markers supplied by the manufacturers were used to read the results obtained. Electrophoresis was used to identify rotaviral dsRNA segments and to visualize the bands of specific segment sizes for different viral genotypes. We also studied the distribution and diversity of RNA electrophoretotypes. Negative and positive control templates were included in the reaction in addition to the gene marker loaded in a hole in the gel to act as a standard for the evaluation of the electrophoresis results.

Results

A systematic stool sample of 71 children admitted with symptoms of acute diarrhea in the

Randle General Hospital, Surulere, Lagos, Nigeria was examined for rotavirus infection.

Out of the 71 gastroenteritis patients studied between August 23, 2012 and September 23, 2012, 55 (77.5%) of them were rotavirus negative while 16 (22.5%) of them were rotavirus positive.

Clinical and other features	Rotavirus gastroenteritis patients (n=16) No. (%)	Non-rotavirus gastroenteritis patients (n=55) No. (%)
Diarrhea	16 (22.5)	55 (77.5)
Vomiting	8 (11.3)	30 (42.3)
Fever	5 (7.0)	18 (25.4)
Dehydration	11 (15.5)	44 (62.0)
Lower respiratory tract infections	2 (2.8)	12 (16.9)
Hospital stay (>7 days)	9 (12.7)	12 (16.9)

Table 1. Clinical and other features of gastroenteritis patients.

Vomiting was less frequent in rotavirus positive patients 8 (11.3%) compared to rotavirus negative gastroenteritis patients 30 (42.3%). A total of 5 (7%) of the patients that were rotavirus positive had fever while 18 (25.4%) of the non-rotavirus gastroenteritis patients had fever. Dehydration featured in 11 (15.5%) of the rotavirus positive cases compared to 44 (62%) in the rotavirus negative cases. A total of 2 (2.8%) of the rotavirus positive patients had lower respiratory tract infections (manifested by cough), compared with 12 (16.9%) of the rotavirus negative patients.

The patients that were hospitalized for more than 7 days were 9 (12.7%) of the rotavirus positive patients and 12 (16.9%) of the non-rotavirus gastroenteritis patients. The results are illustrated in Table 1.

A total of 12 (75%) males were rotavirus positive patients while 4 (25%) females were positive for rotavirus gastroenteritis with most cases (7, 43.8%) distributed to the 13-24 months

age group, followed closely by the 1-6 months age group, with 6 cases, 37.5% (Table 2).

Age group (months)	Number of rotavirus positive patients (n=16)	Male No. (%)	Female No. (%)
1-6	6 (37.5)	4 (25.0)	2 (12.5)
7-12	2 (12.5)	2 (12.5)	0 (0.00)
13-24	7 (43.8)	5 (31.3)	2 (12.5)
25-36	0 (0.0)	0 (0.0)	0 (0.0)
37-48	0 (0.0)	0 (0.0)	0 (0.0)
49-60	1 (6.3)	1 (6.3)	0 (0.0)
Total	16 (100%)	12 (75)	4 (25.0)

Table 2. The distribution of rotavirus infection by gender and age group.

The rotavirus genotypes circulating in the hospital were the G1 and G2 strains (Table 3).

Rotavirus genotype (n=16)	Number of patients No. (%)
G1	6 (37.5)
G2	10 (62.5)
Total	16 (100)

Table 3. Rotavirus genotypes circulating in the hospital.

Figure 1. Gel electrophoresis of dsRNA: UV illuminator photodocumentation of the rotavirus samples. Samples 1 through 23. M Marker; PC positive control; NC negative control

The agarose gel electrophoresis results shown in Figure 1 indicate that lanes 21, 22, and 23 are genotypes G1, G2 and G2 rotavirus positive respectively (Table 4). Other lanes in this figure are rotavirus negative. The band size of lanes 21,

22, and 23 were 180, 248 and 244 bp respectively.

Serial Number (S/N)	Band Size (bp)	Genotype	Result
21	180	G1	Positive
22	248	G2	Positive
23	244	G2	Positive

Table 4. Gel electrophoresis of dsRNA interpretation. Samples 1 through 23.

Figure 2. Gel electrophoresis of dsRNA: UV illuminator photodocumentation of the rotavirus samples. Samples 24 through 46. M Marker; PC positive control; NC negative control

The agarose gel electrophoresis results shown in Figure 2 indicate that lanes 24, 27, and 30 are genotypes G2 rotavirus positive respectively (Table 5). Other lanes in this figure are rotavirus negative. The band size of lanes 24, 27, and 30 were 251, 253 and 255 bp respectively.

Serial Number (S/N)	Band Size (bp)	Genotype	Result
24	251	G2	Positive
27	253	G2	Positive
30	255	G2	Positive

Table 5. Gel electrophoresis of dsRNA interpretation. Samples 24 through 46.

Figure 3. Gel electrophoresis of dsRNA: UV illuminator photodocumentation of the rotavirus samples. Samples 47 through 56. M Marker; PC positive control; NC negative control.

The agarose gel electrophoresis results shown in Figure 3 indicate that lanes 48, 50, 54, 55, and 56 are genotypes G2, G1, G1, G1, G2 and G2 rotavirus positive respectively. Other lanes in this figure are rotavirus negative. For positive lanes, the band size is presented in Table 6.

Serial Number (S/N)	Band Size (bp)	Genotype	Result
48	332	G2	Positive
50	210	G1	Positive
51	225	G1	Positive
54	230	G1	Positive
55	320	G2	Positive
56	315	G2	Positive

Table 6. Gel electrophoresis of dsRNA interpretation. Samples 47 through 56.

Figure 4. Gel electrophoresis of dsRNA: UV illuminator photodocumentation of the rotavirus samples. Samples 57 through 71. M Marker; PC positive control; NC negative control

The agarose gel electrophoresis result shown in Figure 4 indicate that lanes 65, 69, 70 and 71 are genotypes G1, G1, G2 and G2 rotavirus positive respectively. Other lanes in this figure are rotavirus negative. For positive lanes, the band size is presented in Table 7.

Serial Number (S/N)	Band Size (bp)	Genotype	Result
65	209	G1	Positive
69	215	G1	Positive
70	322	G2	Positive
71	310	G2	Positive

Table 7. Gel electrophoresis of dsRNA interpretation. Samples 57 through 71.

Discussion

In previous studies on the prevalence of rotavirus diarrhea, rotavirus was responsible for 21% of gastroenteritis cases in Nigeria.³ A percentage of 22.5% in this study showed that rotavirus disease burden is still increasing in developing countries like Nigeria. Our observation of a higher prevalence in children within the age brackets below 24 months of age and a lower prevalence in the groups of 24 to 60 months of age may mean that our children are

infected much earlier in life but go on to develop resistance to reinfection, unlike the findings in previous studies where Altindis et al. found a prevalence of 7.14% in the 0-12 months age bracket.⁴ This might help in determining the optimum age for vaccination.

This conflicts with findings from the study by Parashar et al. where rotavirus infection was uncommon under 3 months of age, interpreted as a possible protective role of placental transferred maternal antibodies among infants in this age bracket.⁵ However, Cicirello and co-workers in 1994 had documented high infection rates in newborns in six hospitals in India and a predisposition of these infants to infection with unusual rotavirus strains.⁶

Also, the prevalence of asymptomatic rotavirus infection has been documented in literature to be common among the under 5 months of age group and adults, although parents of children with rotavirus diarrhea, immunocompromised patients (including those with HIV), the elderly and travelers to developing countries may occasionally develop illness.⁵

While rotavirus gastroenteritis remains a serious public health problem in the developing world and given the extraordinary diversity of the virus in some countries, determined efforts will be needed to establish rotavirus disease surveillance systems that are adequately sensitive and specific.

In our study, vomiting and fever were less frequent among patients positive with rotavirus than in those with non-rotavirus diarrhea. Surprisingly, dehydration was less frequent in the rotavirus group as well, possibly explained through the fact that all children were monitored and treated in the hospital. A larger number of boys were admitted to the hospital than girls.

In our study, rotavirus was most commonly found among gastroenteritis patients aged 1 to 23 months. This finding agrees with the studies carried out in Singapore² and Sao Paulo, Brazil⁷ but differs from the observations made by Audu and co-workers in Nigeria.⁸

Rotavirus infection occurs throughout the year in Nigeria because of the relatively less defined seasonal variation, constant temperature and humidity in the tropics. However, this

infection is known to show a characteristic seasonal pattern: in developed countries with temperate climates, peak incidence occurs in the winter; however, in developing countries with tropical and subtropical climates, the virus appears to circulate the year round.⁹

Therefore, one of the limitations of this study was the short time span of sample collection, leading to the impossibility of evaluating the seasonal differences. Other limitations of the study were that we did not perform immunological screening tests on fecal samples prior to PCR testing and that we were not able to perform P genotyping. Nonetheless, seeing as the results of this study positively correlate with other similar studies in field literature, as described above, we consider the results of this study to be representative for the children population in Lagos, Nigeria.

Recommendations

Through sustained efforts of basic, applied and operational research carried out essentially in the developed countries, data on rotavirus diarrhea in children have been defined. Tools have been developed for the diagnosis. The natural history and clinical presentation of the disease in children have been elucidated. The role of rotavirus as a major cause of gastroenteritis, dehydration and death in children has been documented and disseminated to the people through effective communication channels. Significant achievements have been made in the area of drug development and research for the treatment of infected subjects. Attempts have been made to develop and administer vaccines for the prevention of rotavirus diarrhea infection in children below five years of age.

However, because the strains differ in various geographical areas, a global epidemiological intervention is hard to design. Data obtained from studies in the developed countries have led to a better understanding of the epidemiology and trend of the disease within their various populations.

Based on a clear understanding of the dynamics of the disease in these developed countries, sustainable, well-targeted, cost-effective

strategies for the control of the disease were developed and implemented.

The resultant impact is a decrease or stabilization of the incidence of the disease in most of these developed countries. On the contrary, the incidence of the disease in most of the developing countries is still increasing at an alarming rate despite the fact that in the past several years, strategies for the control of the disease have been in place in developing countries.

The fact remains that in trying to develop control strategies for rotavirus diarrhea in children, data generated from populations in the developed countries are often extrapolated to populations in the developing countries. This is mainly due to the fact that very little information is available on the disease in developing countries and where available, accessibility to them is often difficult. Control strategies developed on extrapolated data will most often not be effective because the biological and epidemiological factors of rotavirus diarrhea in children have been documented to be region or country-specific. More so, human, viral and other microbiological factors which determine transmission and disease progression rates differ from one country to another.

For the disease to be effectively controlled, it is therefore imperative that each developing country generate and articulate relevant data and information that are essential for a better understanding of the role of rotavirus diarrhea in children in their respective countries. Such generated and collated data will provide the much needed scientific basis for the formulation of more sustainable, cost-effective control strategies that will yield the desired results in their various communities.

A continuous collation of such data will not only monitor the trend of the disease in our communities but will also help give an indication of which components of the control strategies need periodic strengthening.

There is need to evaluate the incidence of pediatric diarrhea in Nigeria and also collate and articulate reliable quantitative and qualitative national epidemiological data.

As a result of the vast nature of the country, budget and logistic reasons, the project for the determination of the role of rotavirus in pediatric diarrhea in a hospital setting was carried out as a first phase but it should serve as springboard for other similar surveys in other communities in Nigeria.

It is envisaged that the collated data will give a clear picture of the true epidemiology and trend of the disease in the country over the years.

In Nigeria, several diarrhea control programs and studies have been going on in the past several years but despite this, there is presently no authoritative national epidemiological or clinical data on pediatric diarrhea in Nigeria and there has been no concerted effort to collate, update and coordinate these data. This has informed the urgent need to pull together existing epidemiological data on various aspects of the epidemic in Nigeria as a basis for producing a definite up-to-date and persuasive document on the scale, distribution, dimension and impact of pediatric diarrhea in the country which will comprise the generation of Epi-Surveillance data and bibliography database.

Generating a national baseline data for rotavirus diarrhea in the country is highly needed because of the paucity of information regarding the prevalence of rotavirus gastroenteritis in developing countries. There is also need for meaningful planning and evaluation of the rotavirus pediatric gastroenteritis control program as the few studies that have so far been carried out are limited to a few hospitals and do not give any idea about the actual prevalence in the general population.

In Nigeria, the awareness, utilization and impact of the Rotarix vaccine (GlaxoSmithKline, UK) is quite low as it has only been in use in this country for a few years. The level of awareness regarding vaccination should increase among caregivers of children. The cost of the vaccine should be subsidized to make it affordable, if not free, to caregivers.

A national compendium of all stakeholders on rotavirus diarrhea should be established and produced for general circulation to serve as a resource document on rotavirus response activities in the country.

Conclusion

Data obtained from this study have provided updated information on the incidence and trend of diarrhea in children in the study area. This information will be disseminated to relevant bodies through a circulation of this paper.

The distribution of diarrhea cases in children by age and gender will give a clearer picture of the impact of diarrhea in children.

This study will help develop the next phase of the National program for the prevention, control and management of diarrhea in children in Nigeria. Results from this study will also serve as reference information for dissemination to various interest groups on the role of rotavirus in diarrhea in children which will be known and will provide an insight into the situation to facilitate the development of appropriate strategies and health reforms for the effective control of the disease. This will also serve as a springboard for other similar surveys in other communities in Nigeria.

Conflicts of interest All authors – none to declare.

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