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Molecular Detection of Astrovirus, Rotavirus (A and B) in Children Less than 5 Years with Gastroenteritis in Aljazeera State, Sudan

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ABSTRACT

Background: Diarrheal diseases represent a major worldwide public health problem particularly in developing countries. Each year, at least four million children under five years of age die from diarrhea. Although rotavirus is a leading cause, other viruses including astrovirus are also important, but have been the subject of limited studies. The objective of this study was to estimate the Rotavirus and astrovirus gastroenteritis among children less than 5 years.

Methods: This study was active surveillance cross sectional study included a total of 66 diarrhea specimens collected from children less than 5 years with gastroenteritis in Aljazeera Children's Hospital and Omdurman children Hospital during January to May2017.From all specimens RNA was extracted, followed by Real Time-PCR amplification to detected Rotavirus and astrovirus was performed. Results:

From 66 samples, one positive in Aljazeera samples (1,5%) while no positive in Khartoum samples for Rotavirus infection by RT-PCR. While for Astrovirus there is one positive in Aljazeera samples (1.5%) and one positive in Khartoum samples (1.5%) for Astrovirus infection by RT-PCR.

Conclusion: The current study showed that infection with astroviruses may be an important cause of gastroenteritis, as well as rotavirus, these findings highlight the need to implement rotavirus and astrovirus detection assays in clinical diagnosis and the nosocomial prevention of gastroenteritis viral infections in pediatric departments. It is recommended to conduct genotyping of Rotavirus on large samples before starting vaccination in the country.

Keywords : Rotavirus, Astrovirus, Diarrhea, Vomiting.

I. INTRODUCTION

Acute gastroenteritis is a common disorder in young children, and the associated dehydration is a leading cause of admission to hospital in industrialized countries and major sources of mortality in developing countries [1]. Although global deaths from diarrheal disease have decreased from 2.6 million to 1.3 million between 1990 and 2013], it remains a major health concern, particularly in Africa [2] Young children are known to be the most affected population, and there were an estimated 450 million diarrheal episodes among children <5 years of age in 2010 in Africa [2] Enteric viruses have been recognized as the most significant etiological agents of the disease, and four categories of viruses are being considered

clinically relevant: group A rotavirus (family: Reoviridae), norovirus (family Caliciviridae), adenovirus 40/41 (subgenus F), and astrovirus [18,19,20] Astrovirus (AstVs) are enteric viruses that can cause gastroenteritis in children and a severe disease in immune compromised and elderly people [3, 4]. Astrovirus belong to the family Astroviridae; the non-enveloped virion is small, with a diameter of 28 nm, and contains a single stranded positive-sense RNA of 6.8 kb. The viral nucleic acid consists of open reading frames (ORF), including ORF1a, ORF1b and ORF2 [5]. The prevalence rate of human astrovirus (HastV) infection ranged from 2 % to 9 % among children with diarrhea [6]. The morbidity varies depending on the season, with higher infection during the winter in temperate climates and the rainy season in tropical regions [7]. The main symptom of astrovirus infection is watery diarrhea, which is often associated with vomiting, fever, and abdominal pain [4].

Rotaviruses are non-enveloped, double stranded RNA viruses with 11 gene segments under Reoviridae family having seven major groups (A-G) [8]. Group A Rotaviruses are the major causes of diarrhea among the infants and young children all over the world [9].
Rotavirus is the most common cause of severe diarrhea in children, resulting in the hospitalization of approximately 55,000 children each year in the United States [9, 10]. In the developing world rotavirus may account for 1 million childhood deaths as well as significant morbidity each year [11]. However, in developing countries, rotavirus gastroenteritis account for more than 800,000 childhood deaths per year due to poor nutrition and health care [12].

Children in the poorest countries account for 82% of rotavirus deaths [13].The main symptoms of rotavirus gastroenteritis(RVGE) are fever, abdominal pain, lethargy; diarrhea and vomiting that may lead to hypo volemic shock and dehydration [14,15]. Severe cases may lead to death [16].

II. MATERIAL AND METHOD

Study area and sample collection

Type and duration of this study was active surveillance cross sectional study, aiming to determine the astrovirus and rotavirus among children less than 5 years of age visiting Aljazeera Children's Hospital in Aljazeera state and Omdurman children Hospital in Khartoum state, Sudan was conducted. The study was carried out during January to May 2017.A total number of66 stool specimens was collected from children less than 5 years with acute diarrhea, using sterile clean containers. Then 1mL of diarrhea sample placed into sterile tube containing 5mL phosphate buffered saline, the suspensions were centrifuged for 20 min, then filter the supernatant in clean tube and stored at -2o°C until used.

RNA extraction:

Total RNA was extracted by using the QIAamp Viral RNA Mini spin according to the protocol of the manufacturer (Qiagen, Germany). Briefly, 140 µl of diarrhea suspended sample was added to 560 µl buffer AVL containing carrier RNA, and then incubated at room temperature for 10 minutes. Subsequently, 560 µl of ethanol (96- 100%) was added to the sample after which 630 µl of the resulting solution was applied to a column. A volume of 500 µl of AW1 and AW2 was added for washing and the nucleic acids were eluted with 60 µl AVE buffer and stored at -80°C until used.

Real time RT-PCR

Real-time one step RT-PCR was done to detect viral RNA by using a commercial kit following the manufacturer's instructions (One-Step Real-Time RT-PCR Master Mixes Kit, In vitrogen, genesig standard kit).

With the primer/probe set 1, RNaseP was used as an internal control and real-time PCR was carried out following the protocol provided by manufacturer's instructions. The real time PCR master mix for one

reaction was prepared as follows: 10 µl of 2XqRT-PCR Mastre mix (consisting of a proprietary buffer system, MgSO4, dNTPs, and stabilizers),1 µl of primer/probe, 4 µl of RNAse/DNAse free water. The final volume was 15 µl for a single reaction. The reaction was performed in an automated 7500 real-time PCR (AB Applied Biosystems, USA). The thermal cycling conditions were 10 minutes at 55°C for reverse transcription, 2 minutes at 95°C for enzyme activation and, 10 seconds at 95°C for denaturation and 60 seconds at 60°C for annealing and extension. A sample whose growth curve crossed the threshold line within 40 cycles (Ct < 40) was considered as positive.

III. RESULT

From 66 sample there is one +ve in Aljazeera samples and no positive in Khartoum samples for Rotavirus infection by RT-PCR(1.5%) while (98.5%) were not, belonged to geno groups A and B and the dominant genotype is A in Aljazeera.

And for Astrovirus from the 66 samples there is one +ve in Khartoum samples and one positive in ljazeera samples for Astrovirus infection by RT-PCR (3.03%) and (98.96%) were not. And (50%) were male and (50%) were female among 2 Astrovirus positive patient.

Table 1. distribution of virus type of the patients

Total Sample	Rota Virus (A)	Rota Virus (B)	Astro Virus
66	1	0	2
100%	1.5 %	0 %	3 %

Table 2. Distribution of symptoms among rotavirus and Astrovirus infected children

Symptoms	Number	Percent %
Children with diarrhea, vomiting and fever	14	21.2 %
Children with diarrhea and vomiting	16	24.2 %
Children with diarrhea and fever	20	30.4 %
Children with diarrhea only	14	21.2 %
Children with vomiting only	2	3 %
TOTAL	66	100 %

Table 3. Distribution of children according to age and gender

Gender / Age	Male	Female	Total
0 up to 12 months	6 (20 %)	10 (27.8 %)	**16 (24.2%)**
More than 12 up to 36 months	16 (53.3%)	14 (38.9%)	**30 (45.5%)**
More than 36 up to 60 months	8 (26.7%)	12(33.3%)	**20 (30.3%)**
Total	30 (45.4%)	36 (54.6%)	**66 (100%)**

Table 4. distribution of Rotavirus in infected children

	Rotavirus(A)	No of cases. (%)
Gender	Male	1 (1.5%)
Age	3 years	1 (1.5%)
Symptom	Diarrhea-vomiting fever	1 (1.5%)

Table 5. distribution of Astrovirus in infected children

	Astrovirus	No of cases. (%)
Gender	M\F	2 (3%)
Age	(1-3) years	2 (3%)
Symptom	Diarrhea-vomiting fever.	1 (1.5%)
	Diarrhea-vomiting	1 (1.5%)

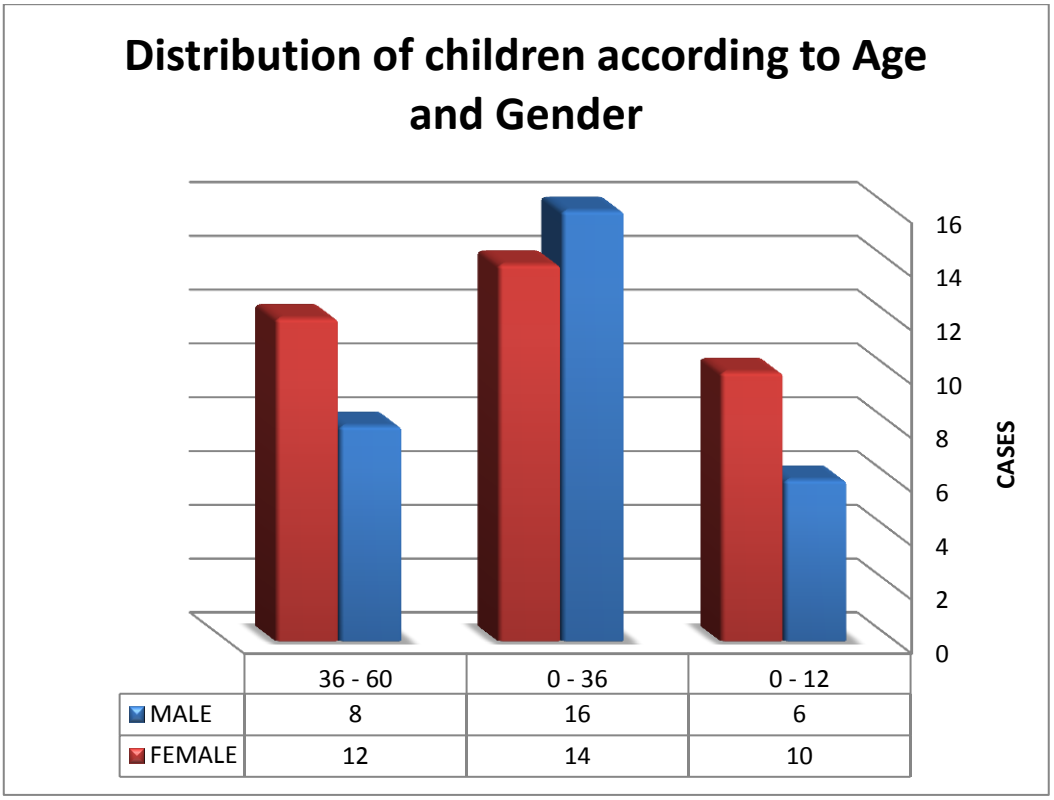

Figure 1. Distribution of children according to Age and Gender

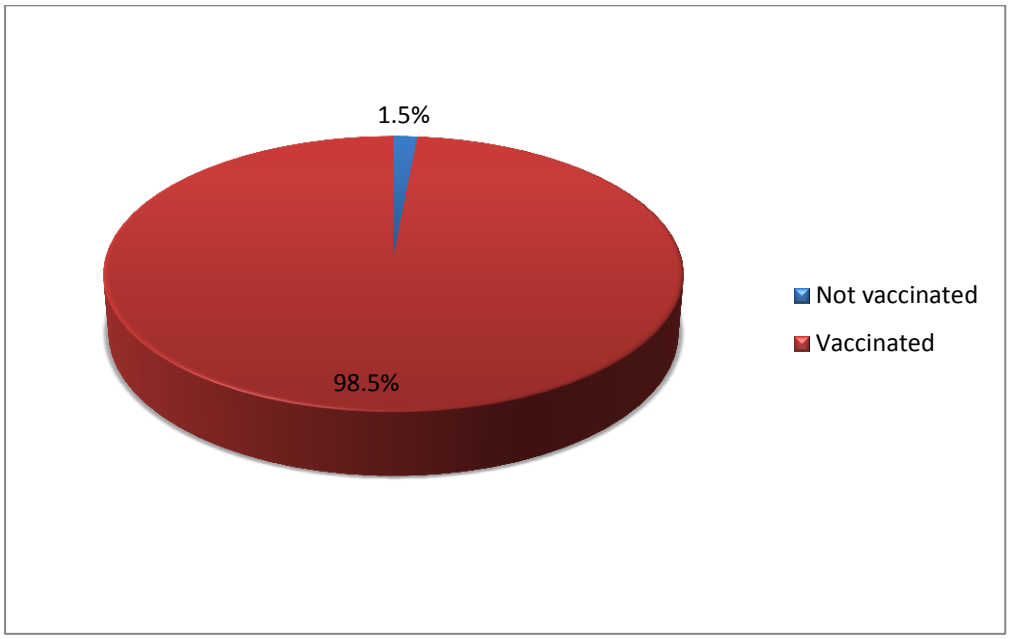

Figure 2. Distribution of Rotaviruses genotype

IV. DISCUSSION AND CONCLUSION

Diarrhea is major causes of morbidity in developed and developing countries. In addition, diarrheal illnesses account for an estimated 12,600 deaths each day in children in Asia, Africa, and Latin America. The causes of diarrhea include a wide range of viruses, bacteria, and parasites, many of which have been recognized only in the last decade [17].gastroenteritis is a common disease associated with significant morbidity, mortality and costs in the Middle East and North Africa. The results of this study may be useful as background information to the planning and implementation of efficient vaccination programs. A vaccine with broad and consistent serotype coverage would be important to help decrease the burden of gastroenteritis in the Middle East and North Africa.
The present study indicates that Astroviruses and Rotavirus are relatively important gastroenteritis viral infections in children less than 5 years with gastroenteritis in Aljazeera Children's Hospital and Omdurman children Hospital (3% and 1.5% respectively).

Out of the 66 patients, viral pathogens were detected in only 3 cases (5%). These findings highlight the need to implement rotavirus and astrovirus detection assays in clinical diagnosis and the nosocomial prevention of gastroenteritis viral infections in pediatric departments.

In several international reports, it was evident that viral pathogens are the most common cause of gastroenteritis in developed countries Worldwide [21, 22-24]. Gastrointestinal rotavirus infections result in an estimated 440,000 deaths in children fewer than five years of age [25].

In several countries, researches revealed a higher prevalence than in our study using the different or the same detection procedure.

Detection of rotavirus infection in Sudanese children has been reported in Melut district (nowadays belongs to Republic of South Sudan) but the rate of infection was not stated [27]. In agreement of our prevalence 16% Parashar et al reported 18% of diarrhea in clinical settings due to rotavirus [26]. In contrast to regional and international reports our result is similar to the 17% prevalence reported from Tunisia [28] and Kenya [29] but higher than the 13% prevalence rate reported from Libya [30].

In Study reported in Tunisia the study shows prevalence of astrovirus (4 %) in children higher than our results [32]. as well as in other countries such as in France (6 %), Italy (3.1 %), Spain (4.9 %) and India (5.8 %) [27].

Since this study is hospital-based, the 5% prevalence rate may not reflect the true prevalence among Sudanese children, thus a community-based surveillance is needed. The limitation of this study, we are not genotyping the Sudanese Rotavirus and Astrovirus to look for the specific typing of the viruses.

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VI. REFERENCES

[1]. Parkin, PC., CMacarthur, AKhambalia, RDGoldman, and JN.Friedman1 June 2009Clinical and laboratory assessment of dehydration severity in children With acute gastroenteritisClinPediatr49:235–239[Epub ahead of print.]

[2]. GBD 2013 Mortality and Causes of Death CollaboratorsGlobal, regional, and national agesex specific allcause and causespecific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013Lancet2014Epub 2014/12/23doi: 10.1016/s01406736 (14)616822 PMID: 25530442.

[3]. Walter JE, Mitchell DKAstrovirus infection in childrenCurr Opin Infect Dis.2003;16(3):247–53.

[4]. Mendez E, Arias CFAstrovirusesIn: Knipe DM, Howley PM, Cohen JI, Griffin DE, Lamb RA, Martin MA, Racaniello VR, Roizman B, editorsFields virology5th edPhiladelphia: LippincottWilliams and Wilkins; 2007p981–1000.

[5]. Astrovirus Gastroenteritis in Children in Taipei HsiaoChuan Lin,1 ChuanLiang Kao,2 LuanYin Chang,3 YuChia Hsieh,3 PeiLan Shao,3 PingIng Lee,3 ChunYi Lu,3 ChinYun Lee,3 LiMin Huang3.

[6]. Bosch A, Pintó RM, Guix SHuman astrovirusesClin Microbiol Rev2014; 27(4):1048–74.

[7]. Nguyen TA, Hoang L, le Pham D, Hoang KT, Mizuguchi M, Okitsu S, et alIdentification of human astrovirus infections among children with acute gastroenteritis in the Southern Part of Vietnam during 2005–2006J Med Virol2008;80(2):298–305. 2009/Accepted 25 February 2010.

[8]. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, Jha P,Campbell H, Walker CF, Cibulskis R, et al: Global, regional, and nationalcauses of child mortality in 2008: a systematic analysisLancet 2010,375(9730):1969–1987.

[9]. Widdowson MA, Bresee JS, Gentsch JR, Glass RI: Rotavirus disease and itspreventionCurr Opin Gastroenterol 2005, 21:26–31.

[10]. Bines JE: Rotavirus vaccines and intussusception riskCurr OpinGastroenterol 2005, 21:20–25.

[11]. Kane EM, Turcios RM, Arvay ML, Garcia S, Bresee JS, Glass RI: Theepidemiology of rotavirus diarrhea in Latin AmericaAnticipatingrotavirus vaccinesRev Panam Salud Publica 2004, 16:371–377.

[12]. WHO: Rotavirus and other viral diarrheasBull WHO 2007, 58(2):183–198.

[13]. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI: Global illness and deaths caused by rotavirus disease in childrenEmerg Infect Dis 2003,9(5):565–572.

[14]. Diggle L: Rotavirus diarrhoea and future prospects for preventionBr JNurs 2007, 16(16):970–974.

[15]. Grimwood K, Lambert SB: Rotavirus vaccines: opportunities andchallengesHum Vaccin 2009, 5(2):57–69.

[16]. Parashar UD, Gibson CJ, Bresse JS, Glass RI: Rotavirus and severe childhood diarrheaEmerg Infect Dis 2006, 12(2):304–306.

[17]. Guerrant RL, Hughes JM, Lima NL, Crane J: Diarrhea in developed anddeveloping countries: magnitude, special settings, and etiologiesRevInfect Dis 1990, 12(Suppl 1):S41–S50.

[18]. de Wit, MA., MPKoopmans, LMKortbeek, WJWannet, JVinje, F.van Leusden, AIBartelds, and YTvan Duynhoven2001Sensor, apopulationbased cohort study on gastroenteritis in the Netherlands: incidenceand etiologyAmJEpidemiol154:666–674

[19]. Levidiotou, S., CGartzonika, DPapaventsis, CChristaki, EPriavali, N.Zotos, EKapsali, and GVrioni2009Viral agents of acute gastroenteritis in hospitalized children in GreeceClinMicrobiolInfect15:596–598

[20]. Oh, D.Y., GGaedicke, and ESchreier2003Viral agents of acute gastroenteritis in German children: prevalence and molecular diversityJMedVirol71:82–93

[21]. Mertens TE, Wijenayake R, Pinto MR, Peiris JS, Wijesundera MD, Eriyagama NB, Karunarathne KG, Ranaweera LR: Microbiological agents associated with childhood diarrhea in the dry zone of Sri LankaTrop Med Parasitol 1990, 41(1):115–120.

[22]. McIver CJ, Hansman G, White P, Doultree JC, Catton M, Rawlinson WD: Diagnosis of enteric pathogens in children with gastroenteritisPathology 2001, 33:353–358.

[23]. Lopman BA, Reacher MH, Duijnhoven V, Hanon YF, Brown XD, Koopmans M: Viral gastroenteritis outbreaks in Europe, 1995–2000Emerg Infect Dis 2003, 9:90–96.

[24]. Simpson R, Aliyu S, IturrizaGomara M, Desselberger U, Gray J: Infantile viral gastroenteritis: on the way to closing the diagnostic gapJ Med Virol 2007, 70:258–262.

[25]. Parashar UD, Breseee JS, Gentsch JR, Glass RI: RotavirusEmerg Infect Dis 1998, 4:561–570.

[26]. Bryce J, BoschiPinto C, Shibuya K, Black REWHO estimates of the causes of death in childrenLancet2005; 365 (9465): 114752

[27]. Sixl W, SixlVoigt B, Stünzner D, Arbesser C, Reinthaler F, Mascher F, Rosegger H, Schneeweiss W, Schuhmann GInvestigations in the problem of diarrhoea in the Melut district, South Sudan (19811982)J Hyg Epidemiol Microbiol Immunol1987; 31(4): 4869

[28]. Trabelsi A, Peenze I, Pager C, Jeddi M, Steele DDistribution of rotavirus VP7 serotypes and VP4 genotypes circulating in Sousse, Tunisia from 1995 to 1999: emergence of natural human reassortantsJ Clin Microbiol2000; 38(9):34159

[29]. Kiulia NM, Peenze I, Dewar J, Nyachieo A, Galo M, Omolo E, Steele AD, Mwenda JMMolecular characterisation of the rotavirus strains prevalent in Maua, Meru North, KenyaEast Afr Med J2006; 83(7): 360365

[30]. Rahouma A, Klena JD, Krema Z, Abobker AA, Treesh K, Franka E, Abusnena O, Shaheen HI, El Mohammady H, Abudher A, Ghenghesh KSEnteric pathogens associated with childhood diarrhea in TripoliLibyaAm J Trop Med Hyg2011; 84(6): 886891

[31]. Monastiri et alBMC Public Health (2016) 16:57 DOI 10.1186/s1288901627265.

[32]. Bosch A, Pintó RM, Guix SHuman astrovirusesClin Microbiol Rev2014; 27(4):1048–74.