ISSN No:-2456-2165

Genotypes of Rotavirus among Yemeni Children with Acute Diarrhea

Najla N. Al-Sonboli[1,2], PhD; Mabrook A. Bin Mohnna[1], M.D.;
Nasher A. Al-Aghbari[3], PhD; Zekra Al-Shaekh[2], M.Sc.; Basheer Abo Asba[4], M.Sc.
[1] Paediatrics Department, Medical Faculty of Medicine, Sana'a University, Sana'a, Yemen.
[2] Al-Sabeen Hospital for Maternity and Children, Sana'a, Yemen.
[3] Al-Thawra General Modern Hospital, Sana'a, Yemen.
[4] Ministry of Health and Population, Sana'a, Yemen.

Abstract:-

- ***Background***

Rotavirus gastroenteritis is an essential cause of morbidity and mortality among under five years children in the developing countries.

- ***Aim:***

Determination of its incidence and serotypes.

- ***Methods:***

It is a prospective cross-sectional study conducted in a tertiary hospital in Sana' as part of regional surveillance for rotavirus. Children <5 years with acute diarrhea were eligible for enrollment. The stool samples were collected and tested according to standard WHO guideline. Rotavirus was tested using enzyme linked immunoassay and the serotypes by PCR at the Regional Rotavirus Reference Laboratory, Cairo, Egypt. The data was processed and analyzed using Epi info program (version, 2008).

- ***Results:***

The incidence rate of Rotavirus was 24.4%. G12 constitutes 45.5%, G2 constitutes 19.5%, G1, and G9 14% each and G3 2.6% of the samples. The genotyping of P gene showed that P[8] constitutes 78% of the samples, P[4] 17%, P[6] 4%, and the un-typable (UT) only 1% of the samples. The rate of G-P combinations showed that G12P[8] in thirty-four samples; G2P[4] in thirteen samples, G1P[8] in eleven samples; G9P[8] in ten samples ; G[UT] P[8] in three samples; G2P[6] and G3P[8] in two samples each; and G9P[6] in one sample.

- ***Conclusion:***

The incidence rate of Rotavirus in Sana'a didn't change much despite the introduction of the vaccine. This could be attributed to the emergence of new serotypes such G12 and G9 that were not covered by the Rotarix vaccine. There will be a strong need to develop a new vaccine that cover these new emerging serotypes.

Keywords:- *Rotavirus, Children, Serotypes, Sana'a.*

I. INTRODUCTION

Rotavirus gastroenteritis (RVGE) is the most common cause of severe childhood acute gastroenteritis (AGE) worldwide and of diarrheal mortality in developing countries[1]. The highest infection rates for rotavirus are in children below five years of age, with 95% of children between the age of three and five years affected[2]. Rotavirus is transmitted by the fecal-oral route[3]. There is seasonality to rotavirus infection, with the majority of cases in temperate climates occurring in the winter months between November and February[3]. Seasonality in tropical and developing countries is less marked[2].

Diarrhea and vomiting are the main symptoms of RVGE that may lead to dehydration and shock. Severe dehydration may lead to death[2]. Other symptoms include fever and abdominal pain may be found[3,4]. The World Health Organization (WHO) estimates that 215,000 children under the age of five years die of rotavirus disease each year[5].

A, B, C, D, E, F, G, and H were the eight species of rotavirus[6]. Species A (mainly), B and C infect humans[7,8]. The most dominant, group A, causes diarrheal diseases worldwide[3]. Within rotavirus A there are different strains, called serotypes[9]. Rotavirus was classified based on two proteins on the surface of the virus. The G serotypes defined by the glycoprotein VP7 and the P serotypes defined by the protease-sensitive protein VP4[10]. The two genes that determine G-types and P-types can be passed on independently to progeny viruses; this led to different G-P combinations [11].

The incidence rate of RVGE in the Middle East ranged from 16% to 61%[12]. The epidemiology of rotavirus and the seroprevalence of G-P serotypes in Yemen and mainly Sana'a are unclear due to the secrecy of the studies. There was only one study in Sana'a. It was before the rotavirus vaccine introduction and reported an incidence rate of 27%[13]. Thus, there is a great need for this study to re-assess the epidemiology of rotavirus in Sana'a and the circulated serotypes. Also to evaluate the effect of the rotavirus vaccine that was introduced in Yemen in 2012.

ISSN No:-2456-2165

➢ *Aim:*

To determine the incidence of rotavirus and its genoypes among Yemen in children with acute diarrhea.

II. SUBJECTS AND METHODS

This study was a prospective cross-sectional study conducted for a period of 18 months, from April 2014 to December 2015. The Ministry of Health and Population (MOH&P) in Yemen implemented surveillance of AGE (including RVGE) in children less than 5 years of age at two sentinel hospitals in Taiz and Aden in 2004. Al-Sabeen Hospital for Maternity and Children in Sana'a, the capital, was selected as a third sentinel hospital in April 2014. It is a tertiary referral hospital with a capacity of 110 beds for children, 30 beds of them were dedicated to the infectious diseases including AGE. It has an extensive outpatient clinic, emergency unit, and oral rehydration corner. It serves mainly, Al-Amanah and Sana'a provinces with a population of more than two million. Sana' climate features the very rare mild version of a cold semi-arid climate[14], due to its high elevation (2,300 metres, 7500 ft), temperatures are much more moderate than many other cities on the Arabian Peninsula; average temperatures remain relatively constant throughout the year in Sana'a, with its coldest month being January and its warmest month in July. The city seldom experiences extreme heat or cold. Accordingly, it was hard to define the four seasons and for the practicality, we defined three seasons according to the daily mean of the temperature and the rain fall that recorded during 2014[14]. The period from November to April was assigned as "the cold season", May and October "the intermediate season" and the period from June to September "the hot season". The WHO Eastern Mediterranean Regional Office (EMRO) support these sentinel hospitals technically, and the standard operating procedures (SOPs) are described in the WHO generic protocol for rotavirus surveillance[15]. After informed consent, any child under 5 years old with a history of acute diarrhea (>3 times watery or loose stool per day for less than 7-10 days) attending the hospital was enrolled, consecutively. A standard case form was filled for each participant by a trained pediatrician on duty. The collected data include age (months), sex, duration of symptoms, diarrhea and/or vomiting (days), the frequency of episodes per day before the visit, and whether fever was present. Details of dehydration status (classified as mild, moderate and severe according to the WHO guideline), treatment was given, duration of hospitalization, and treatment outcome on discharge were also collected. Within 24-48 hours after enrollment, stool samples were collected from each patient in labeled containers according to the WHO–EMRO guidelines and sent immediately to the hospital's laboratory or kept under - 4 °C for over-night in the hospital laboratory then, in the morning they were tested using enzyme linked immunoassay (ELISA) using kits that supplied by the WHO (IDEIA™, DAKO Diagnostics, United Kingdom). For purpose of quality assurance, 10% of the samples were re-tested in the National Central Laboratory, Sana'a. According to SOP guidelines rotavirus-positive specimens were stored at −20 °C until sent to the Regional Rotavirus Reference Laboratory, the Egyptian Holding Company for Biological Products and Vaccines (VACSERA), 51, Wezarat El Zeraa St. Dokki, Cairo, Egypt for G and P genotyping using PCR. MOH&P team made monthly visits to the hospital and check the reports and the data quality. Mid-yearly and annual meetings of the hospital and the MOH&P teams were conducted to discuss the data and check its accuracy. A sample size of 467 was collected during the study period. All the clinical and laboratory data were transferred to a Microsoft Office Excel 2007 spread sheet and analyzed using Epi Info software version 3.5.1 (2008). Pearson's chi-square test was used for categorical variables and student's test for continuous variables with equal variance not assumed. The P value <0.05 was considered significant.Ethical approval from the MoH&P and Al-Sabeen hospital Ethical Committees were taken. Verbal consent after explanation to the legal guardians of the participants were taken before enrolment in the study. All the collected data was kept confidentially with limited access to the research team only.

III. RESULTS

The total number of enrolled children was 467. Figure 1 showed the number of children enrolled and those who found to be rotavirus positive according to the season. The cold season was the predominant season for enrollment (208) and getting positive rotavirus diarrhea. The incidence rate of rotavirus GE was 24.4%.

The clinical characteristics of the enrolled children were shown in Table 1. The mean (SD) of their age was 8.9 moths (7.1) with a range of 1-50 moths. There was the predominance of the male gender (63%). Forty five percent of the children were in the age group of 1-6 months, 37% in the age group of 7-12 months, 15% in the age group of 13-24 months while only 3% above 24 months of age. The mean duration of the symptoms was 4.5 days and the mean frequency of diarrhea per day was 6.7. The majority of children (270, 59%) had some dehydration while 154 (33.4%) children had severe dehydration. Vomiting and fever were found in 72% and 58% of the children, respectively. The majority of the children (352, 79%) were improved and 22 (5%) died. On comparing these clinical parameters according to the season it was found that there were no statistically significant differences, only the mean duration of symptoms and the mean duration of diarrhea were statistically significantly shorter during the intermediate season than the cold and hot seasons (p value <0.05 for both). However, more children (40%) who were enrolled during the cold season had severe dehydration than those during the intermediate (24.4%) and the hot season (30%) (p value <0.05). There was no statistical difference in the outcome according to the season (Table 1).On comparing the general and the clinical characteristics of children with positive rotavirus and those with negative rotavirus, it was found that there were no significant differences (p>0.5) a part from the mean frequency of diarrhea/day and mean frequency of vomiting/day (p<0.05) (table 2).

ISSN No:-2456-2165

Four hundred and five (87%) children gave stool samples and all were tested by ELISA. Out of those 98 (24%), samples were positive for rotavirus. However, only 77 (78.6%) samples were tested by PCR. All 77 samples were confirmed to be positive rotavirus. The genotyping of G gene showed that the G12 constitutes almost half the samples (45.5%), G2 constitutes 19.5%, G1 and G9 14.3% each, GUT (4%), and G3 2.5% of the samples (Figure 2). The genotyping of P gene of these samples revealed that the majority of the samples (78%) were of P[8] genotyping, while P[4] constitutes 17%, P[6] 4%, and P[untypable (UT)] only 1% of the samples (Figure 3). On analyzing the combinations of the two genes, it was found that G12P[8] in 34 (44.2%) samples; G2P[4] in 13 (17%) samples, G1P[8] in 11(14.3%) samples; G9P[8] in 10 (13%) samples; G[UT] P[8] in 3 (4%) samples; G2P[6] and G3P[8] in 2 (2.6%) samples each; and G9P[6] and G12 P[UT] in 1 (1%) sample each (Table 4 & Figure 4). G9 P[UT] combination wasn't found. Table 3 showed also the frequency of the G and P genotypes according to the season. It was found that G1 was isolated mainly during the cold season, while G2 and G3 predominantly during the hot season. The two new emerging genotypes G9 and G12 were isolated predominantly during the cold season. Regarding the P genotype, P[4] genotype was predominantly isolated from samples during the hot season. P[6] was isolated only during the cold season. P[8] isolated mainly during both the cold and intermediate seasons (82% and 81.3%) respectively, while it was isolated from only 55% of the samples during the hot season($p=0.05$).

IV. DISCUSSION

Each year rotavirus causes millions of cases of diarrhea in developing countries, almost 2 million of which result in hospitalization[16].

In the current study it was found that the mean (SD) age of children with RVGE was 8.3 (6.1) months which is in consistency with others[17] who reported a median age of 9 months; however another study from Yemen reported a mean age of 12.2 month[18]. The present study also showed that almost half of the children were below 6 months of age, 37% in the age group of 7-12 moths, 15% in the age group of 13-24 months while only 3% above 24 months of age. These results in agreement with others[19,20]. We reported that the majority of the children with RVGE were in the age group of 1-6 months, on the contrary, Al-Badani et al.(2014)[17] reported that the higher frequency of rotavirus diarrhea was in infants between 7 and 11 months (32.1%) and the lower frequency were in infants between 1 and 6 months (29.6%). However, others reported that children between 13 and 24 months of age have the highest rate of rotavirus infection ($P<0.005$) [21, 22].

The current study showed the predominance of the male gender (63%) among children with AGE. This was in line with others[23, 24]. There were no statistical significant differences between the percentage of male gender among children with RVGE (69.4%) and none-RVGE (63%) ($p >0.05$) and this in agreement with others [17; 25].

There was no significant difference between the rate of hospitalization of children with RVGE (80.6%) and none-RVGE (81%). This in concordance with other studies [18,17].

Although the mean duration of diarrhea and vomiting was not statically significant between RVGE and none-RVGE, but their frequency per day was statically significantly more in RVGE. The frequent vomiting and diarrhea per day lead to the risk of dehydration in rotavirus infection more than other diarrheal infections. These support the findings of Soriano-Gabarro et al (2006) who reported that rotavirus is most frequently associated with severe dehydrating diarrhea, substantial hospitalization, increased emergency room visits, and increased costs for both hospitals and parents[26]. The present study reported a third of the children had severe dehydration, 40% of them were enrolled during the cold season. Dehydration is more common in rotavirus infection than in other pathogens and is the main cause of death in rotavirus infection [27].

In this study, rotaviruses were detected in 24.4% of the patients with AGE, which is consistent with the rates reported in the Middle East; RVGE was identified in 16-61% of all cases of AGE[12]. In comparison to other studies from Yemen, our findings are also in line with the results of Kirby et al,(2011)[13] who reported 27% rate in the same region but before the introduction of the rotavirus vaccine. The reported rate in this study was lower than what was reported from Taiz region during the vaccination era (45%)[17], however, another study[18] reported by Banajeh and Abu-Asba, (2015) 41.6% rate during the pre-vaccination era and 19.9% during the post-vaccination era in Taiz and Aden. These differences in the three Yemeni studies may be due to the differences in the geographical nature of each region where Aden situated in a coastal area and Taiz at 1200 meter above sea level and both have hot weather most of the year, while Sana'a located at a higher altitude, 2300 meter above sea level with cool weather most of the year.

The genotyping of G gene showed that the new emerging genotype G12 constitutes almost half the samples (45.5%), G2 constitutes 19.5%, G1 and G9 14% each and G3 2.6% of the samples. The rate of rotavirus genotypes in the pre and post-vaccination period in Taiz and Aden were compared[18]. It was found that the most prevalent G types were G1 and G2, and while the percentage of G1 did not change in the post-vaccine period, G2 was dramatically reduced in the post-vaccine period. They also documented that G9 increased from 6.3 % before, to 29.2 % after rotavirus vaccine introduction in Yemen[18].

The genotyping of P gene of the study samples revealed that the majority of the samples (78%) were of P[8] genotyping while P[4] constitutes 17%, P[6] 4%, and UT only 1% of the samples. However, another Yemeni study[18] reported that the P genotypes showed similar variation, with P[4] accounting for 33.8 % of the pre-vaccination strains, but became less frequent, comprising 14.3 % of the post-vaccine strains. P[8] strains increased in frequency from 26.3 to 51 % after the vaccine introduction.

ISSN No:-2456-2165

Interestingly, G12, a recently emerging serotype detected in this study was also detected in Europe, Asia, and the Americas[28- 30], but has not been reported in any of many studies in the Middle East captured in a review by Khoury et al. (2011) [12].

On analyzing the combinations of the two genes, it was found that the most common combination was G12P[8] in thirty-four samples (44%); followed by G2P[4] in thirteen samples (17%), G1P[8] in eleven samples (14%); G9P[8] in ten samples (13%), G[UT] P[8] in three samples (4%); G2P[6] in two samples (2.5%) , G3P[8] in two samples (2.5%), G9P[6] in one sample (1%) and G12P[UT] in one case (1%).

Apart from G12 and G9 the results of the current study were similar to the results of the study reported from Taiz where the most common genotypes were G2P[4] (55%), followed by G1P[8] (15%) [17], similarly in the Saudi Arabia [31] and other Middle East countries[12]. Khoury et al (2011) was found that G2P[4] was the most prevalent genotype combination in four countries (Egypt, Jordan , Oman and Yemen) with a range from 26% to 48%[12]. These genotype combinations are also predominant in Western Europe[32]. However, in a previous study in Sana'a but during the pre-vaccination period, Kirby et al (2011)[13] reported that the most common genotype combination was G1P[8] (55%), followed by G9P[8] (21%), G2P[4] (12%), G2P[6] (5%) with other combinations.

The following table showed the effect of the vaccine in the evolution of the rotavirus serotypes in the study area. Some combinations decreased, others increased, some disappeared and others emerged.

Genotypes G-P combinations	Kirby et al [13] (pre-vaccination period)	Current study (post vaccination period	comments
G1P[8]	55%	14%	Decreased
G9P[8]	21%	13%	Decreased
G2P[4]	12%	17%	Increased
G2P[6]	5%	2.5%	Decreased
G9P[4]	1%	Zero	Disappeared
G9P[NT]	1%	Zero	Disappeared
G12P[6]	1%	Zero	Disappeared
G12P[8]	1%	44%	Significantly increased
G3P[8]	Zero	2.5%	Appeared
G9P[6]	Zero	1%	Appeared
G12P[NT]	Zero	1%	Appeared

Table 5:- Comparison of the G-P Combinations Pre-and Post Vaccination Period in Sana'a

Banajeh and Abu-Asba,(2015)[18] reported that G1P[8] increased in frequency from 45.5 % to 87.5 %, while G2P[4] decreased from a frequency of 76.5 % to the detection in only two strains after vaccine introduction in Taiz and Aden. These results were opposite to our findings in Sana'a where G1P[8] decreased after the introduction of the vaccine while G2P[4] increased slightly. These differences could be due to the differences in the geographical characteristics of the two regions.

This study reported that the genotyping in Sana'a region is different and distinguished by the presence of the two new G12 mainly and G9 genotypes that were not present in the other two different regions (Taiz and Aden). G12 is not covered by either of the two rotavirus vaccines. Since G12 was responsible for almost half the RVGE in Sana'a this may explain why the rate of RVGE didn't change significantly despite the introduction of the vaccine in 2012. The rate was 27% during the pre-vaccine introduction[13] and 24.4% in the post-vaccine introduction.

The present study showed an increase in RVGE during the cold season and this is line with what was identified in some countries where RVGE peaked in the winter season[33-37,21]. On the contrary, Al-Badani et al (2014)[17]reported higher frequency (53%) for RVGE in Taiz during the summer season while another study [18] reported by Banajeh and Abu-Asba,(2015) showed similar results to ours from Taiz and Aden only during the pre-vaccine period, but during the post-vaccination period, the seasonal variation became opaque.

The incidence of infection with particular group A rotavirus serotypes and genotypes varies between geographical areas during a rotavirus season, and from one season to the next[38]. The current study showed that G2 and G3 and P[4] genotype was predominantly isolated from samples during the hot season and G1 and P[8] isolated mainly during both the cold and intermediate seasons and this in accordance with results of Al-Badani et al (2014)[17]. G9 and G12 and P[6] were isolated predominantly during the cold season. However, G9 and G12 were not isolated from Taiz region according to results of Al-Badani et al (2014)[17], although another study isolated G9 in small percentages[18].

In 2013, an estimated 215,000 children less than five died from rotavirus, 90% of whom were in the developing countries[39] and Yemen is the poorest Arabic state and thus the determination of RVGE rate is important to help policy-makers in planning and improving the preventive strategies.

V. CONCLUSION

The epidemiology of rotavirus in Sana'a is unique, as there was a predominance of the new emerging serotypes G12 and G9. These two serotypes were not recovered in Taiz or Aden. This may explain the insignificant reduction of RVGE rate during the post-vaccination period in Sana'a region. Thus there may be a need to develop a new vaccine that involves these two serotypes. Indeed the national surveillance for rotavirus need to be continued to report the changes in the prevalence of the rotavirus serotypes. Also this surveillance need to be extended to other regions as it may discover other differences in the different Yemeni regions that characterized by a variety of different geographical characteristics.

REFERENCES

[1]. *Widdowson MA, Steele D, Vojdani J, Wecker J, Parashar UD: Global rotavirus surveillance: preparing for the introduction of rotavirus vaccines. J Infect Dis. 2009, 200 (Suppl 1): S1-S8. 10.1086/605061.*
[2]. *Parashar UD, Gibson CJ, Bresse JS, Glass RI: Rotavirus and severe childhood diarrhea. Emerg Infect Dis. 2006, 12 (2): 304-306.*
[3]. *Diggle L: Rotavirus diarrhoea and future prospects for prevention. Br J Nurs. 2007, 16 (16): 970-974.*
[4]. *Grimwood K, Lambert SB: Rotavirus vaccines: opportunities and challenges. Hum Vaccin. 2009, 5 (2): 57-69. 10.4161/hv.5.2.6924.*
[5]. WHO, (2013). http://www.who.int/immunization/monitoring_surveillance/burden/estimates/rotavirus/en/
[6]. "Virus Taxonomy: 2014 Release". International Committee on Taxonomy of Viruses (ICTV).
[7]. Wakuda M, Ide T, Sasaki J, Komoto S, Ishii J, Sanekata T, Taniguchi K. "Porcine rotavirus closely related to novel group of human rotaviruses". Emerging Infectious Diseases. 2011, 17 (8): 1491–3.
[8]. Marthaler D, Rossow K, Culhane M, Goyal S, Collins J, Matthijnssens J, Nelson M, Ciarlet M . "Widespread rotavirus H in commercially raised pigs, United States". Emerging Infectious Diseases. 2014, 20 (7): 1195–8.
[9]. O'Ryan M . "The ever-changing landscape of rotavirus serotypes". The Pediatric Infectious Disease Journal. 2009, 28 (3 Suppl): S60–2.
[10]. Patton JT. "Rotavirus diversity and evolution in the post-vaccine world". Discovery Medicine. 2012, 13 (68): 85–97.
[11]. Desselberger U, Wolleswinkel-van den Bosch J, Mrukowicz J, Rodrigo C, Giaquinto C, Vesikari T. "Rotavirus types in Europe and their significance for vaccination". Pediatr. Infect. Dis. J. 2006, 25 (1 Suppl.): S30–41.
[12]. Khoury H, Ogilvie I, El Khoury AC, Duan Y, Goetghebeur MM. Burden of rotavirus gastroenteritis in the Middle Eastern and North African pediatric population. BMC Infectious Diseases. 2011 Jan 7;11:9. doi: 10.1186/1471-2334-11-9
[13]. Kirby A, Al-Eryani A, Al-Sonboli N, Hafiz T, Beyer M, Al-Aghbari N, et al. Rotavirus and norovirus infections in children in Sana'a, Yemen. TM&IH Volume 16, Issue 6, 2011, Pages 680–684.
[14]. "Climate: Sanaa - Climate graph, Temperature graph, Climate table". *Climate-Data.org.* Retrieved 2014,-02-23.
[15]. World Health Organization. Generic protocols for (i) hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children and (ii) a community-based survey on utilization of health care services for gastroenteritis in children. Field test edition. Geneva: World Health Organization; 2002.
[16]. Simpson E, Wittet S, Bonilla J, Gamazina K, Cooley L, Winkler JL. "Use of formative research in developing a knowledge translation approach to rotavirus vaccine introduction in developing countries". BMC Public Health. 2007, 7: 281.
[17]. Al Al-Badani A, Al-Areqi L, Majily A, Al-Sallami S, Al-Madhagi A, Amood Al-Kamarany M. Rotavirus Diarrhea among Children in Taiz, Yemen: Prevalence—Risk Factors and Detection of Genotypes. International Journal of Pediatrics. Int J Pediatr. 2014;2014:928529. doi: 10.1155/2014/928529. Epub 2014 Aug 12.
[18]. Banajeh SM. and Abu-Asba B A. The epidemiology of all-cause and rotavirus acute gastroenteritis and the characteristics of rotavirus circulating strains before and after rotavirus vaccine introduction in Yemen: analysis of hospital-based surveillance data. BMC Infectious Diseases. 2015, 15:418. DOI 10.1186/s12879-015-1165-8
[19]. Ramsay M, Brown D, Desselberger U., Gray, James, eds. Rotaviruses: methods and protocols. Totowa, NJ: Humana Press. 2000, p. 217. ISBN 0-89603-736-3.Free ebook
[20]. Grimwood K, Lambert SB. "Rotavirus vaccines: opportunities and challenges". Human Vaccines. 2009, 5 (2): 57–69. doi:10.4161/hv.5.2.6924. PMID 18838873
[21]. Mousavi Nasab SD, Sabahi F, Makvandi M, Mirab Samiee S, Nadji SA, Ravanshad M. Epidemiology of Rotavirus-Norovirus Co-Infection and Determination of Norovirus Genogrouping among Children with Acute Gastroenteritis in Tehran, Iran. Iran. Biomed. J. 2016 (5): 280-286
[22]. Phua KB, Tee N, Tan N, Ramakrishnan G, Teoh YL, Bock H, Liu Y. A hospital-based surveillance of rotavirus gastroenteritis in children <5 years of age in Singapore. The Pediatric infectious disease journal 2013; 32(12): e426-e431.
[23]. Rheingans RD, Heylen J, Giaquinto C. "Economics of rotavirus gastroenteritis and vaccination in Europe: what makes sense?". Pediatr. Infect. Dis. J. 2006, 25 (1 Suppl): S48–55. doi:10.1097/01.inf.0000197566.47750.3d. PMID 16397429
[24]. Ryan MJ, Ramsay M, Brown D, Gay NJ, Farrington CP, Wall PG. "Hospital admissions attributable to rotavirus infection in England and Wales". J. Infect. Dis. 1996, 174 (Suppl 1): S12–8.

doi:10.1093/infdis/174.Supplement_1.S12. PMID 8752285.
[25]. Nguyen TV, LeVan P, LeHuy C, and Weintraub A, "Diarrhea caused by rotavirus in children less than 5 years of age inHanoi, Vietnam," Journal of Clinical Microbiology, 2004, 42 (12),5745–5750.
[26]. *Soriano-Gabarro M, Mrukowicz J, Vesikari T, Verstraeten T: Burden of rotavirus disease in European Union countries. Pediatr Infect Dis J. 2006, 25 (Suppl 1): S7-S11.*
[27]. Maldonado YA, Yolken RH. "Rotavirus". Baillière's Clinical Gastroenterology. 1990, 4 (3): 609–25. doi:10.1016/0950-3528(90)90052-I. PMID 1962726
[28]. *Matthijnssens J, Rahman M, Ciarlet M, Van Ranst M: Emerging Human Rotavirus Genotypes. Viruses in the Environment. Edited by: Palumbo E, Kirkwood C., Kerala, India: Research Signpost, 2009, 171-219.*
[29]. *Banyai K, Bogdan A, Kisfali P, Molnar P, Mihaly I, Melegh B, et al, Emergence of serotype G12 rotaviruses, Hungary. Emerg Infect Dis. 2007, 13 (6): 916-919.*
[30]. *Grassi T, De Donno A, Guido M, Gabutti G: G-genotyping of rotaviruses in stool samples in Salento, Italy. J Prev Med Hyg. 2006, 47 (4): 138-141.*
[31]. Kheyami AM, Nakagomi T, Nakagomi O, Dove W, Hart CA, and Cunliffe NA, "Molecular epidemiology of rotavirus diarrhea among children in Saudi Arabia: first detection of G9 and G12 strains," Journal of Clinical Microbiology,2008, 46 (4), 1185–1191.
[32]. *Van Damme P, Giaquinto C, Maxwell M, Todd P, Van der WM: Distribution of rotavirus genotypes in Europe, 2004-2005: the REVEAL Study. J Infect Dis. 2007, 195 (Suppl 1): S17-S25. 10.1086/516715.*
[33]. Hassine-Zaafrane M, Sdiri-Loulizi K, Ben Salem I, Kaplon J, Ayouni S, Ambert-Balay K, Sakly N, et al.. The molecular epidemiology of circulating rotaviruses: three-year International Journal of Pediatrics 9 surveillance in the region of Monastir, Tunisia. BMC Infectious Diseases. 2011, 11 (266), 1–6, 2011.
[34]. Kargar M, Zare M, Najafi A. Molecular epidemiology of rotavirus strains circulating among children with gastroenteritis in Iran. Iranian journal of pediatrics 2012; 22(1): 63-69.
[35]. Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, et. al. Global mortality associated with rotavirus disease among children in 2004. Journal of infectious diseases 2009; 200 (Suppl 1): S9-S15.
[36]. Atchison CJ, Tam CC, Hajat S, van Pelt W, Cowden JM, Lopman BA. "Temperature-dependent transmission of rotavirus in Great Britain and The Netherlands". Proceedings of the Royal Society B: Biological Sciences. 2010, 277 (1683): 933–42. doi:10.1098/rspb.2009.1755. PMC 2842727. PMID 19939844.
[37]. Levy K, Hubbard AE, Eisenberg JN. "Seasonality of rotavirus disease in the tropics: a systematic review and meta-analysis". International Journal of Epidemiology. 2009, 38 (6): 1487–96. doi:10.1093/ije/dyn260. PMC 2800782. PMID 19056806.
[38]. *Iturriza-Gomara M, Green J, Brown DW, Ramsay M, Desselberger U, Gray JJ: Molecular epidemiology of human group A rotavirus infections in the United Kingdom between 1995 and 1998. J Clin Microbiol. 2000, 38 (12): 4394-4401.*
[39]. Tate, Jacqueline E.; Burton, Anthony H.; Boschi-Pinto, Cynthia; Parashar, Umesh D. "Global, Regional, and National Estimates of Rotavirus Mortality in Children <5 Years of Age, 2000–2013". Clinical Infectious Diseases. 2016, 62 (Suppl 2): S96–S105.

ISSN No:-2456-2165

	Total No.(%) **467 (100%)**	**Cold season** **208 (44.5%)**	**Intermediate Season** **87 (18.6%)**	**Hot season** **172 (36.8%)**	**Chi square**	**P value**
Mean of age (SD)[range] (months)	8.9 (7.1)[1-50]	8.7 (7)[1-43]	8.3 (6.9)[2-50]	9.6 (7.4) [1-55]		0.06
Male	293 (63%)	132 (64%)	60 (69%)	101 (59%)	2.7	0.3
Female	167 (37%)	76 (36%)	27 (31%)	71 (41%)		
Age groups:						
• 1-6 months	209 (45%)	99 (48%)	46 (53%)	64 (37%)	8.6	0.1
• 7-12 months	174 (37%)	71 (34%)	27 (31%)	76 (44%)		
• 13-24 months	71 (15%)	31 (15%)	13 (5%)	27 (16%)		
• >24 month	13 (3%)	7 (3.4%)	1 (1%)	5 (3%)		
Mean Duration of symptoms (days) (SD)[range]	4.5 (2)[1-10]	4.7 (2)[1-10	3.9 (1.8)[1-10]	4.5 (2)[1-9]		**0.03**
Mean frequency of diarrhea/ day (SD)[range]	6.7 (3.2)[1-25]	6.5 (3.2)[1-25]	6.9 (2.9)[1-15]	7 (3.2)[1-20]		0.2
Mean Duration of diarrhea (SD)[range]	4.7 (2.5)[1-15]	5 (2.6)[1-14]	4 (1.9)[1-10]	4.6 (2.6)[1-14]		**0.02**
Vomiting	337 (72%)	146 (72%)	64(74%)	127(74%)	0.7	0.7
Mean Duration of vomiting (days) (SD)[range]	4 (2.3)[1-14]	4.3 (2.2)[1-12]	3.6 (1.9)[1-10]	4.2 (2.6)[1-14]		0.08
Mean frequency of vomiting/ day (SD)[range]	5 (3.4)[1-20]	4.9 (3.2)[1-20]	5.7 (3.9)[1-20]	4.9 (3.3)[1-15]		0.3
Fever	271 (58%)	112 (54%)	47 (54%)	112(65%)	5.6	0.06
Status of dehydration						
• Severe	154 (33.4%)	83(40%)	21(24.4%)	50 (30%)	12.8	**0.01**
• Some	270 (59%)	114(55%)	55(64%)	101 (60%)		
• none	37 (8%)	9 (4.4%)	10 (11.6%)	18 (10.7%)		
Outcome						
• Improved	352 (79%)	151(77.4%)	70(81.4%)	131(78.4%)	4.4	0.6
• Death	22(5%)	8(4%)	6(7%)	8(5%)		
• Transferred	13(3%)	8(4%)	2(2.3%)	3(2%)		
• DAMA	61 (13.6%)	28(45.9%)	8(9.3%)	25(15%)		

Table 1:- The clinical characteristics of the study population in general and by season

ISSN No:-2456-2165

	RV positive 98 (24.4%)	RV negative 303 (75.6%)	Chi square	P value
Site of entry: • OPD • ER • Ward	 12(12.2) 7 (7%) 79(80.6%)	 32(10.6) 25(8.2%) 246(81.2%)	1	0.5
Mean of age (SD)[range]	8.3 (6.1)[1-30]	9.1 (7.5) [1-55]		0.2
Male	68 (69.4%)	191 (63%)	1.3 (0.8-2.2)	0.1
Age groups:				
• 1-6 months	52 (53%)	132(43.5%)	5.3	0.2
• 7-12 months	27 (27.6%)	118 (40%)		
• 13-24 months	17(17.3%)	43(14.2%)		
• >24 month	2(2%)	10(3.3%)		
Mean Duration of diarrhea	4.5(2.5) [1-14]	4.8(2.5) [1-14]		0.3
Mean frequency of diarrhea/ day	7.6(3.5)[2-20]	6.5(3.1)[1-25]		**0.008**
Mean Duration of vomiting	3.7(2)[1-14]	4.3(2.3)[1-13]		0.3
Mean frequency of vomiting/ day	6(4)[1-20]	4.6(3)[1-15]		**0.01**
Status of dehydration • Severe • some • None	 34(35%) 57(59%) 6(6%)	 102(34%) 171(57%) 27(9%)	1.01	0.06
Fever	56(57%)	182(60.1%)	0.8(0.5-1.4)	0.3
Outcome				
• Improved	78 (82.1%)	228 (79.2%)	1.5	0.6
• Death	4 (4.2%)	13 (4.5%)		
• Transferred	3 (3.2%)	10 (3.5%)		
• DAMA	10 (10.5%)	37 (13%)		

Table 2:- Clinical characteristics of the study population and by Rotavirus positivity

	No.(%) 467 (100%)	**Cold season 208 (44.5%)**	**Intermediate Season 87 (18.6%)**	**Hot season 172 (36.8%)**	**Chi**	**P value**
Frequency of taken stool	405 (87%)	198(95%)	67(77%)	140(81.4%)	24	**<0.0001**
No of positive Rotavirus by ELISA test	98 (24%)	54(27.3%)	20(30%)	24(17%)	11.1	**0.02**
Type P frequency	**N=77**	**N=50**	**N=16**	**N=11**	12.5	**0.05**
• **P[8]**	60 (78%)	41(82%)	13(81.3%)	6(55%)		
• **P[4]**	13 (17%)	6(12%)	2(13%)	5(50%)		
• **P[6]**	3(4%)	3(6%)	0(0%)	0(0%)		
• **P[UT]**	1(1%)	0(0%)	1(6.3%)	0(0%)		
Type G frequency	**N=77**	**N=50**	**N=16**	**N=11**		
• **G12**	35(45.4%)	24(48%)	10(63%)	1(9%)	27.1	**0.002**
• **G2**	15(19.5%)	8(16%)	2(12.5%)	5(45.5%)		
• **G1**	11 (14.3%)	9(18%)	1(6%)	1(9%)		
• **G9**	11(14.3%)	8 (16%)	1(6%)	2(18.2%)		
• **G UT**	3 (4%)	1(2%)	2(12.5%)	0 (0%)		
• **G3**	2(2.5%)	0(0%)	0(0%)	2(18.2%)		

Table 3:- The laboratory characteristics of the study population in general and by season

ISSN No:-2456-2165

G-P Serotypes combination	No.(%) 77 (100%)	Cold season 50 (65%)	Intermediate Season 16 (21%)	Hot season 11 (14%)
G12 P[8]	34 (44.2%)	24(48%)	9(56%)	1(9%)
G2 P[4]	13(17%)	6(12%)	2(12.5%)	5(45%)
G1 P[8]	11 (14.3%)	9 (18%)	1(6.2.5%)	1(9%)
G9 P[8]	10 (13%)	7(14%)	1 (6.25%)	2(18%)
Gut P[8]	3 (4%)	1(2%)	2(12.5%)	0(0%)
G2 P[6]	2 (2.6%)	2(4%)	0(0%)	0(0%)
G3 P[8]	2 (2.6%)	0(0%)	0(0%)	2(18%)
G9 P[6]	1 (1%)	1(2%)	0(0%)	0(0%)
G12 P[UT]	1(1%)	0(0%)	1(6.25%)	0(0%)
G9P[UT]	0 (0%)	0(0%)	0(0%)	0(0%)

Table 4:- Frequency of the G-P genotype combination by season

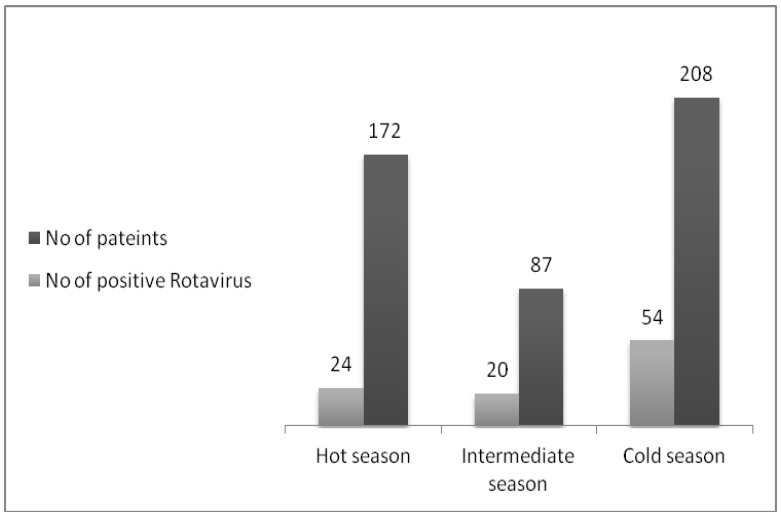

Fig 1:- Frequency of Enrolled Children and Positive Rotavirus by Season

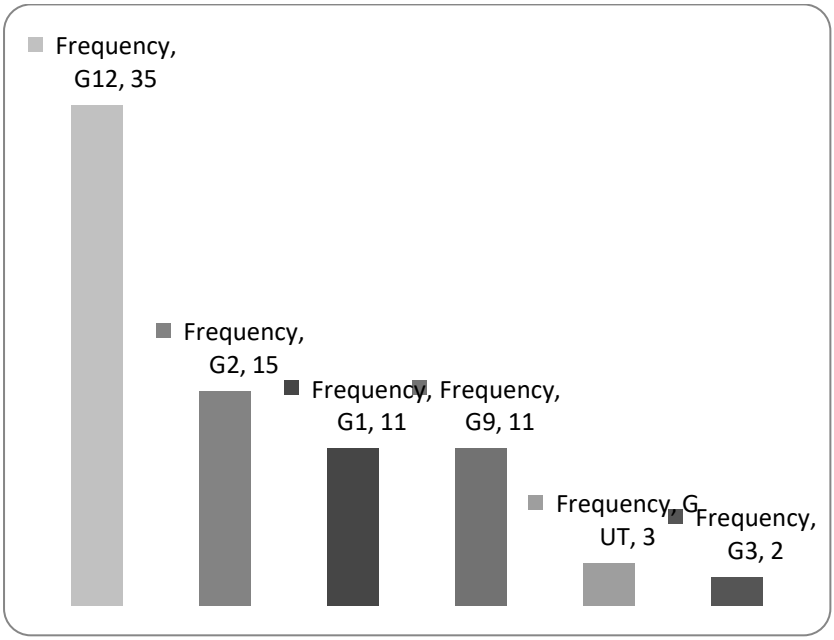

Fig 2:- Frequency the Genotype G

ISSN No:-2456-2165

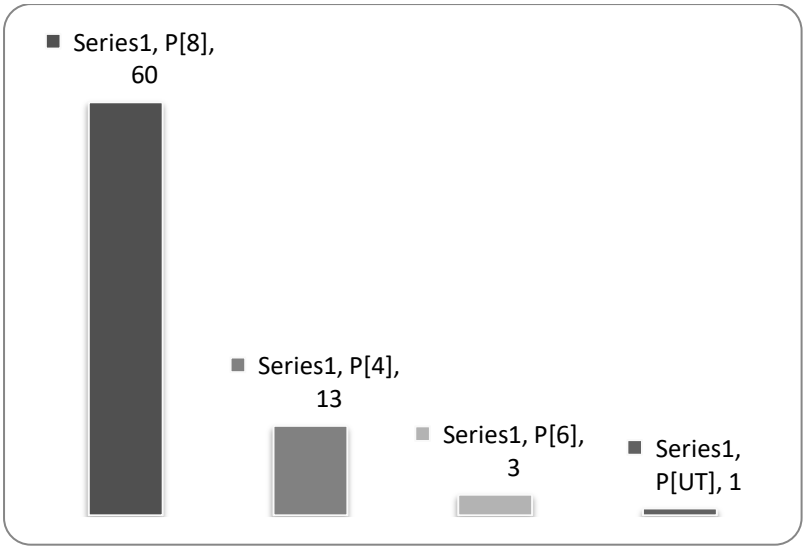

Fig 3:- Frequency of the Genotype P

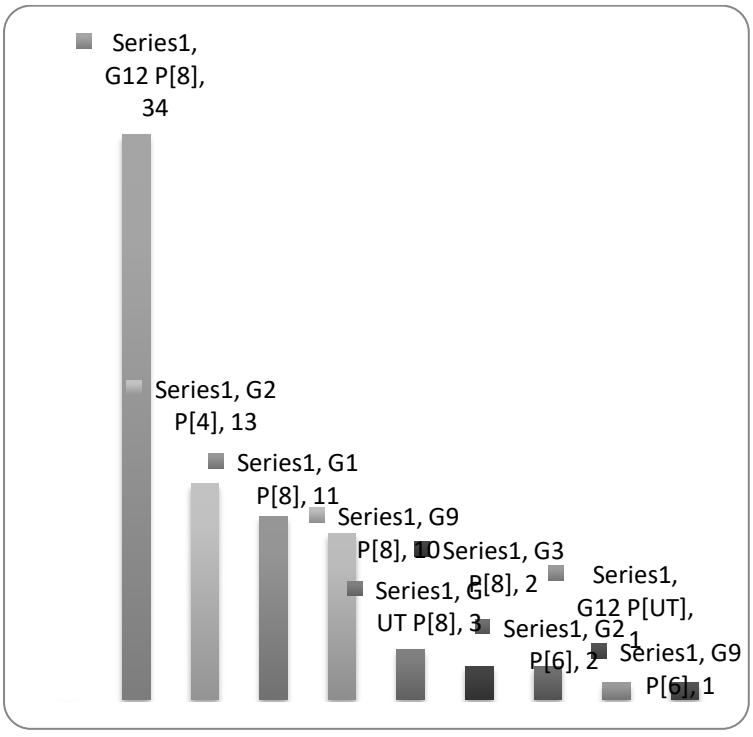

Fig 4:- Frequency of Genotypes P and G Combinations