Etiology of acute gastroenteritis among pre-school children in Peshawar-KP Pakistan, a case control study.

Running Title: Etiology of acute gastroenteritis.

Farid Ullah Shah[1], Jahangir Anjum[2], Muhammad Daud[3], Wasim Ahmad [4,*]

ABSTRACT

Objective: This study aimed to investigate the etiology of acute viral gastroenteritis among preschool children in Peshawar, Khyber Pakhtunkhwa, Pakistan.

Methodology: A total of 420 stool samples were gathered from children under five, displaying symptoms like diarrhea, vomiting, nausea, and abdominal cramps. Recruitment took place at a Peshawar tertiary care hospital, approved by the institute's IRB. The cohort was split evenly into 210 cases and 210 controls. Healthy controls\ originated from the outpatient department with no diarrhea within the last 10 days, while cases were from the emergency, inpatient, and outpatient units. Diagnosis criteria encompassed at least one vomiting episode in the last 24 hours and over three loose stools daily, enduring less than 5 days. Moderate to severe cases included indicators like skin turgor loss, sunken eyes, IV treatment, dysentery, or gastroenteritis-related hospitalization. A 2-month follow-up tracked complications and mortality, with parental consent secured.

Results: Among participants, 60% (n=126/210) were male and 40% (n=84/210) were female. The average age was 20 months (range 1-50 months). Clinical symptoms were evident during enrollment; cases displayed watery diarrhea (4 episodes on average in 24 hours, lasting 2.50 days), and 48% (n=101) experienced vomiting (3 episodes on average in 24 hours). Acute gastroenteritis symptoms persisted for an average of 4.0 days. Severe and mild dehydration were observed in 12% (n=25) and 28% (n=58) of patients, respectively. Additional symptoms included fever (8%, n=17), abdominal pain (8%, n=17), and dysentery (5%, n=10). The majority (90%, n=189) received intravenous fluids, while 10% (n=21) were treated orally.

Conclusion: The common causative organism for childhood diarrhea is adenovirus.

Key words: Acute gastroenteritis, Acute diarrhea, Vomiting, Nausea, Abdominal Cramps

Introduction:

Gastroenteritis is a short-lived, watery-diarrheal ailment that typically lasts less than a week. Its symptoms encompass nausea, reduced appetite, vomiting, fever, and abdominal spasms. Among children under the age of five, acute gastroenteritis poses a significant risk for illness and even death. In regions with lower to middle incomes, this condition contributes to about 446,000 annual fatalities.[1] It stands as the second leading cause of death among preschoolers. The burden of this disease is especially pronounced in areas lacking access to clean water, hygiene, and sanitation, exacerbating the situation.[2]

Acute gastroenteritis typically stems from viral or bacterial infections, causing inflammation of the stomach and intestines. Viruses like norovirus and rotavirus are common culprits, often spread through contaminated food, water, or close contact. Bacterial pathogens such as Escherichia coli (E. coli), Salmonella, and Campylobacter are also frequent causes, usually transmitted via undercooked food or poor hygiene practices. In some cases, parasites like Giardia or Cryptosporidium can trigger gastroenteritis when ingested. The condition's rapid onset of symptoms, including diarrhea, vomiting, and abdominal discomfort, highlights its acute nature.

The origins of the illness range from moderate to severe and vary across different geographical locations due to varying environmental factors and hosts. Some case-control studies have detailed the causes of moderate to severe diarrhea in Asia and Africa.[3-6] The risk of acute gastroenteritis in preschool children varies worldwide. While around 25 different protozoa and bacteria can lead to a similar clinical syndrome, viral gastroenteritis proves more persistent than bacterial or parasitic types. Research indicates that viruses account for over 75% of cases. Notably, the prevalence of rotavirus, norovirus, and human adenovirus has been linked to acute gastroenteritis in preschool children. The introduction of the monovalent rotavirus vaccine has contributed to reduced hospitalization rates in various parts of the world.[7-10]

Human adenoviruses are medium-sized, non-enveloped, icosahedral particles measuring 70-90 nanometers. They carry linear DNA in their genomes and belong to the Adenoviridae family. So far, 103 genotypes of this virus have been identified. Among these, both non-enteric genotypes like HAdV-3, HAdV-7, HAdV-8, HAdV-31, and enteric genotypes such as HAdV-40 and HAdV-41 are notably associated with acute gastroenteritis in preschool children.[11-13] Since effective treatments for viral gastroenteritis are limited to the rotavirus vaccine, having regional and local epi-

1: Department of Biochemistry, Pak International Medical College Peshawar-KP

2: Department of Medicine, Mohtarma Benazir Bhutto Shaheed Medical College, Mirpur, Azad Kashmir

3: Department of Gastroenterology, Jinnah Medical College, Peshawar

4:Department of Allied Health Sciences, SHS-Peshawar

=corresponding author : vazim4847@gmail.com

demiological data on adenovirus infections is crucial for healthcare practitioners and officials.[14-18] This information helps in developing and implementing appropriate vaccination and infection control strategies. Several studies have highlighted diverse viral agents causing gastroenteritis in children in Pakistan.

Objective:

To investigate the etiology of acute gastroenteritis in preschool children from Peshawar district of KP.

Methodology:

From January 2022 to December 2022, a case-control study with age-matched participants was carried out at a private tertiary care hospital in Peshawar-KP. The primary objective was to investigate the underlying causes of moderate to severe acute gastroenteritis (viral origin) among preschool children. The study received ethical approval from the hospital's institutional review board. The sample size consisted of 420 individuals, determined using the WHO online calculator. The participants were evenly split into two categories: cases and healthy controls, each comprising 210 subjects. Control participants were chosen from the outpatient department (with no diarrhea within the last 10 days), while cases were selected from the emergency, inpatient, and outpatient departments. The criteria for diagnosing acute gastroenteritis included at least one episode of vomiting within the last 24 hours and a minimum of three instances of loose stools per day, lasting less than 5 days. Moderate to severe cases were defined as those exhibiting signs such as skin turgor loss, sunken eyes, IV treatment, dysentery, or hospitalization due to acute gastroenteritis. A follow-up period of 2 months was implemented to monitor any health-related complications and mortality. Prior to their child's participation, the parents or guardians provided informed consent. Pre-school children, presence of acute, moderate-to-severe diarrheal episodes and vomiting within the past 5 days was inclusion criteria whereas, children older than five years of age and presence of chronic diarrhea (>5 days) was the exclusion criteria.

The study participants' demographic information was documented, including details about their water and food sources, medical and nutritional backgrounds, as well as epidemiological factors. Stool samples were gathered from the participants (cases) on the day they joined the study. These samples were then promptly transferred to Chughtai Lab within a span of four hours to undergo testing. The identification of different viruses within the DNA extracted from stool samples involved the use of Reverse Transcription Polymerase Chain Reaction (RT-PCR). Human adenovirus presence in these samples was determined through cell culture and ELISA techniques. The accuracy of this discovery was then affirmed through subsequent PCR procedures. We analyzed the data using statistical software SPSS version 22. We presented descriptive stats as frequency, mean and percentage. To determine if there were any notable differences, a chi-square test was utilized. Statistical significance was recognized for p-values below 0.05.

Results:

Overall, 420 participants were enrolled, however 210 served as age, gender matched control. Among cases (n=210) 60% (n=126/210) were male, and 40% (n=84/210) were female. The mean age of study participants was 20 months, ranging from 1 to 50 months. At presentation all children from case group were having watery discharge/ diarrhea, with an average of 4 diarrheal episodes in the last 24 hours (range 3-7). The mean duration of diarrhea was 2.50 days (range 2-5 days). Vomiting was reported in 48% (n=101) of patients, with an average of 3.0 episodes per 24 hours (range 2-6). The symptoms of acute gastroenteritis (AGE) persisted for an average of 4.0 days (range 3-6). Among the patients, 12% (n=25) were severely dehydrated, while 28% (n=58) showed mild dehydration. Only 8% (n=17) experienced fever, 8% (n=17) had abdominal pain, and 5% (n=10) had dysentery. The majority of study participants (90%, n=189) received intravenous fluids, while 10% (n=21) were treated orally. The results have been summarized in table 1.

Table No 1: Demographics of the study participants

Characteristics	No of pts	%	Mean	Range
Gender				
Male Female	126 84	60 40	NA NA	NA NA
Age (months)			20	1-50
Duration of symptoms (days)			4	3-6
Clinical manifestations				
Vomiting episodes	101	48	3	2-6
Vomiting duration (days)	101	48	2.5	1-5
Diarrheal episodes	210	100	4	3-7
Diarrheal duration (days)	210	100	2.5	2-5
Fever	17	8	NA	NA
Abdominal pain	17	8	NA	NA
Dysentery	10	5	NA	NA
Dehydration status				
Severe Mild	25 58	12 28	NA NA	NA NA
Treatment given				
IV fluids	189	90	NA	NA
Oral	21	10	NA	NA

The presence of adenovirus was identified through the utilization of the cell culture approach. Subsequently, the confirmation of results was carried out via ELISA, followed by validation using the nested PCR method. HEp-2 cell lines were employed to inoculate the stool supernatant, resulting in 20% (n=42) of the study participants exhibiting a positive cytopathic effect. Correspondingly, 11% (n=23) of the children displayed a positive confirmation of adenovirus antigen. Furthermore, 15% (n=15) of the children tested positive for adenovirus through the application of nested PCR. The results are displayed in figure 1.

During this study, we categorized the participants into five distinct age groups. Furthermore, the findings indicated that 72.00% of children infected with HAdV were 24 months old or younger. A significant distinction among the five age groups was revealed through Pearson's chi-square statistical analysis, with a calculated p-value of 0.005.

Table No 2: The gender, age and seasonal variation of the AGE among the Adeno virus positive and negative patients

Characteristics	Adeno +ve	Adeno -ve	Total	*p* value
Gender				
Male	9	117	126	0.001*
Female	6	76	84	
Age group (months)				
1-6	5	28	34	0.005*
7-12	14	34	48	
13-18	8	18	26	
19-24	6	40	46	
Older than 24	10	46	56	
Season				
Winter	14	42	56	0.001*
Summer	6	50	56	
Spring	6	42	48	
Autumn	20	30	50	

*p<0.05 as significant

Discussion:

Acute gastroenteritis (AGE) is a prevalent and significant health concern among pre-school children, characterized by inflammation of the stomach and intestines, leading to symptoms such as vomiting, diarrhea, abdominal pain, and dehydration. The etiology of acute gastroenteritis in this population is multi-faceted, involving various infectious agents and environmental factors. Bacterial pathogens such as Rotavirus and Norovirus have been identified as leading causes of AGE among pre-school children.[13,15] These viruses are highly contagious and can spread easily in settings where children are in close proximity, such as daycares and preschools. Additionally, bacterial infections attributed to Escherichia coli, particularly enterotoxigenic and enteropathogenic strains, as well as Campylobacter and Salmonella, contribute to the etiology of AGE in pre-school children.[16,17] These pathogens are often transmitted through contaminated food, water, or contact with infected individuals. Poor hygiene practices and inadequate sanitation facilities in settings with young children can further exacerbate the transmission of these infectious agents.[18] Furthermore, environmental factors play a significant role in the etiology of acute gastroenteritis among pre-school children. Crowded living conditions, suboptimal water and sanitation systems, and lack of access to clean drinking water can amplify the spread of infectious agents.[19] Socioeconomic disparities also contribute to the burden of AGE, as children from lower-income families may have limited access to healthcare services and preventive measures such as vaccinations against rotavirus.[20] In current study, in contrast to certain researchers' findings, we discovered a notable gender-related prevalence of adenovirus infection both across general and urban areas. Our findings indicated a higher occurrence of adenovirus infections in males compared to females, which aligned with earlier research conducted in Bangladesh and Northwestern Nigeria.[21-23] This observation can be credited to the physiological differences between the two sexes, where males frequently demonstrate a comparatively weaker immune response to viral infections compared to females, as previously noted by other rese archers.[24] The current trial also revealed that a significant proportion of HAdV-infected patients were aged ≤2 years, while the infection rate markedly decreased in children older than two years. Among various age brackets, the peak incidence of HAdV was noted in children aged 13–18 months. Other studies have shown reduced susceptibility in children aged 1-6 months, corroborating the concept that maternal antibodies offer substantial protection against viral infections during the initial six months of a child's life. Similar to previous research, this study possesses several constraints. Among these, one limitation was the exclusion of asymptomatic individuals from the control group.

Consequently, the findings lack applicability to broader rural and urban regions, as well as hospitals spanning the entire province and country. Additionally, we did not explore alternative sources of diarrheal infections, apart from adenovirus, among the participants of this study

Conclusion:

The etiology of acute gastroenteritis among pre-school children is complex and multifaceted, involving a range of viral pathogens; environmental and socioeconomic factors also play some role.

Competing Interest

None to declare

Funding

No funding for this study.

References:

1. GBD Diarrhoeal Diseases Collaborators. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis. 2017;17(9):909-48. doi: 10.1016/S1473-3099(17)30276-1, PMID 28579426.
2. Polman K, Becker SL, Alirol E, Bhatta NK, Bhattarai NR, Bottieau E, et al. Diagnosis of neglected tropical diseases among patients with persistent digestive disorders (diarrhoea and/or abdominal pain 14 days): Pierrea multi-country, prospective, non-experimental case-control study. BMC Infect Dis. 2015;15:338. doi: 10.1186/s12879-015-1074-x, PMID 26282537.
3. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. 2013;382(9888):209-22. doi: 10.1016/S0140-6736(13)60844-2, PMID 23680352.
4. Walker CLF, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA, et al. Global burden of childhood pneumonia and diarrhoea. Lancet. 2013;381(9875):1405-16. doi: 10.1016/S0140-6736(13)60222-6, PMID 23582727.
5. Platts-Mills JA, Babji S, Bodhidatta L, Gratz J, Haque R, Havt A, et al. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). Lancet Glob Health. 2015;3(9):e564-75. doi: 10.1016/S2214-109X(15)00151-5, PMID 26202075.
6. Schnee AE, Haque R, Taniuchi M, Uddin MJ, Alam MM, Liu J, et al. Identification of etiology-specific diar-

rhea associated with linear growth faltering in Bangladeshi infants. Am J Epidemiol. 2018;187(10):2210-8. doi: 10.1093/aje/kwy106, PMID 29767678.
7. Direccio´n de Epidemiologı´a y Demografı´a,Ministerio de Salud y protección social. ANALISIS de SITUACIO´ N De Salud (ASIS) Colombia, 2020. [Lo´pez-Medina E, Parra B, Da´valos DM, Lo´pez P, Villamarı´n E, Pelaez M. Acute gastroenteritis in a]. Imprenta Nac Colomb. 2016;8.
8. López-Medina E, Parra B, Dávalos DM, López P, Villamarín E, Pelaez M. Acute gastroenteritis in a pediatric population from Cali, Colombia in the post rotavirus vaccine era. Int J Infect Dis. 2018;73:52-9. doi: 10.1016/j.ijid.2018.06.006, PMID 29908961.
9. Cotes-Cantillo K, Paternina-Caicedo A, Coronell-Rodríguez W, Alvis-Guzmán N, Parashar UD, Patel M, et al. Effectiveness of the monovalent rotavirus vaccine in Colombia: a case-control study. Vaccine. 2014;32(25):3035-40. doi: 10.1016/j.vaccine.2014.03.064, PMID 24699470.
10. Farfán-García AE, Imdad A, Zhang C, Arias-Guerrero MY, Sánchez-Álvarez NT, Iqbal J et al. Etiology of acute gastroenteritis among children less than 5 years of age in Bucaramanga, Colombia: A case-control study. PLOS Negl Trop Dis. 2020 Jun 30;14 (6):e0008375. doi: 10.1371/journal.pntd.0008375, PMID 32603324.
11. Manrique-Abril FG. Tigne y Diane B, Bello SE, Ospina JM. [Diarrhoea-causing agents in children aged less than five in Tunja, Colombia]. Rev Salud Publ (Bogota). 2016;8:88-97.
12. Ryoo E. Causes of acute gastroenteritis in Korean children between 2004 and 2019. Clin Exp Pediatr. 2021;64(6):260-8. doi: 10.3345/cep.2020.01256, PMID 32972055.
13. Matthijnssens J, et al. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch Virol. 2019;156(10):1627-37.
14. Bartsch SM, et al. The potential economic value of a hypothetical Zika vaccine to prevent Latin American epidemics. Vaccine. 2018;34(46):5796-806.
15. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. 2013;382(9888):209-22. doi: 10.1016/S0140-6736 (13)60844-2, PMID 23680352.
16. Degiuseppe JI, Stupka JA. Genotype distribution of Group A rotavirus in children before and after massive vaccination in Latin America and the Caribbean: systematic review. Vaccine. 2020 Jan 22;38(4):733-40. doi: 10.1016/j.vaccine.2019.11.017, PMID 31771863.
17. Troeger C, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis. 2017;18(11):1211-28.
18. Lopman BA, Pitzer VE, Sarkar R, Gladstone B, Patel M, Glasser J, et al. Understanding reduced rotavirus vaccine efficacy in low socio-economic settings. PLOS ONE. 2012;7(8):e41720. doi: 10.1371/journal.pone.0041720, PMID 22879893.
19. Meqdam MM, Thwiny IR. Prevalence of group a rotavirus, enteric adenovirus, norovirus and astrovirus infections amongchildren with acute gastroenteritis in Al-Qassim, Saudi Arabia. Pak J Med Sci. 2017;23:551.
20. Aminu M, Ahmad AA, Umoh JU, De Beer MC, Esona MD, Steele AD. Adenovirus infection in children with diarrhea disease inNorthwestern Nigeria. Ann Afr Med. 2007;6(4):168-73. doi: 10.4103/1596-3519.55702, PMID 18354941.
21. Jarecki-Khan K, Tzipori SR, Unicomb LE. Enteric adenovirus infection among infants with diarrhea in rural Bangladesh. J Clin Microbiol. 1993;31(3):484-9. doi: 10.1128/jcm.31.3.484-489.1993, PMID 8458940.
22. Schmttz, H; Wigand, R; Heinrich, W. Worldwide epidemiology of human adenovirus infections. Am J Epidemiol. 2019;117:455-66.
23. Klein SL. Sex influences immune responses to viruses, and efficacy of prophylaxis and treatments for viral diseases. BioEssays. 2012;34(12):1050-9. doi: 10.1002/bies.201200099, PMID 23012250.
24. Akihara S, Phan TG, Nguyen TA, Hansman G, Okitsu S, Ushijima H. Existence of multiple outbreaks of viral gastroenteritisamong infants in a day care center in Japan. Arch Virol. 2005;150(10):2061-75. doi: 10.1007/s00705-005-0540-y, PMID 15841336.