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Original Article

Age and Gender Distribution of Enteric Adenovirus and Rotavirus Infections among Pediatric Population and Determination the Level of IL-6, IL-8 and IL-10

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Abstract:

The onset of acute gastroenteritis due to viral infection mostly occurs during early childhood. Two viruses namely rotavirus and enteric adenovirus serve as the leading agents which trigger acute gastroenteritis in children and infants. The objective of this retrospective study involved evaluating demographic information alongside the frequency of rotavirus and enteric adenovirus antigen testing in hospitalized pediatric patients at our medical facility. Medical reviewers examined all hospital patients between October 2023 and March 2024 who presented with diarrhoea symptoms until their stool samples received testing. Stool antigen testing for rotavirus and enteric adenovirus occurred through the use of an immunochromatographic assay. The rotavirus stands as the leading factor in child acute gastroenteritis according to Babylon Hospital for Women and Children. A proper diagnosis of gastroenteritis depends on frequent testing for rotavirus and adenovirus antigens in recent stool samples. Viruses lead to most cases of gastroenteritis among children during their childhood. The Babylon Governorate area of our hospital underwent analysis revealing that rotavirus and adenovirus act as the two primary microorganisms causing gastroenteritis infections particularly prevalent among children in their 5-24 months period during autumn-winter months. Knowledge about the gastroenteritis origin in young children under two years old would promote swift patient recovery and reduce unnecessary healthcare expenses. The study of these conditions serves as a crucial requirement to spread understanding about the condition among impoverished populations who reside in Babylon Governorate. The scientists determined the lymphoblast fraction compared to complete lymphocytes based on transformation rates between study groups and controls (62.91 ± 11.45).

Keywords: Enteric Adenovirus, Rotavirus, IL-6, IL-8, IL-10

Introduction:

Viral gastroenteritis shows a wide range of clinical outcomes which range between undetected infections to dangerous fluid loss. Identifying between bacterial and viral causes of gastroenteritis remains difficult to determine by clinical means in human patients. The therapeutic steps differ between bacterial and viral causes of illness. The correct administration of specific antibiotics becomes essential for bacterial invasive gastrointestinal infections in order to decrease the risks of mortality and illness. Bacterial agents typically get identified through stool culture growth procedures according to research [1, 2]. The outcome of such diagnostic tests requires multiple days until results are available and these tests prove ineffective for bacterial infection identification. Clinical diagnosis of intestinal inflammation in infectious gastroenteritis remains controversial because of faecal leukocytes detection. Acute gastroenteritis affects anyone regardless of age yet its underlying causes and disease intensity depend on the age of the individual. Multiple studies verify that rotavirus and enteric adenovirus exist as the main virus contributors to nonbacterial gastroenteritis among children younger than five years old. Most cases of gastroenteritis in children develop due to viral infections. Children become infected by rotavirus numerous times throughout their lives with the entire child population generally experiencing infections before turning five years old [3-5]. The fast transmission rate of gastroenteritis among children occurs because the illness incubation period lasts less than 48 hours. Symptoms due to rotavirus infection start within 24-48 hours post-infection showing mild shock experience and dehydration with vomiting and watery diarrhea along with periodic fever. Infections leading to diarrhoea stand as one of the principal reasons responsible for child mortality [6]. This research aimed to measure IL-6 IL-8 and IL-10 concentrations together with investigating the gender ratios within children infected with enteric adenovirus and rotavirus. Our study includes epidemiological provincal data which shows enteric adenovirus and rotavirus agent distributions among genders and aged groups infected with viral gastroenteritis.

Patients and Methods:

This study included data from October 2023 to March 2024 that investigated hospitalized children at our pediatric outpatient clinic who experienced diarrhoea and had watery stool samples with negative parasite findings through microscopic examination. The analysis excluded both parasite specimens (including Giardia intestinalis, Entamoeba histolytica adhesin antigen, etc.) and cases with hemorrhagic stool results. The analysis involved reviewing the clinical records of patients from ages 0 to 15 who presented positive results for rotavirus and adenovirus antigens on their fresh stool samples. The study documented essential demographic data about these subjects. Our microbiology laboratory used standardized operating procedures for tests that checked recent stool specimens for rotavirus and adenovirus through immunochromatographic methods. The testing devices for rotavirus achieved a sensitivity level of 99.1 percent and the ones for adenovirus reached a sensitivity level of 99.9 percent based on manufacturer tests. SPSS Inc.'s version 12.0 of Statistical Package for the Social Sciences performed the data analysis of collected information. A chi-square test was applied for statistical analysis of the obtained results. The analysis required statistical significance when P values measured lower than 0.05.

Blood Sample Collecting:

- The researcher collected ten milliliters of venous blood from each study participant through a sterile test tube before placing a screw cap to seal it. This tube container already contained 200 units of heparin including no preservatives at a concentration of 20 units per milliliter. The obtained sample requires this division into specific sections:
- The measurement of 3H* -thymidine takes place in a 7-milliliter blood specimen by using either "separated lymphocyte" or "whole blood" methods in the lymphocyte transformation test.

Viability and Lymphocyte Separation Assay

Studies confirmed that Ficoll-Hypaque density gradient technique served as the primary method for extracting PBMs from peripheral blood. The mixture required equal volumes of Heparinised blood along with Hanks Balance Salt Solution before the combination process began. A sterile Pasteur pipette applied lymphoprep on top of the 2:1 mixed solution. The 2000 rpm centrifugation process of the tube lasted thirty minutes under ambient environmental conditions. The research team obtained the buffy interface mixture with lymphocytes and a few monocytes which they subsequently cleaned twice using HBSS [9, 10]. The last step required a neubar hemocytometer to measure 1 ml of complete RPMI-1640 medium for dilution until the cells reached 106 cells/ml.

To determine the viability of trypano blue dye exclusion test follow this procedure:

The reaction was halted when 5 microlitres of cell suspension were mixed with 200 microlitres of 0.2% trypan blue dye. when waiting 1-3 minutes, cells were counted using a hemocytometer until a minimum of 100 cells were obtained. No staining of living cells was observed using trypano blue dye.

Viability = Viable cells counted / Total cells count (viable + dead cells) × 100

At all times, we consider the viability to be higher than 95%.

Separated Lymphocytes for Lymphocyte Transformation Assay

a reaction that caused T-lymphocytes to proliferate was established. We started by finding the best concentration for lymphocyte proliferation using a different concentration of PHA (250µg/ml). Then, we created the serial dilution as follows: concentration range in milligrams/milliliter This RPMI-1640.

Transformation of Lymphocytes with the Use of Whole Blood and Culture Method

In a lymphocyte transformation assay, whole blood was mixed with RPMI-1640 medium (which does not contain FCS) at a ratio of 1:15 (v:v).

Using the Stain Method for Lymphocyte Transformation Assay

The following procedure had been followed:

- Using two separate tubes for each test, mix 250 µl of heparinized blood with 2.5 ml of full RPMI-1640 medium in sterile silicon coated tubes.
- One tube was treated with 250 □l of mitogen (PHA) from the Saddam center for cancer and molecular genetics research in Iraq (the test tube), while the other tube was left untreated (the control tube).
- The tubes were incubated at 37°C with 5% CO2 in a humidified environment for 72 hours; daily shaking was required.
- Centrifugation at 2000 rpm for 10 minutes was used to separate the cells at the end of the incubation time.
- The sediment cells were treated with 5 milliliters of KCl, a hypotonic solution, and left to incubate at 37 degrees Celsius for half an hour. - ACentrifugation was then performed for 10 minutes at 2000 rpm.
- After adding 5 milliliters of fixation solution to the sediment cells, they were refrigerated at 4 degrees Celsius for 15 minutes.
- A colorless suspension of sediment cells was achieved by washing the sample three to four times with fixing solution and centrifugation.
- After transferring a single drop of sediment cells onto two separate slides using a Pasteur pipette, allowing them to dry at room temperature, staining them with giemsa for 10 minutes, washing them with D.W., and finally, counting 200 cells under a microscope using an oil immersion lens, the process was inspected under a microscope.
- This equation had been used to determine the transformed cell ratio:

  Transformed cells % = No. of transformed cells / Total no. of cells counted.

Results and Discussion:

Health professionals consider diarrhea as one of the main health concerns particularly in developing nations. Analyzing child diarrhea experiences helps explain the prevalence since every child under five years faces two episodes

yearly. The main reason infants and older children need hospital treatment due to severe diarrhea-related dehydration stems from viral gastroenteritis in both developing and developed countries where the illness leads to infant mortality as well [11, 12]. The majority of these patients develop their symptoms due to rotavirus infections. The major significance of viral gastroenteritis exists in its cause of excessive hospitalization rates. From this perspective the healthcare expenses related to the disease represent a considerable financial strain for both families and nations. The distribution of cases and rate of rotavirus (+) and adenovirus (+) antigen positivity according to gender (43.59 and 41.94) respectively for male and (56.41 and 58.06) for female. The distribution of cases and rate of rotavirus (+) and adenovirus (+) antigen positivity according to age [(0-10), (11-20), (21-40) and (41-60) months] recorded (17.94%, 20.51%, 35.90% and 25.64%) in male and (16.13%, 22.58%, 35.48% and 25.81%) in female. Research shows the rotavirus presents itself as the leading agent behind viral gastroenteritis in literature. Among all contributing factors the rotavirus along with rotavirus-adenovirus combined to make up 17% of cases. The study conducted by Bayraktar et al. analyzed 348 specimens which showed rotavirus antigen at 23.7% while adenovirus antigen detected at 1.5% findings. Coban et al. performed a study which revealed out of all gastroenteritis cases tested both rotavirus- and adenovirus-associated incidents reached 13.6% and 49.3%, accordingly. In their study Bates et al. discovered that rotavirus gastroenteritis affected 78.3% of subjects whereas adenovirus gastroenteritis affected 17.8%. Research proves that rotavirus vaccines successfully protect up to 85% of patients against rotavirus diarrhea and prevent all instances of severe diarrhea in 85% - 100% of cases. We contacted families who had children with rotavirus-diagnosed illness for an inquiry about their child's vaccination status where we discovered that none of the children received vaccines. The study aimed to offer proper recommendations about protecting from viral gastroenteritis to our provincial physicians whose families include children [13-16]. The studies show uniform results indicating gender similarity when it comes to viral gastroenteritis occurrence. Studies from Iraq reveal that gender does not affect the occurrence of disease among the population. This scientific investigation failed to reveal any important connection ($P > 0.05$) regarding gender and viral antigen detection results. Data shows that rotavirus and adenovirus gastroenteritis occur mainly in children who are under two years of age [18-21]. Children experience adenovirus gastroenteritis throughout a larger time span from the ages 17 to 27. Study results from Bayraktar et al. demonstrated that among 348 viral-antigen–positive patients rotavirus affected half of the people at age 2. This study showed that the youngest population under 2 years old consisted of 187 of 327 (57.1%) rotavirus cases while the age ranges from 2 to 5 years old had 88 of 327 (26.9%) rotavirus cases and ages from 5 to 15 years old had 52 of 327 (15.9%) rotavirus cases. Adenovirus prevalence showed 53 of 327 (16.2%) cases in children younger than 2 years old along with 19 of 327 (5.8%) cases in children aged 2 to 5.

Table 1. The distribution of cases and rate of rotavirus (+) and adenovirus (+) antigen positivity according to age and gender.

Rotavirus (+), n=39			Adenovirus (+), n= 31		
Gender	Male	17(43.59)	Gender	Male	13(41.94)
	Female	22(56.41)		Female	18(58.06)
Age groups (Months)	0-10	7(17.94)	Age groups (Months)	0-10	5(16.13)
	11-20	8(20.51)		11-20	7(22.58)
	21-40	14(35.90)		21-40	11(35.48)
	41-60	10(25.64)		41-60	8(25.81)

Stain Method for Lymphocyte Transformation: The lymphoblast percentage from the total lymphocytes was determined by measuring the morphological change of lymphocytes under the oil immersion lens of the light microscope. The results were expressed as an assay. The transformed cells (Mean ± S.D) were significant less P<0.05. Lymphoblast percentage from the total lymphocytes was determined by measuring the morphological change of lymphocytes (Lymphocyte Transformation) in rotavirus (+) (33.09 ± 6.17) and adenovirus (+) (40.00 ± 6.29) than those of control group (62.91 ± 11.45) Figure 1. These findings agreed with the previous studies reporting that the capacity of lymphocyte to be transformed as response to rotavirus (+), and those with adenovirus (+) all have lower PHA levels than the control group. Changes in plasma lipoproteins are a prevalent cause of the poor T-cell response to mitogens as PHA in chronic liver disease, according to some writers. Because there are fewer lymphocytes in the peripheral blood of patients with rotavirus and adenovirus, the T lymphocyte response to PHA is diminished. Immunosuppressive factors, linked to high viral replication levels, were responsible for the decreased lymphocyte PHA [22, 23] capacity in rotavirus and adenovirus; these findings demonstrated the function of these factors in the immunological pathogenic processes. Studies have shown that healthy carriers have a normal lymphocyte response to PHA, which means that there is no major liver damage and no overall impairment of the immune system's cellular response. Utilizing flow cytometry for a more precise assessment of the expression on the surface of several activation antigens, he conducted the proliferative response with more targeted T-cell activation techniques.

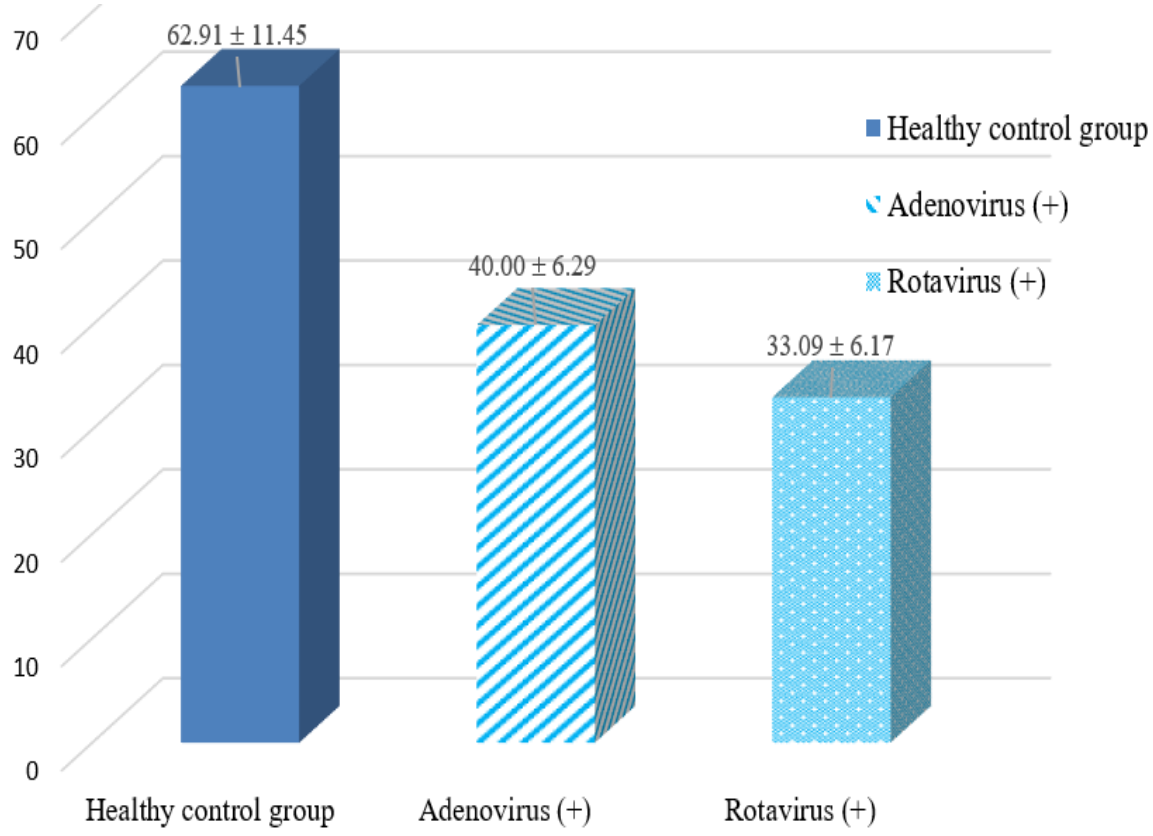


Figure 1. Lymphoblast percentage from the total lymphocytes was determined by measuring the morphological change of lymphocytes (Lymphocyte Transformation) in Rotavirus (+), Adenovirus (+) and healthy control group.

Figure 2. The distribution of cases and rate of rotavirus (+) antigen positivity according to gender

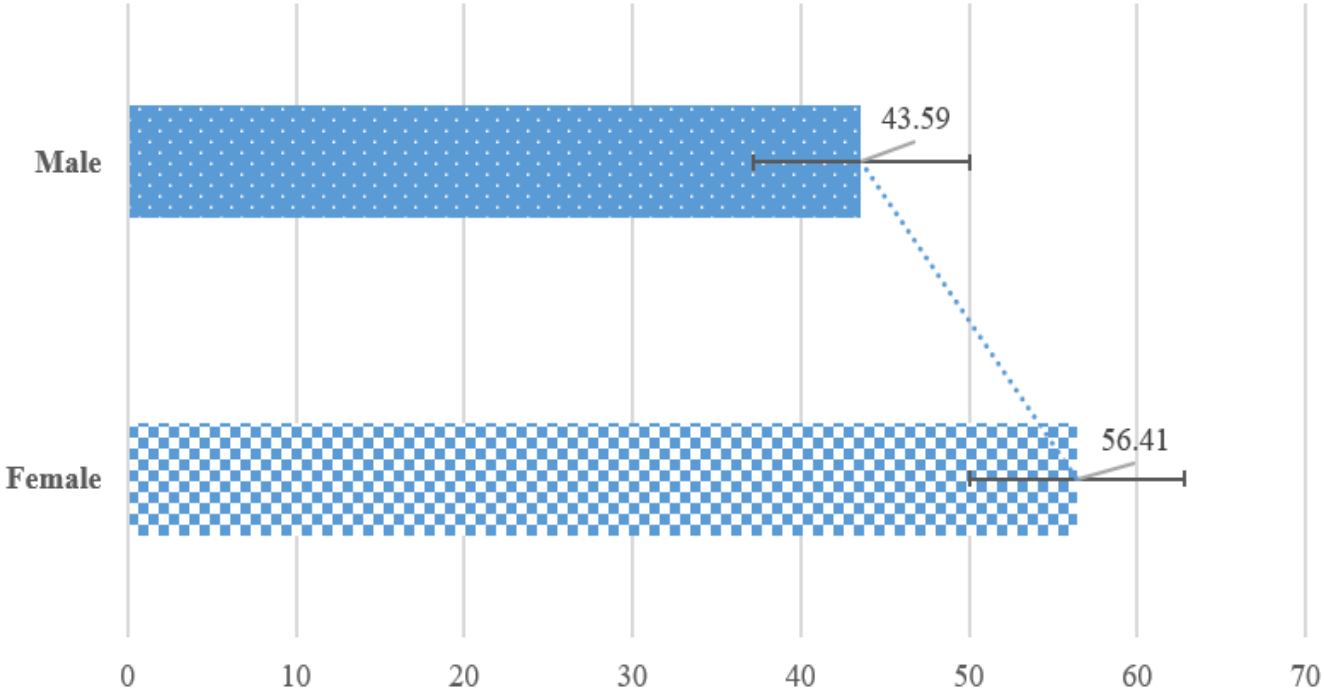


Figure 3. The distribution of cases and rate of adenovirus (+) antigen positivity according to gender

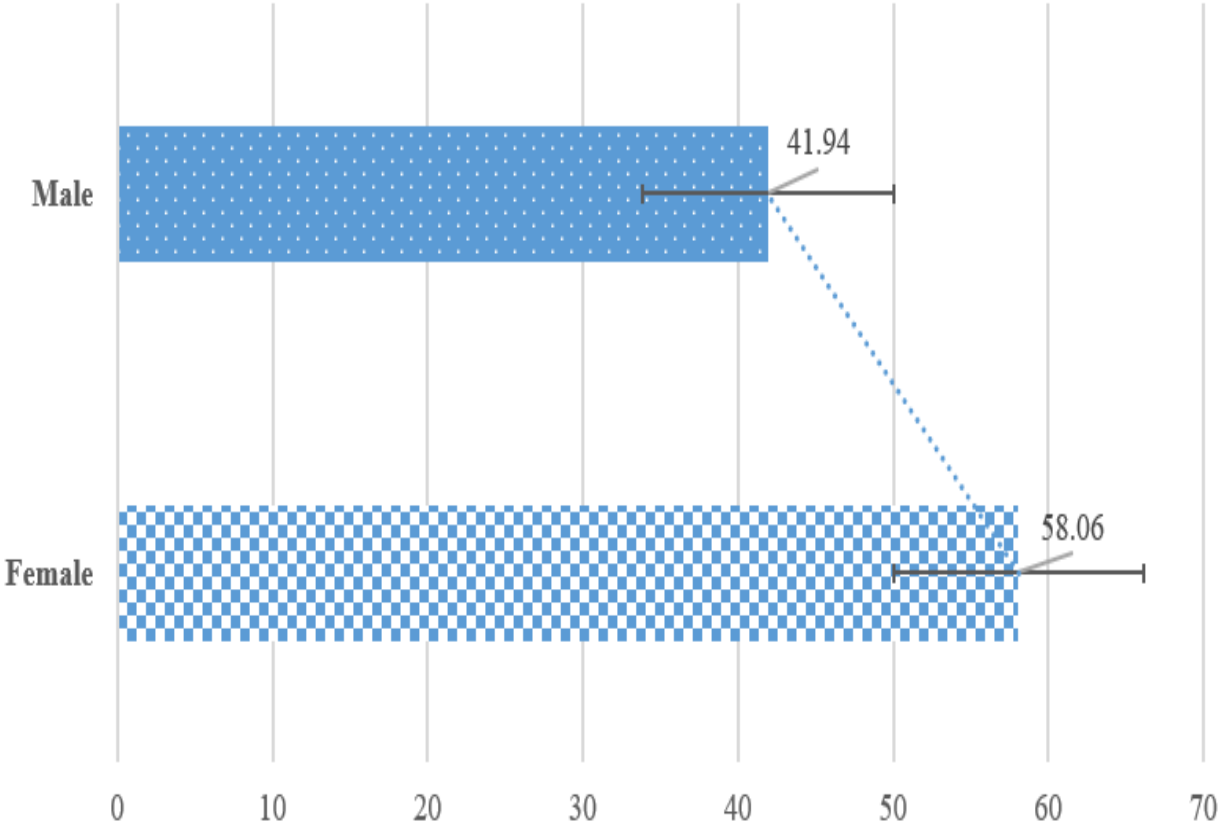


Figure 4. The distribution of cases and rate of rotavirus (+) antigen positivity according to age group.

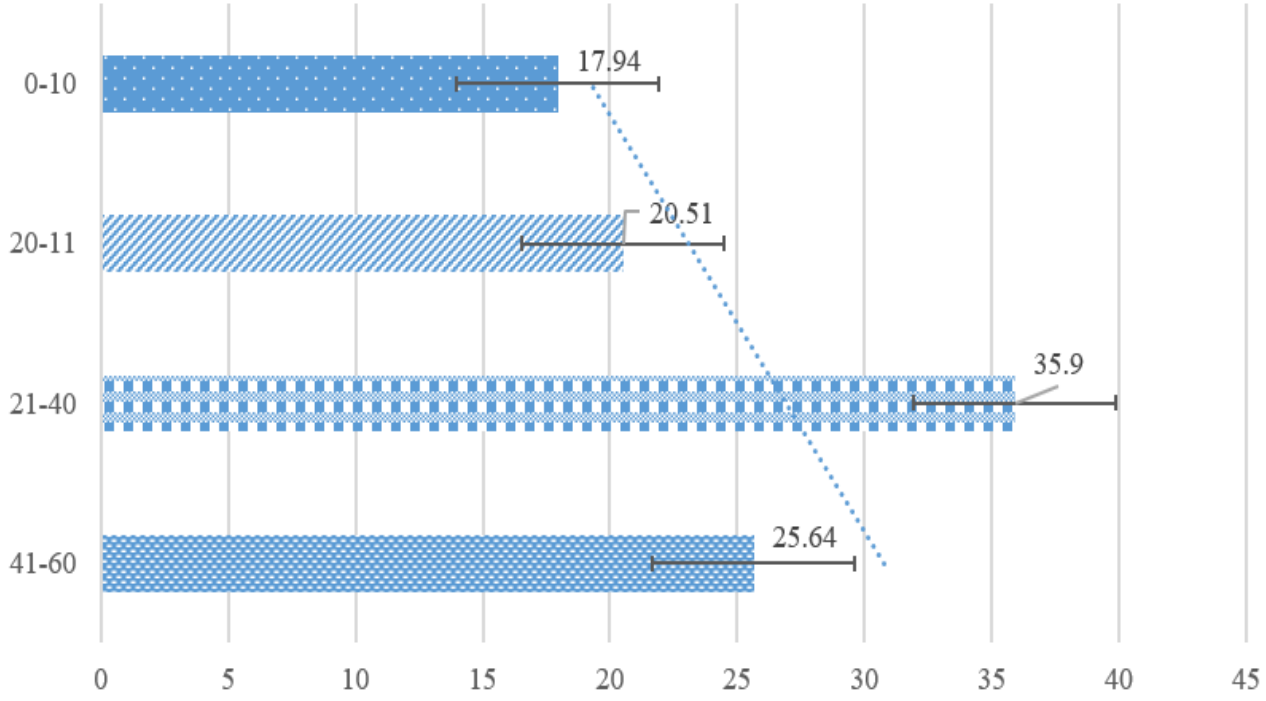
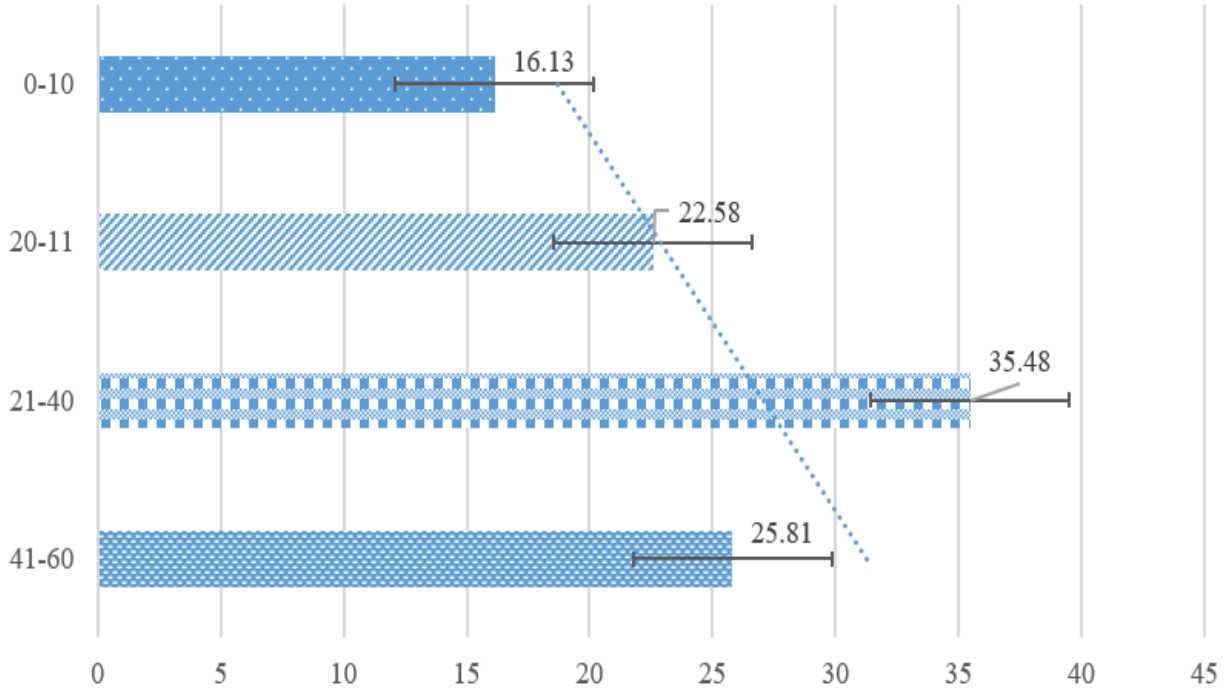


Figure 5. The distribution of cases and rate of adenovirus (+) antigen positivity according to age group.



The endemic nature of rotavirus persists throughout all seasons in tropical regions yet it primarily causes outbreaks in winter and early spring periods in both temperate and our country. Recent studies from the literature show that rotavirus epidemiology has experienced modifications mainly because of the implementation of rotavirus vaccines. Some research showed that rotavirus showed positive results during winters but adenovirus displayed higher positivity rates in springtime. Research shows rotavirus tends to be detected often in the

winter season and adenovirus emerges in both autumn and winter periods. The pattern of seasonal variation does not exist for adenoviruses since these viruses have been found to cause acute gastroenteritis throughout all seasons. The study revealed that spring and winter produced the highest frequency of rotavirus positivity yet failed to detect statistical evidence showing seasonal patterns for adenovirus. Studies that provide essential epidemiological data about rotavirus infections have significantly grown in our nation since the disease's severity received stronger public appreciation. The identification of viral gastroenteritis pathology allows healthcare providers to avoid prescribing antibiotics unnecessarily to patients. Routine service laboratories cannot conduct viral culture tests since these methods require extensive time and prove difficult to implement. Immunochromatographic tests serve as a standard technique for stool-based rotavirus and enteric adenovirus antigen detection because their short test duration and high detection accuracy go together with their ability to process multiple agents. Researchers have mainly used ELISA and immunochromatographic methods to detect rotavirus infections in their studies. The tests will deliver their best performance results if used during symptomatic times of patient experiences.

Conclusion:

Viral agents must be included in the analysis of causative factors for acute gastroenteritis which affects children younger than five years. Rotavirus and enteric adenoviruses act as major infectious agents throughout the period of seasonal migration in our hospital's service area during winter months. The occurrence of rotavirus gastroenteritis surpassed adenovirus gastroenteritis infections. Rapid diagnostic tests using immunochromatographic methods hold potential benefits for the prevention of wrong antibiotic administration and severe complications because they provide early diagnosis for proper treatment selection. The study about enteric adenovirus and rotavirus agents in viral gastroenteritis will advance our province's epidemiological data through gender and age analysis.

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