Original Article

*Iraqi National Journal of Medicine* 1st June 2019, vol. 1, no. 2

# Human Astrovirus among Children with Cancer in Basrah

**Morroge A. Jassim (MBChB),** [1] **Janan G. Hasan (CABP),** [2] **Hassan J. Hasony (PhD).** [3]

[1] **Morroge Abdulaali Jassim (MBChB)**

[2] **Janan Ghalib-Hasan (CABP)** Professor, Department of Pediatrics, College of Medicine, Basrah University

[3] **Hassan J. Hasony (PhD)** Department of Microbiology, College of Medicine, Basrah University

**Correspondence:**
**Janan Ghalib-Hasan**
Email:
jenan_ah03@yahoo.com

**ABSTRACT**

**Background.** The impairment of the T-lymphocyte system leads to reduced viral clearance, resulting in intensified disease and the possibility of prolonged infection.(2) The poor functioning of B lymphocytes makes the host susceptible to bacterial and viral infections, especially in cases of malignant disease.

**Aim of the study.** The present study set out to determine the frequency of human astrovirus infection among patients who suffered from malignancies who were being treated in the Oncology Center of the Basrah Children's Specialty Hospital.

**Patients and Method.** A cross-sectional study was approved for a population of children with cancers during the period from October 1, 2015 through the end of January, 2016. Forty-five children (24 females and 21 males), all with cancers, were admitted to the Oncology Center of Basrah Children's Specialty Hospital. Their ages ranged from under 1 year to 15 years. According to the results of a specially designed questionnaire, data were obtained from patients who were either symptomatic or asymptomatic for human astrovirus infections, including 3 newly diagnosed cases (before chemotherapy) and 38 cases (during chemotherapy), with the remaining 4 cases admitted after chemotherapy. Ninety stool samples were collected at day 0 and day 4 after admission, and all were tested using astrovirus antigen enzyme-linked immuno sorbent assay (ELISA) kits (EIA-4456).

**Results.** On both days, the rate of astrovirus infections was 15.6% in the hospitalized children with cancers, and there were no statically significant differences between hematological malignancies and solid tumors (P value was 0.857). Among hematological malignancies, astrovirus was detected at a significant rate (the P value was 0.0001) in patients with acute myeloblastic leukemia, while patients with solid tumors exhibited significant expression of rhabdomyosarcoma (the P value was 0.001).

Astrovirus infection was more prevalent in females (85.8%) than in males (the P value was 0.001). Infection was most prevalent in the age group of >1–5 years (57%), and most of the infected patients (85.8%) were from rural areas (the P value was 0.012). In most of the symptomatic infected cases (71.4%) the symptom was acute diarrhea (the P value was 0.05), and this was typically during chemotherapy.

**Conclusion.** Astrovirus infections occur at significant rates in acute myeloblastic leukemia, among hematological malignancies, and in patients with rhabdomyosarcoma, in the solid-tumor category. Therefore, astrovirus screening should be done for all children with cancers, and especially for patients with acute myeloblastic leukemia or rhabdomyosarcoma.

**Keywords:** Cancer in children, human astrovirus

## INTRODUCTION

Childhood cancers include a variety of malignant tumors. The incidence worldwide is around 160,000 cases per year, with annual mortality rates typically around 90,000. (1) Human astrovirus (HAstVs) is considered to be the second most-common cause of gastroenteritis in children worldwide, right behind rotavirus. (2,3) Little is known about the prevalence of astrovirus gastroenteritis in immune-compromised patients with hematological malignancies,(4) which has been regularly associated with hospital-acquired gastroenteritis and the

shedding of HAstVs for sustained periods of time. [5]

In the last 3 decades, the health status of the Iraqi people has suffered serious setbacks. The tragically unfortunate circumstances of wars and economic sanctions have damaged many aspects of life in Iraq, with adverse effects on physical health, nutritional status, and psychological wellbeing, in addition to a rising incidence of both communicable and non-communicable diseases, including cancer.

Children's cancer accounts for a higher percentage of cancer in developing countries than in high-income countries, owing to the greater proportion of children in poorer nations. Iraq is no exception in this regard. [6] Cancer is one of the leading causes of morbidity and mortality, and it is the second-leading cause of death in Iraq, according to the 2011 results of the Iraqi registry. The distribution of the top ten cancers in Iraqi children is ranked here according to their frequency up to fourteen years of age. Leukemia comprises about 33.11% of all childhood cancer, 26.8% of them from children in Basrah alone. [7] Acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) are the two most recurrent types of leukemia found in children. Central nervous system (CNS) cancers are the next-most recurrent cancers in Iraqi pediatric oncology, accounting for about 17.34% of all Iraqi childhood cancers. Lymphomas represent the third-most common type of childhood cancer in Iraq, according to the Iraqi cancer registry. Hodgkin's lymphoma (HL) accounts for 6.89% of all childhood cancers, and non-Hodgkin's lymphomas (NHL) account for 13.92% of all childhood malignancies. Bone cancers (including osteosarcoma and Ewing's sarcoma) occur with a frequency of 5.26%; kidney cancers, 4.85%; soft-tissue cancers, 3.62%; eye cancers, 2.46%; adrenal gland cancers, 1.64%; and finally, liver cancers, 1.16%. These data represent all cases of Iraqi pediatric oncology. [7]

The immune-competent host has a number of complex, non-specific (innate) and specific (adaptive) immune defenses against viruses. Rare defects of the innate immune system are associated with increased susceptibility to viral infections. These include deficiencies of complement, the interferon system, natural killer (NK) cells, and phagocytic cells.[8] There are several mechanisms of immunosuppression described in patients burdened by cancer, categorized into two main subgroups: (1) those indirectly caused by malignant cells through recruitment or expansion of suppressive cells that are not themselves malignant, and (2) immunosuppressive effects that are directly caused by the de facto malignant cells.[9]

In cancer, immune-suppression is mediated by immature myeloid cells (IMCs) that have been activated to become myeloid D-derived suppressor cells (MDSCs). In healthy individuals, IMCs constitute around 0.5% of circulating cells, developed in the bone marrow and readily differentiated into mature myeloid cells. However, in many forms of cancer, MDSCs accumulate as a result of a block in myeloid differentiation, and they up-

regulate immunosuppressive mechanisms, such as arginase activity, nitric oxide synthase (NOS) activity, and reactive oxygen species (ROS) production.[10] The impairment of the T lymphocyte system leads to reduced viral clearance, resulting in intensified disease and, possibly, prolonged infection. Poor functioning of B lymphocytes makes the host susceptible to bacterial infections. Defects of the adaptive immune system can be either primary or secondary, and leukemia is considered to be a secondary cause. [8]

Increased risk for infection is also associated with damaged skin or mucous membranes, indwelling catheters, and malnutrition. Invasive procedures present a risk for infections in an immunosuppressed host. Long hospital stays and widespread use of antibiotics also increase the risk of infections.[8] The risk of infection is further affected by the cause of the neutropenia—i.e., whether it is caused by bone marrow suppression or by increased consumption of neutrophils because of infection, or by some other cause. Infections are categorized into neutropenic and non-neutropenic infections, and the treatment of infection is influenced by the neutrophil count.[11]

Human astroviruses (HAstVs) were first identified in 1975 via electron microscopy, in stool samples of newborns with gastroenteritis; they have been isolated from humans as well as from animal species.[13] HAstVs have eight serotypes (HAstV-1 through HAstV-8), built on their reactivity to polyclonal antibodies and on a study by immunofluorescence assays, neutralization assays, and immune electron microscopy (IEM).[2] Astrovirus-specific antibodies (as determined by IEM) are associated with shielding immunity. It is undetermined whether humans develop neutralizing antibodies to astrovirus, as has been established in rabbits immunized with astroviruses 1, 3, and 5.[15]

Epidemiological studies conducted worldwide have reported the occurrence of HAstV infection among children hospitalized with diarrhea, as well as in the context of community-based studies. Overall, prevalence rates have ranged from 2% to 16% in hospital-based studies, and from 5% to 17% in community-acquired gastroenteritis. [16,17] Another study in Maya reported a prevalence of up to 26%. [18]

Astrovirus-associated gastroenteritis is most prevalent among children under 2 years of age (and less prevalent among adults), in daycare centers,[19] in immunocompromised patients, and in the elderly in nursing homes.[20] It has also been found to be a common cause of nosocomial diarrhea, and was shown to be more severe among patients with HIV infection.[21]

Gastroenteritis associated with astrovirus, like rotavirus, occurs in both developing and developed nations, suggesting that improvements in water and sanitation are unlikely to decrease disease incidence. The age distribution of HAstV infections may differ, depending on the scientific settings, geographic location, and the age span of the population studied.

In Iraq, particularly in Basrah, HAstVs were detected in 2.6% of acute diarrheal

cases of symptomatic hospitalized children, with ages ranging from 1 to 3 years old, and were not detected in children under 1 year of age or above 3 years. [19]

The seasonality of HAstV infections is debated and seems to differ by geographic region. In temperate environments, astrovirus diarrhea cases peak in winter,[22] while the seasonality is less clear in tropical locations. [19] Studies demonstrated that 34% to 60% of diarrhea cases among children in the United States, France, and Finland have a viral relevant agent appearing in late winter and spring. [23] In Korea, the majority of HAstV infections among hospitalized children occur in winter, simultaneously with the highest number of rotavirus infections,[24, 25] though astrovirus infections can also occur in the summer months. [26] HAstV infection can originate during any period of the year but mainly occurs during the cold season, from October to January. [27] In Iraq, particularly in Basrah, astrovirus infections were found in the winter and not detected in other seasons. [19] Infection with HAstV-2 was most common (42%),[28] while Madagascar reported a high incidence of the unusual HAstV-8 strain. [29]

Transmission in children happens typically from person to person. [5] Astrovirus, like other enteric viruses, is spread mainly through the fecal-oral route (including food- and water-borne transmission); sporadically, it is also transmitted by aerosols. [15] Children may transmit the virus for 1–2 days prior to illness and 4–5 days following illness; nonetheless, transmission for as long as 3 weeks has been reported.[30,31] Asymptomatic secretion can occur in neonates and young children, and this is a significant cause of infection in nurseries, childcare centers, and hospitals. [32]

The clinical features of HAstV infections may also rest on several factors. Associated diarrhea has been categorized by a median period of 3 days (with a range of 1 to 21 days), with a median of 4 stools (ranging from 1 to 10 stools a day), throughout the first 24 hours. HAstV-associated diarrhea was less severe than rotavirus-induced diarrhea, as measured by the number of stools and duration of mild, watery diarrhea,[5] vomiting, and fever, associated with other symptoms, such as anorexia, abdominal pain,[13] dehydration, and headache. [5] No recurrence of symptomatic infections was detected.

Few HAstV-infected children (3%) were hospitalized. AstV-MLB1 was not connected with diarrhea. However, other novel astroviruses were not assessed. Astroviruses can be associated with asymptomatic infections, sometimes requiring hospitalization, especially in 6- to 12-month-old babies, or they may be complicated for several weeks by a malabsorption syndrome. [15] Hepatitis, nephritis, and neurological complications were also reported. [33]

The sensitivity of the astrovirus ELISA was 91%, and the specificity was 96%; the new ELISA provides a sensitive and precise means for the detection of astrovirus gastroenteritis [34] HAstV is commonly associated with hospital-acquired gastroenteritis; it is also found in immunocompromised patients who

are known to excrete HAstVs for prolonged periods of time, especially in malignancy. [34] The purpose of this study, therefore, is to define the incidence of astrovirus infection among patients with malignancy and to identify the relationship between types of malignancies and human astroviruses.

## PATIENTS AND METHOD

A cross-sectional study was carried out on 45 patients with childhood cancers in the Oncology Center of Basrah Children's Specialty Hospital. The study included 3 newly diagnosed cases with malignancy, before chemotherapy; 38 known cases of childhood cancers, during chemotherapy; and 4 cases after chemotherapy. Patient ages ranged from ≤ 1 year to 15 years, with 24 females and 21 males. Ninety stool samples were collected at day 0 and day 4 of hospitalization, from patients who were either symptomatic or asymptomatic for human astrovirus infection in the period between October 1st, 2015, and the end of January 2016. All cases involved inpatients in the Oncology Center.

In this study, 25 cases involved hematological malignancy (15 cases of ALL and 5 cases of AML, 3 cases of HL and 2 cases of NHL); 20 cases involved solid tumors (5 cases each of neuroblastoma, brain tumors, and bone tumors, 2 cases of germ-cell tumors, 2 cases of rhabdomyosarcoma, and 1 case of Wilms' tumor). Using a questionnaire designed specifically for the purpose of this study (see Appendix I), the following information was taken: name, gender (male or female), residence (urban or rural), date of birth, and age group. With regard to age group, all children were classified into one of four categories: (≤1, >1–5,> 5–10, and >10–15). Education levels for patients' mothers and fathers were determined and classified into five categories: illiterate, primary, elementary, secondary, and academy. The type of water supply was ascertained (tap water or reverse osmosis (RO) water), as was the type of feeding: breast, bottle, or mixed (for ages ≤ 2 years), adult diet (for ages >2–15 years), or mixed. For the purposes of this study, the autumn season was considered to be the months from September through November, and the winter season was considered to be the months from December through February. Also noted were the date of diagnosis and the type of cancer diagnosed (specifically, whether the cancers were solid tumors or hematological malignancies). With regard to clinical manifestations, the questionnaire noted the presence of diarrhea and its duration, along with the presence of fever, vomiting, anorexia, abdominal pain, dehydration, and headache. The chemotherapy status noted whether the stool samples were collected before, during, or after chemotherapy. Blood investigations included WBC counts and neutrophil counts, classified into three neutropenia categories: severe (<500 cell/µl), moderate (500–1000 cell/µl), and mild (1000–1500 cell/µl). [35]

A total of 90 stool samples were collected from 45 children on day 0 and day 4 of their inpatient stays in the Oncology Center of the Basrah Children Specialty Hospital. To ensure the most accurate results, the samples were

immediately transmitted to the Laboratory department within approximately 5 minutes, and were then stored in a deep freezer at a temperature of -20°C for more than three months. All stored frozen samples were transported within 20 minutes from the Laboratory Unit of the Basrah Children's Specialty Hospital to the Virology Unit of the Microbiology Laboratory in Basrah Medical College by a special incubator box.

At Basrah Medical College, an analysis was conducted using astrovirus antigen enzyme-linked immune sorbent assay (ELISA) kits from DRG International Instruments in Germany, which measure the astrovirus antigen in fecal samples (EIA-4456). Astrovirus AG ELISA is a one-step enzyme immunoassay on the source of polyclonal and monoclonal antibodies, contrasted to astrovirus antigens. Frozen samples are quickly thawed, warmed to room temperature, and mixed well. The astrovirus ELISA was performed with specimens diluted at a 1:11 ratio. Diluted stool samples and horseradish peroxidase (HRP), considered monoclonal anti-astrovirus antibodies, were distributed simultaneously into the wells of microtitration plates coated with polyclonal anti-astrovirus antibodies. After an evolution time of 60 minutes at room temperature, unbound components were removed by a washing step. HRP converts the subsequent extra colorless substrate solution of 3, 3`, 5, 5` tetramethylbenzidine (TMB) within a 10-minute response time at room temperature, protected from light, into a blue product. The enzyme response is ended by distributing sulfuric acid into the wells, changing the solution from blue to yellow. The optical density (OD) of the solution read at 450/≥ 620 nm is straight, relative to the specifically bound volume of astrovirus, using an Huma Reader HS from the Human Company.

All statistical analyses were carried out using the Numerical Packages for Social Sciences (SPSS) software, version19. Data were expressed by the mean ± standard deviation. Comparisons of proportions were performed by cross-tab, using a chi-square test, when each cell had an expected frequency of 5 or more; when one or more of the cells had an expected frequency of less than 5, Fisher's exact test was used in a 2×2 table. Binary logistic relapse analysis was also done for the analysis of different variables, and binary logistical regression analysis was done for the analysis of different variables; odd's ratio (OR) and 95% self-confidence breaks (CI) were assessed. For all tests, a P-value of <0.05 was considered to be statistically significant.

## RESULTS

The classification by cancer type of the 45 cases included in this study is shown in Table 1. The population of the study consisted of 45 patients selected from among children with cancer. The breakdown of cancer types was as follows: hematological malignancies accounted for 55.6%, with ALL comprising 33.3% overall cancers; the remaining 44.4% of cases involved solid tumors, with the majority being made up of neuroblastomas, brain tumors, and bone tumors (11.1%), and

with the least prevalent cancer being Wilms' tumor (2.2%).

The demographic characteristics of pediatric oncology cases are presented in Table (2).

**Table 1.** Distribution of cases according to cancer types

| Types of cancer | | Cases | |
|---|---|---|---|
| | | NO. | % |
| Hematological malignancies | ALL | 15 | 33.3 |
| | AML | 5 | 11.1 |
| | HL | 3 | 6.7 |
| | NHL | 2 | 4.4 |
| Total | | 25 | 55.6 |
| Solid tumors | Neuroblastoma | 5 | 11.1 |
| | Brain tumors | 5 | 11.1 |
| | Bone tumors | 5 | 11.1 |
| | Germ cell tumors | 2 | 4.4 |
| | Rhabdomyosarcoma | 2 | 4.4 |
| | Wilms' tumor | 1 | 2.2 |
| Total | | 20 | 44.4 |
| Total | | 45 | 100 |

| Demographics | | Cases (Total 45) | |
|---|---|---|---|
| | | Number (NO.) | Percentage (%) |
| Sex | Males | 21 | 46.7 |
| | Females | 24 | 53.3 |
| Age (years) | ≤1 | 1 | 2.2 |
| | >1-5 | 22 | 48.9 |
| | >5-10 | 14 | 31.1 |
| | >10-15 | 8 | 17.8 |
| Residence | Urban | 15 | 33.3 |
| | Rural | 30 | 66.7 |
| Mother's education | Primary | 11 | 24.4 |
| | Elementary | 34 | 75.6 |
| Father's education | Illiterate | 3 | 6.7 |
| | Primary | 16 | 35.6 |
| | Elementary | 10 | 22.2 |
| | Secondary | 4 | 8.9 |
| | High Education Levels | 12 | 26.6 |
| Season | Autumn | 25 | 55.6 |
| | Winter | 20 | 44.4 |
| Water supply types | Both RO and Tap water | 45 | 100 |
| Dietary History | Bottle for≤2 years of age | 1 | 2.2 |
| | Adult diet for >2–15 years of age | 40 | 88.9 |
| | Both | 4 | 8.9 |

**Table 2.** Description of cases according to demographic variables

Females made up a greater portion of the study participants than males (53.3% and 46.7%, respectively). As regards age, most of the cases fell into the >1–5year age group, while the lowest number of participants were in the ≤ 1-year group. The largest percentage of cases came from rural areas, with fewer coming from urban areas. Educationally, most of the patients' mothers had an elementary-level education, while most of the fathers had a primary-level education; the smallest group being those who were illiterate. More patients were tested in autumn than in winter. Most were on an adult diet, and patients utilized both tap and RO water supplies.

Classification of cases in terms of diarrheal manifestation as related to chemotherapy status at the time of stool sample collection is shown in Table 3.

**Table 3.** Chemotherapy in relation to clinical diarrhea manifestation and its duration

| Patients' groups | | Chemotherapy | | | | | | Total | | P-Value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Before | | During | | After | | | | |
| | | NO. | (%) | NO. | (%) | NO. | (%) | NO. | (%) | |
| Diarrhea | Yes | 0 | 0 | 18 | 47.4 | 0 | 0 | 18 | 40 | 0.05 |
| | No | 3 | 100 | 20 | 52.6 | 4 | 100 | 27 | 60 | |
| Total | | 3 | 6.7 | 38 | 84.4 | 4 | 8.9 | 45 | 100 | |
| Diarrhea duration | ≤1week | 0 | 0 | 18 | 100 | 0 | 0 | 18 | 100 | ----- |
| | >1 week | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Total | | 0 | 0 | 18 | 100 | 0 | 0 | 18 | 100 | |

(P value was assessed by Fisher's exact test.)

This table shows that 40% of cases involved acute diarrhea that was statistically significant in relation to chemotherapy (the P value was 0.05). All of these cases had stool samples collected during chemotherapy. The remaining 60% of cases did not involve diarrhea and had stool samples collected mostly during chemotherapy. The classification of neutrophil counts relating to chemotherapy status is seen in Table 4. This table shows that most of the tested patients (60.5%) who had neutrophil counts in the range of >1000–1500 cell/µl were undergoing chemotherapy, while the lowest number (11.1%) represents neutrophil counts of ≤500 cell/µl, where there was no statistically significant relationship between chemotherapy and the neutrophil count (the P value was 0.7). The frequency of astrovirus infection in all 45 tested cases of children with cancer is shown in Table 5. In this table, we see that the rate of astrovirus infection in the selected group of children with cancers was 15.6%. Astrovirus infection was detected by Astrovirus Ag. ELISA (EIA-4456) done on stool samples, and similar positive results were found on day 0 and day 4 of hospitalization.

**Table 4.** Neutrophil count in relation to chemotherapy

| Investigation | Chemotherapy | | | | | | Total | | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | Before | | During | | After | | | | |
| | NO. | (%) | NO. | (%) | NO. | (%) | NO. | (%) | |
| Neutrophil counts ≤500 | 0 | 0 | 4 | 10.5 | 1 | 25 | 5 | 11.1 | 0.7 |
| >500–1000 | 1 | 33.3 | 11 | 29 | 0 | 0 | 12 | 26.7 | |
| >1000–1500 | 2 | 66.7 | 23 | 60.5 | 3 | 75 | 28 | 62.2 | |
| **Total** | 3 | 6.7 | 38 | 84.4 | 4 | 8.9 | 45 | 100 | |

**Table 5.** Frequency of astrovirus infection among children with cancers

| Astrovirus Ag. ELISA tests | Day 0 | Day 4 |
|---|---|---|
| | No. (%) | No. (%) |
| Positive | 7<br>15. 6 | 7<br>15. 6 |
| Negative | 38<br>84.4 | 38<br>84.4 |
| Total | 45<br>100 | 45<br>100 |

The frequency of astrovirus infection with different types of malignancies is shown in Table 6.

**Table 6.** Distribution of astrovirus infection according to types of childhood cancers

| Types of cancers | | Tested NO. | Astrovirus Ag. ELISA | | | | Total +ve (%) | P-value |
|---|---|---|---|---|---|---|---|---|
| | | | +ve | | -ve | | | |
| | | | NO. | (%) | NO. | (%) | | |
| Hematological malignancy | ALL | 15 | 1 | 6.7 | 14 | 93.3 | 14.3 | |
| | AML | 5 | 2 | 40 | 3 | 60 | 28.5 | 0.001 |
| | HL | 3 | 1 | 33.3 | 2 | 66.7 | 14.3 | |
| | NHL | 2 | 0 | 0 | 2 | 100 | 0 | |
| | Total | 25 | 4 | 16 | 21 | 84 | 57.1 | 0.857 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Solid tumors** | Neuroblastoma | 5 | 1 | 20 | 4 | 80 | 14.3 | 0.001 |
| | Brain tumors | 5 | 0 | 0 | 5 | 100 | 0 | |
| | Bone tumors | 5 | 1 | 20 | 4 | 80 | 14.3 | |
| | Germ cell tumors | 2 | 0 | 0 | 2 | 100 | 0 | |
| | Rhabdomyosarcoma | 2 | 1 | 50 | 1 | 50 | 14.3 | |
| | Wilms' tumor | 1 | 0 | 0 | 1 | 100 | 0 | |
| | Total | 20 | 3 | 15 | 17 | 85 | 42.9 | |
| | Total | 45 | 7 | 15.6 | 38 | 84.4 | 100 | |

*( P value was assessed by Fisher's exact test.)

According to the classification in this table, in hematological malignancies, the frequency of astrovirus infection was predominant and statistically significant (the P value was 0.001) in cases involving AML, while in patients with solid tumors, astrovirus infections were more prominent and statistically significant (the P value was 0.001) in children with rhabdomyosarcoma. There were no statistically significant differences for astrovirus infections between hematological and solid tumors (the P value was 0.857).

The effect of demographic factors on the distribution of astrovirus infection among children with malignancy are shown in Table 7. This table shows that the rate of astrovirus infections was statistically significant in females (the P value was 0.001) and in patients who come from rural areas (the P value was 0.012), while it was not statistically significant for other demographic indices included in the study. Association of diarrheal manifestation, neutrophil counts, and chemotherapy with astrovirus infection in this selected group is presented in Table 8.

**Table 7.** Distribution of astrovirus infection among children with cancers by demographic indices

| Demographics | | Tested NO. | Astrovirus Ag ELISA +ve NO. (%) | Astrovirus Ag ELISA -ve NO. (%) | Total +ve (%) | P-value |
|---|---|---|---|---|---|---|
| Gender | Males | 21 | 1 4.8 | 20 95.2 | 14.2 | |
| | Females | 24 | 6 25 | 18 75 | 85.8 | 0.001 |
| Age (year) | ≤1 | 1 | 0 0 | 1 100 | 0 | |
| | >1–5 | 22 | 4 18.2 | 18 18.8 | 57% | |
| | >5–10 | 14 | 2 14.3 | 12 85.7 | 28.8 | 0.627 |
| | >10–15 | 8 | 1 12.5 | 7 87.5 | 14.2 | |
| Residence | Urban | 15 | 1 6.7 | 14 93.3 | 14.2 | |
| | Rural | 30 | 6 20 | 24 80 | 85.8 | 0.012 |
| Season | Autumn | 20 | 2 10 | 18 90 | 28.6 | |
| | Winter | 25 | 5 20 | 20 80 | 71.4 | 0.068 |
| Mother's education | Primary | 11 | 2 18.2 | 9 81.8 | 28.6 | |
| | Elementary | 34 | 5 14.7 | 29 85.3 | 71.4 | 0.782 |
| Father's education | Illiterate | 3 | 1 33.3 | 2 66.14 | 14.3 | |
| | Primary | 16 | 2 12.5 | 14 87.5 | 28.5 | 0.623 |
| | Elementary | 10 | 1 10 | 9 90 | 14.3 | |
| | Secondary | 4 | 0 0 | 4 100 | 0 | |
| | Academy | 12 | 3 25 | 9 85.3 | 42.9 | |
| Water supply types | Both RO and Tap water | 45 | 7 15.6 | 38 84.4 | 100 | ---- |
| Feeding type | Bottle for ≤2years age | 1 | 0 0 | 1 100 | 0 | |
| | Diet of adults for >2–15years age | 40 | 6 15 | 34 85 | 85.7 | 0.782 |
| | Both | 4 | 1 25 | 3 75 | 14.3 | |
| Total/demographic | | 45 | 7/45 15.6 | 38/45 84.4 | 100 | |

* ( P value was assessed by Fisher's exact test.)

**Table 8.** Distribution of astrovirus infection among children with cancers by selected variables

| Patient groups | | Tested NO. | Astrovirus Ag ELISA | | | | Total +ve% | P-value |
|---|---|---|---|---|---|---|---|---|
| | | | +ve | | -ve | | | |
| | | | NO. | % | NO. | % | | |
| Diarrhea | Yes | 18 | 5 | 27.8 | 13 | 72.2 | 71.4 | |
| | No | 27 | 2 | 7.4 | 25 | 92.6 | 28.6 | 0.05 |
| Neutrophil counts | ≤500 | 5 | 0 | 0 | 5 | 100 | 0 | |
| | >500–1000 | 12 | 3 | 25 | 9 | 75 | 42.9 | 0.413 |
| | >1000–1500 | 28 | 4 | 14.3 | 24 | 85.7 | 57.1 | |
| Chemo -therapy | Before | 3 | 0 | 0 | 3 | 100 | 0 | |
| | During | 38 | 7 | 18.4 | 31 | 81.6 | 100 | 0.480 |
| | After | 4 | 0 | 0 | 4 | 100 | 0 | |
| Total | | 45 | 7 | 15.6 | 38 | 84.4 | 100 | |

*( P value was assessed by Fisher's exact test.)

Table 8 shows that most of the cases of infection (71.4%) had the manifestation of diarrhea at a statistically significant level (the P value was 0.05), while the remainder of infected patients (28.6%) were asymptomatic (i.e., had no diarrhea). Astrovirus infections were presented mainly (57.1%) in patients with neutrophil counts >1000–1500 cell/µl, with results that were not statistically significant (the P value was 0.413); while none had been detected in patients with neutrophil counts ≤ 500 cell/µl. All of the infected cases were found during chemotherapy and were not statistically significant (the P value was 0.480). The associated clinical manifestations in the 5 symptomatic infected children are shown in Table 9.

**Table 9.** Frequency of clinical manifestations in symptomatic astrovirus infections among pediatric oncology patients

| Clinical manifestations | NO./Total | (%) |
|---|---|---|
| Diarrhea | 5 | 100 |
| Anorexia | 5 | 100 |
| Fever | 4 | 80 |
| Abdominal pain | 4 | 80 |
| Vomiting | 3 | 60 |
| Headache | 2 | 40 |
| Dehydration | 0 | 0 |

The rates of clinical manifestations associated with symptomatic astrovirus infections in pediatric cancers are shown in Table 9. Both diarrhea and anorexia were registered in all patients. In most cases, these were accompanied by fever and abdominal pain. Vomiting was less common, and the least commonly reported symptom was headache; dehydration was not detected in the study. The selected variables included in the study were subjected to logistical regression analysis to adjust the possible confounders and determine the risk factors associated with astrovirus infection in pediatric cancer, as seen in Table 10.

**Disclaimer.** The author has no conflicts of interest to declare.

**Table 10.** Logistic regression analysis for selected variables related to astrovirus infection among children with cancers

| Independent variable | 95% confidence interval | | OR | P-value |
|---|---|---|---|---|
| | Lower bound | Upper bound | | |
| Female gender | 0.190 | 0.890 | 0.190 | 0.02 |
| Rural residence | 1.202 | 5.400 | 1.473 | 0.024 |
| Acute myeloblastic leukemia | 1.031 | 2.324 | 1.521 | 0.029 |
| Season | 2.590 | 11.384 | 4.302 | 0.358 |
| Age | 0.173 | 4.077 | 0.840 | 0.946 |
| Mother's education | 0.340 | 1.728 | 0.767 | 0.522 |
| Father's education | 0.139 | 8.622 | 0.345 | 0.371 |
| Feeding type | 0.012 | 4.545 | 0.229 | 0.785 |
| Neutrophil count | 0.265 | 5.575 | 1.210 | 0.411 |
| Chemotherapy | 0.023 | 17.733 | 0.632 | 0.788 |

Three independent risk factors of astrovirus infection are shown here to have highly significant results: first is female gender; this is followed by rural residence; third is acute myeloblastic leukemia.

## DISCUSSION

In childhood cancers, immune suppression may be induced either by cancer itself or by cancer treatments, leading to increased susceptibilities to infection by various pathogens. These, in turn, may not only contribute to prolonged hospitalization but even to mortality. [(8)] The seriousness of astrovirus gastroenteritis in this area was not previously well recognized, and little was known about its prevalence

among patients who complained of immune-compromising hematologic malignancies, and who were found to defecate human astrovirus for prolonged periods of time. [4]

In the present study, the frequency of human astrovirus infection was 15.6% among hospitalized patients on day 0 and day 4 of their hospitalization, in comparison to the general population. These findings were similar to the results of a study undertaken in the town of Ahvaz (Iran) by Mozhgani et al.,[36] among children hospitalized with gastroenteritis who were non-cancer patients. The similarity in the results may be the result of unfortunate unhygienic conditions. In contrast, the results of previous studies on human astrovirus infection rates were 2.6% in Iraq, as reported by Thewiny et al.,[19] and 9.2% in Saudi Arabia, as reported by Meqdam et al.[37] Among children with gastroenteritis in the general population, while a higher rate of astrovirus infection (19.8%) was reported by Chen et al.[38] in northern Taiwan among children with serious gastroenteritis, this increase in the rate of astrovirus infections among patients with cancers might be the result of an impairment of immunity induced either by the cancers themselves or by the cancer treatments, leading to an increased susceptibility to infection.

In this study, the rate of human astrovirus infection was higher in patients with hematological malignancies than in those with solid tumors, but the difference was not statistically significant. Within hematological malignancies, the rate of human astrovirus infection was significantly higher among leukemic children who had AML, while in solid tumors the rate of human astrovirus infection was significant in patients with rhabdomyosarcoma. Previously, there was only one case, reported by Coppo et al.,[4] in which severe astrovirus gastroenteritis was found in a 56-year-old male patient in France who had chronic lymphocytic leukemia.

As regards gender, astrovirus infections were significantly more common in females than in males. This finding was similar to the analytical result of another study, conducted in Korea by Ham et al.,[39] among children of the general population. This is, however, in contrast with previous results, reported in Iraq by Thewiny et al.,[19] and in India by Bhattacharya et al.[40] In these latter studies, gender was not shown to have a significant effect on the rate of astrovirus infection among children in the general population who suffered from acute diarrhea. These differences leave it unclear why the rate of human astrovirus infection is higher in females than males among pediatric cancer patients.

Regarding age, human astrovirus infection rates were mostly identified among children with cancers in the age group of >1–5 years, though this was found not to be statistically significant. This finding is in agreement with the results of a study in Pakistan, conducted by Phan et al.,[41] in which it was

observed that astrovirus infection could be detected in children of all age groups in the general population, but with a somewhat higher detection rate up to 24 months of age. This finding is in contrast to the studies conducted by Thewiny et al.,[19] and by Guix,[42] in which astrovirus infections were not detected in children over the age of 3 years. Meanwhile, in Saudi Arabia, Meqdam et al. [36] found astrovirus infection most in children aged ≤ 1 year. The results of the present study, which detected human astrovirus infection in children with cancers, may be explained by their lack of immunity against infections, or by re-infection caused either by cancer itself or by chemotherapy.

Human astrovirus infections have been shown, in this study, to have greater prominence among patients from rural areas than those from urban areas, which may be due to poor hygienic conditions. This finding may also suggest that astrovirus is endemic in these areas, as was found in Ahvaz by Mozhgani et al.,[36] and in Egypt by Naficy et al. [43]

With regard to the seasons, the rate of human astrovirus infection was found to be higher during winter than in autumn, though, in patients with cancer, this difference was not statistically significant. This finding was similar to the somewhat higher rate of infection observed in Iran by Maham et al.[14] It is, though, in disagreement with an earlier study in Iraq, conducted by Thewiny et al.,[19] in which all human astrovirus infections were detected in children of the general population with acute diarrhea only in winter. The reason for the different seasonal astrovirus patterns was unclear, though it may be due to the small number of cases detected and the short duration of the study. This left us unable to determine whether the pattern of human astrovirus contamination among children with cancers in Iraq is seasonal or not.

The frequency of human astrovirus infection was not shown to have a statistically significant correlation with the educational levels of patients' parents. This result was in agreement with the findings reported in Nigeria by Maryam et al.,[44] which showed no statically significant relationship between parents' literacy levels and the distribution rate of astrovirus infections.

Regarding the types of water supply and feeding varieties, we found no significant relevance to the propagation of astrovirus infection among children with cancers. This may be due to the fact that it was a hospital-acquired infection. This finding is in agreement with the observations in children of the general population in Georgia made by Dennehy et al.[45] However, it contrasts with a study in Korea conducted by Joeng et al.,[5] in which it was reported that poor food and unsanitary water supplies increased the incidence rate of astrovirus gastroenteritis in children of the general population.

Most cases of infection were significant, with acute diarrhea manifested in all symptomatic infected patients. A

smaller percentage of patients had an asymptomatic infection (i.e., infection without diarrhea), and this should be taken seriously as it may suggest a role in the distribution of the infection among children with cancer, or to others in the general population. It may be due to a failure to produce anti-astrovirus antibodies as a protective mechanism against astrovirus infections (or re-infection) among children with cancers. This finding is in agreement with the analytical results of a study conducted among the general population in Brazil by Gabbay et al.[46] However, it disagrees with the results of Thewiny et al.[19] and Meqdam et al.,[37] who identified astrovirus infections only among symptomatic children of the general population.

Other clinical manifestations were detected in symptomatic infected patients with childhood cancers, including anorexia in 100% of the patients in the study, fever and abdominal pain in 80%, vomiting in 60%, and headache in 40%. Similar findings were recorded in many studies, though differing in frequency. In relation to dehydration semblances, which were not identified among the infected cases in our study, our findings contrast with the results of a study conducted in Iraq by Thewiny et al.,[19] as well as those of a study conducted in Egypt by Naficy et al.[45] These latter two studies reported high rates of dehydration among children of the general population suffering from astrovirus gastroenteritis. The difference in findings may be due to sufficient fluid replacement in hospitalized patients.

Astrovirus infection rates were not related in a statistically significant way to neutrophil counts, because neutrophils played no role in the defense mechanism against astrovirus infections. Neutrophils were only slightly diminished, suggesting T-cell depletion, which had an important role in the defense mechanism against astrovirus infections, especially in cancer patients. [4] This finding is in contrast to the results of the study conducted among human immunodeficiency virus-infected children in Venezuela by Liste et al. [47] In that study, astrovirus gastroenteritis was found to be responsible for prolonged diarrhea, due to impaired T-cell functions.

All of the cases of astrovirus infection were detected during chemotherapy, though this was not statically significant. This finding is in contrast with the results of a case report study done by Coppo et al.,[4] which suggested that astrovirus needs to be considered as an etiological agent of gastroenteritis in immunocompromised patients, particularly in those treated with fludarabine monophosphate.

There were three independent risk factors shown to increase the frequency of astrovirus infection with highly significant results. These were, first, female gender, followed by rural residence, and lastly acute myeloblastic leukemia.

In this study, astrovirus infections among children with cancers were detected at a significant rate in cases of hematological malignancy, especially acute myeloblastic leukemia and rhabdomyosarcoma. Therefore, preventive and control measures are needed to prevent transmission of infections from patients to others. Furthermore, an extended study with large sample size is needed in the future in order to determine the significance of astrovirus infection among children with other types of cancers.

## REFERENCES


1. Alibek K, Mussabekova A, Kakpenova A, Duisembekova A, Baiken Y, Aituov B, et al. Childhood cancers: what is a possible role of infectious agents? Infect Agent Cancer. 2013;8(48):1-9.
2. Guo L, Gonzalez R, Wang W, Li Y, Paranhos-Baccalà G, Vernet G, et al. Complete genome sequence of human astrovirus genotype 6. Virol J. 2010;7(29):1-9.
3. Aminu M, Esona M, Geyer A, Steele A. Epidemiology of rotavirus and astrovirus infections in children in Northwestern Nigeria. Ann Afr Med. 2008;7(4):168-74.
4. Coppo P, Scieux C, Ferchal F, Clauvel J, Lassoued K. Astrovirus enteritis in chronic lymphocytic leukemia patient treated with fludarabine monophosphate. Ann Hematol. 2000;79:43-45.
5. Jeong H, Jeong A, Cheon D. Epidemiology of astrovirus infection in children. Korean J Pediatr. 2012;55(3):77-82.
6. Al-Hadad S, Al-Jadiry M, Al-Darraji A, Al-Saeed R, Al-Badr S, Ghali H. Reality of pediatric cancer in Iraq. Pediatr Hematol Oncol. 2011;33(2):154-56.
7. Alsaraji M, Al-Mukhtar M, Muzahim H, Husain A, Jasim K, Mutleg L, et al. Iraqi cancer registry. Iraq Cancer Board. 2011;2:140-47.
8. Koskenvuo M, Ruuskanan O, Salmi T, Rajantie J, KorppI M, Heikinheimo M. Febrile infections in children with leukemia with special reference to respiratory viral infections. Medica-Odontologica. 2008;1-85.
9. Alizadeh D, Larmonier N. Chemotherapeutic targeting of cancer-induced immunosuppres-sive Cells. Amer Assoc Cancer Res. 2014;74:2663-68.
10. Rabinovich G, Gabrilovich D, Sotomayor E. Immunosuppressive strategies that are mediated by tumor cells. Annu Rev Immunol. 2007;25: 267-96.
11. Cheng C, Chan J, Cembrowski G, Assendelft V. Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. Lab Hematol. 2004;10(1):42-53.
12. Caballero S, Guix S, El-Senousy W, Calicó I, Pintó R, Bosch A.

Persistent gastroenteritis in children infected with astrovirus: association with serotype-3 strains. J Med Virol. 2003;71:245-50.

13. Silva A, Leite E, Assis R, S Majerowicz, Leite J. An outbreak of gastroenteritis associated with astrovirus serotype 1 in a daycare center. Rio de Janeiro. 2001;96(8): 1069-1073.
14. Quaye O, McDonald S, Esona M, Lyde F, Rustempasic S, Roy S, et al. Recently identified novel human astroviruses in children with diarrhea, China. J Emerg Infect Dis. 2013;19(8):1333-35.
15. Koopmans M, Bijen M, Monroe S, Vinje J. Age-stratified seroprevalence of neutralizing antibodies to astrovirus types 1 to 7 in humans in the Netherlands. Clin Diag Lab Immunol. 1998;5(1):33-37.
16. Rodriguez-Baez N, O'Brien R, Qui S, Blass D. Astrovirus, adenovirus and rotavirus in hospitalized children: prevalence and association with gastroenteritis. J Pediatr Gastroenterol Nutr. 2002;35:64-68.
17. Oishi I, Yamazaki K, Kemoto T. A large outbreak of acute gastroenteritis associated with astrovirus among students and teachers in Osaka, Japan. J Infect Dis. 1994;170:439-43.
18. Maldonado Y, Cantwell M, Old M, Hill D, Sanchez ML, Logan L, et al. Population-based prevalence of symptomatic and asymptomatic astrovirus infection in rural Mayan infants. J Infect Dis. 1998;178:334–39.
19. Thewiny H, Hasony H, Salman B. Human astrovirus among hospitalized children under five years of age with acute diarrhea in Basrah, Iraq. Med J of Basrah Univ. 2014;32(2):71-76.
20. Shastri S, Doane A, Gonzales J, Upadhyayula U, Bass D. Prevalence of astroviruses in a children's hospital. J Clin Microbiol. 1998;36(9): 2571–74.
21. Gabbay Y, Linhares A, Cavalcante-Pepino E, Nakamura L, Oliveira D, Silva L, et al. Prevalence of human astrovirus genotypes associated with acute gastroenteritis among children in Belem, Brazil. J Med Vir. 2007;79(5):530-38.
22. Mitchell D, Madson D, Jiang X, Berke T, Monroe S, Carter M, et al. Molecular epidemiology of childhood astrovirus infection in child care centers. J Infec Dis. 1999;180:514–17.
23. Chikhi-Brachet R, Bon F, Toubiana L, Pothier P, Nicolas J, Flahault A, et al. Virus diversity in a winter epidemic of acute diarrhea in France. J Clin Microbiol. 2002;40:4266-72.
24. Kang Y, Park Y, Ahn J, Yeun J, Jee Y. Identification of human astrovirus infections from stool

samples with diarrhea in Korea. Arch Virol. 2002;147:1821-27.

25. Nguyen T, Hoang L, Pham I, Hoang K, Mizuguchi M, Okitsu S, et al. Identification of human astrovirus infections among children with acute gastroenteritis in the southern part of Vietnam during 2005–2006. J Med Virol. 2008;80:298-305.

26. Maham S, Marhamati N, Fallah F, Raheleh Sadat Sajadi R, Atashrazm F. Epidemiology of astrovirus infection in young children hospitalized with gastroenteritis in Iran, over a period of seven years, using reverse transcriptase-polymerase chain reaction (RT-PCR). J Public Health Epidemiol. 2013;5(1):37-42.

27. Fang Z, Sun Y, Ye X, Wang H, Zhang Q, Duan Z, et al. Astrovirus infection among hospitalized children with acute diarrhea in seven regions of China, 1998–2005. Zhonghua Liu Xing Bing Xue Za Zhi. 2006;27:673-76.

28. Walter J, Mitchell D. Astrovirus infection in children. Curr Opin Infect Dis. 2003;16:247-53.

29. Papaventsis D, Dove W, Cunliffe N, Nakagomi O, Combe P, Grosjean P, et al. Human astrovirus gastroenteritis in children, Madagascar, 2004–2005. Emerg Infect Dis. 2008;14:844-46.

30. Monroe S, Glass R, Noah N, Jiang X. Electron microscopic reporting of gastrointestinal viruses in the United Kingdom, 1985–87. J Med Virol. 1991;33(3):193-98.

31. Mendez-Toss M, Griffin D, Calva J, Contreras J, Puerto F, Mota F, et al. Prevalence and genetic diversity of human astroviruses in Mexican children with symptomatic and asymptomatic infections. J Clin Microbiol. 2004;42 (1):151-57.

32. Herrmann J, Nowak N, Perron-Henry D, Hudson R, Cubitt W, and Blackiow N. Diagnosis of astrovirus gastroenteritis by antigen detection with monoclonal antibodies. J Infect Dis. 1990;161(2):226-29.

33. Meliopoulos V, Kayali G, Burnham A, Olshansky C, Thomas P, Gray G, et al. Detection of antibodies against turkey astrovirus in humans. PLoS ONE. 2014;9(5):1-6.

34. Rodriguez-Baez N, O'Brien R, Qiu SQ, Bass D. Astrovirus, adenovirus, and rotavirus in hospitalized children: Prevalence and association with gastroenteritis. J Pediatr Gastroenterol Nutr. 2002;35:64-68.

35. Coates T. Neutrophils. In: Kliegman R, Stanton B, Geme J, Schor N, Behrman R. Nelson Textbook of Pediatrics. 20th ed. Philadelphia: Elsevier; 2016:1048-3473.

36. Mozhgani S, Samarbaf-Zadeh A, Makvandi M, Shamsi-Zadeh A, Parsanahad M, Jalilian S. Relative frequency of astrovirus in children suffering from gastroenteritis referred to Aboozar

Hospital, Ahvaz. JJM. 2011; 4(1):67-70.

37. Meqdam M, Thwiny I. Prevalence of group A rotavirus, enteric adenovirus, norovirus and astrovirus infections among children with acute gastroenteritis in Al-Qassim, Saudi Arabia. Pak J Med Sci. 2007;23(4):551-55.

38. Chen S, Chang Y, Lee Y, Chao H, Tsao K, Lin T, et al. Molecular epidemiology and clinical manifestations of viral gastroenteritis in hospitalized pediatric patients in Northern Taiwan. J Clin Microbiol. 2007;45: 2054-57.

39. Ham H, Oh S, Jang J, Jo S, Choi S, Pak S. Prevalence of human astrovirus in patients with acute gastroenteritis. Ann Lab Med. 2014;34:145-47.

40. Bhattacharya R, Sahoo G, Nayak M, Ghosh S, Dutta P, Bhattacharya M, et al. Molecular epidemiology of human astrovirus infections in Kolkata, India. Infect Genet Evol. 2006;6:425-35.

41. Phan T, Okame M, Nguyen T, Maneekarn N, Nishio O, Okitsu S, et al. Human astrovirus, norovirus (GI, GII), and sapovirus infections in Pakistani children with diarrhea. J Med Virol. 2004;73:256–61.

42. Guix S, Caballero S, Villena C, Bartolome R, Latorre C, Rabella N, et al. Molecular epidemiology of astrovirus infection in Barcelona, Spain. J Clin Microbiol. 2002;40(1): 133-39.

43. Naficy A, Rao M, Holmes J, Abu-Elyazeed R, Savarino S, Wierzba T, et al. Astrovirus diarrhea in Egyptian children. J Infect Dis. 2000;182:685-90.

44. Maryam A, Dewar J, Ahmad A, Umoh J. Molecular epidemiology of rotaviruses, astroviruses, and adenoviruses associated with gastroenteritis in children in northwestern Nigeria. Diarrheal Pathogens Research Unit. 2006;1:1-292.

45. Dennehy P, Nelson S, Spangenberger S, Noel J, Monroe S, and Glass R. A prospective case-control study of the role of astrovirus in acute diarrhea among hospitalized young children. J Infect Dis. 2001;184:1-15.

46. Gabbay Y, NedaLuz C, Costa I, Cavalcante-Pepino E, Sousa M, Oliveira K, et al. Prevalence and genetic diversity of astroviruses in children with and without diarrhea in São Luís, Maranhão, Brazil Mem Inst Oswaldo Cruz, Rio de Janeiro. 2005;100(7):709-14.

47. Liste M, Natera I, Suarez J, Pujol F, Liprandi F, Ludert J. Enteric virus infections and diarrhea in healthy and human immunodeficiency virus-infected children. J Clin Microbiol. 2000;38(8):2873-77