DISCUSSION

This study was conducted on fifty Egyptian infant's patients attending the Alexandria university children's hospital at El-Shatby during the winter/ spring season 2013 and clinically diagnosed with acute bronchiolitis.

In the present study, all of the studied patients were reported to be less than one year in age; 44% of them were in less than 3 months, 34% were between 3 months to 6 months, whereas only 22% of the patients were 6 months or above. The median of age was 3.41 months and mean 4.42 ± 2.99 months. These data suggests that young age (< 6 months) is a significant risk factor for acute bronchiolitis in children. [106]

According to the study of risk factors for respiratory syncital virus (RSV) bronchiolitis in children by Muneera Fadhil Rida (2011) reported that the age group (1-6 months) was the most frequent (60%) age group involved in the study .[107] Study of multiple viral respiratory pathogens in children with bronchiolitis by Hilary E Slempel et al (2009) reported that in fifty-five percent of the study subjects the median age was 6.5 months ,[108] study of impact on parents of bronchiolitis hospitalized infants by Alexandre Lapillone et al (2012) reported that the peak age of infection was three to six months . [109]

According to the official journal of the American Academy of Pediatrics, more than one third of children develop bronchiolitis during the first two years of life; [110, 111] 90% of cases requiring hospitalization occur in infants under twelve months of age. [112] Incidence of the disease peaks at age three to six months. [113]

In the present study, the majority of the studied patients diagnosed with acute bronchiolitis were similarly found to be males (72%). according to a retrospective cohort study by Flaher-man VJ et al. (2012), it was reported that male gender is associated with an increased risk of having a bronchiolitis episode and/or longer duration. [114] study of multiple viral respiratory pathogens in children with bronchiolitis by Hilary E Slempel et al (2009) reported that fifty-five percent of the study subjects were male. [108] In a study of risk factors associated with bronchiolitis in children less than 2 years of age, Ruiz-Charles MG el. (2002) reported that gender, unlike age, didn't show statistically significant association with bronchiolitis. [115]

In the current study, 46%, 40% and 14% of the cases diagnosed with acute bronchiolitis were found in January, February, and March respectively. In the study of season of infant bronchiolitis and estimates of subsequent risk of early childhood asthma by Kecica N. Caroll (2009) reported that bronchiolitis occurred during winter season (November to April) or non winter season (May to October) with predominant period between (December to feburary).[114] In the study of spatiotemporal patteren of infant bronchiolitis by Solan et al (2013) they reported that the peaking of infection primarily between November and march in the northen hemisphere and during april and September in the southern hemisphere .[116]

In the present study, cough was the most common clinical feature found in almost all studied patients (98%). A wheezy chest was the second most common feature after cough, observed in 96% of all cases. A radiograph with a hyper-inflated chest was also found in the majority of patients (94%). Fever was common too, found in 72% of all cases. Tachypnea was also common, where 62% of the infants were found to be tachypneic

according to the WHO criteria. Vomiting and feeding difficulties were present in 56% and 54% of the cases, respectively. 28% of all the patients experienced rhinorrhea, and 18% had dyspnea. 16% of the cases were hypoxemic, whereas 12% had been suffering from grunting as a sign of respiratory distress. Diarrhea was slightly uncommon, found in only 6% of the cases. Otitis media was rare (2%), observed in only one case in the study.

Yahia S. et al .(2012) reported that cough, wheezing, rhinorrhea, fever and chest wall retraction were most frequent presentation (81.2%, 68.8%, 66.7%, 64.6% and 56.3% respectively).[117]

Arabpour M. et al .(2008) reported that cough, coryza, wheezing, fever, rhinitis, pharngitis, abnormal tympanic membrane, vomiting and diarrhea were (95.6%, 82.4%, 76.5%, 75%, 72.1%, 52.9%, 32.4%, 17.6% and 13.2% respectively).[118]

William V. et al. (2004) reported that cough, coryza, rhinitis, fever, abnormal tympanic membrane, pharngitis, diarrhea and vomiting were (90%, 88%, 77%, 52%, 39%, 17% and 10% respectively).[119]

In the present study, different risk factors for acute bronchiolitis were observed among all studied patients; 78% of the studied patients were at young age (<6 months), 32% were bottle-feeders, 82% were exposed to tobacco smoke, 66% had school or child-care siblings, 22% were living in an overcrowded environment and 20% were born with a low birth weight .

Several risk factors are associated with acute bronchiolitis in infants and young children. Infants younger than 6 months old are at increased risk for developing the disease. In a study by Bradley JP et al. (2005), age was found to be a significant factor in the severity of infection. [106] the younger an infant was, the more severe the infection tended to be. In addition to young aged infants, premature infants (i.e., born more than 3 weeks before their due date), infants who have been bottle-fed exclusively, infants who have congenital heart and/or chronic lung diseases, and infants with weakened immune systems are also at a higher risk for developing more serious cases of bronchiolitis. [115]

Exposure to tobacco smoke (passive smoke, particularly maternal) is also a great risk factor for developing bronchiolitis in infants. According to Bradley JP et al., the severity of hRSV bronchiolitis was also affected by cigarette smoke exposure. [106] Similarly, Semple MG et al. (2011) have found that infants admitted to hospital with bronchiolitis from a household where a parent smokes, are twice as likely to need oxygen therapy and five-times as likely to need mechanical ventilation as infants whose parents do not smoke.[120] other environmental and social risk factors [121] associated with acute bronchiolitis includes overcrowding (living in or visiting crowded areas), nursery attendance and the presence of older sibling.

In this present study, 76% of the cases were reported to be positive at least to one or more of seven viruses (RSV, influenza virus A and B, parainfluenza virus types 1-3 and adenovirus). A study by Barenfanger J et al. (2000) screened patients hospitalized with RTIs for common respiratory viruses similarly reported 74% of the tested cases to be positive. [110] Kleines M. el al. (2007) have also screened respiratory samples reported that 60.6% of the tested samples were positive to one of those viruses. [122]

In the present study, hMPV was detected in respiratory secretions of eight out of 50 cases (16%) of infants who were less than 1 year of age and clinically diagnosed with acute bronchiolitis. To our knowledge, this study is the first study to date to report the prevalence of hMPV infections among Children in Alexandria governorate detected by real-time RT-PCR assay.

According to van den Hoogen BG (2001) [36] and Kahn JS (2006) [37], hMPV has been estimated to account for 3% to 19% of bronchiolitis cases. Xepapadaki P. et al. (2004) reported the presence of hMPV in 16% of acute bronchiolitis cases in children. [85] Bouscambert-Du-champ M. et al. (2005) detected hMPV in 6 (6.4%) of 94 consecutive French children hospitalized for acute bronchiolitis from September 2001 to June 2002. [71]

In a study carried out by Shier MK et al. (2008) in Saudi Arabia, hMPV was detected in the respiratory secretions of 6.1 % of infants less than three years of age hospitalized with ARTIs. [123] According to this study, hMPV was the second most common viral agent, after RSV, identified in these children. The highest incidence of hMPV ever reported in the world so far was found in Ahwaz, Iran (54.4%) and was detected among children less than two years of age affected by ARTIs. [118] The variations in hMPV incidence could be attributed to different localities, age group, genetic susceptibility, sampling technique and detection method. [117]

Several retrospective studies have reported the presence of hMPV sequences in 4 to 21% of nasopharyngeal samples of infants with acute bronchiolitis. [38, 85, 124]

In Egypt, a previous study carried out at Al-Mansoura University in 2009 found a prevalence of 13.6% of hMPV but among adult patients with lower respiratory tract infections. [125] The first hMPV prevalence study to be carried out among Egyptian children with acute respiratory infections was carried out in 2011 by Yahia S. el al. in Al-Mansoura University. [117] in their study, nasopharyngeal aspirates from 600 infants and children with symptoms and signs of respiratory infections were collected, and samples were examined by RT-PCR for hMPV. According to their clinic-based study, the overall prevalence of hMPV among the studied children was 8 %.[117]

In the present study, 64% of the studied cases were positive to RSV and/or influenza A/B, parainfluenza types 1-3 or adenovirus by IF, but negative to hMPV by PCR. Twelve percent were positive to hMPV and to at least one of the seven respiratory viruses detected by the IMAGEN® respiratory screen kit (i.e. having a mixed infection). Four percent were positive only to hMPV by PCR, but negative to RSV, influenza A/B, parainfluenza types 1-3 and adenovirus by IF. Twenty percent were negative to hMPV, RSV, influenza A/B, parainfluenza types 1-3 and adeno-virus. So in other words, at least one viral agent (either hMPV or one of the seven most common respiratory viruses mentioned) was detected in 40 cases (80%) of our studied patients. Co-infection with two or more viruses was confirmed in at least 6 cases (12%) of the collected samples, whereas no viral etiology was detected in 10 cases (20%) of the participated patients. The presence of 10 cases of bronchiolitis in which no virus has been detected is probably because those cases are caused by respiratory viruses other than those detected by the IMAGEN® immunofluorescence screen test (rhinoviruses, for example) or may be because new “undiagnosable” pathogens were involved in infection and have not been discovered yet.

In a study of the viral etiologies of lower respiratory tract infections among Egyptian children in 2012, nasopharyngeal aspirate samples from 450 children under 5 years of age who presented with LRTI during a one-year period, were collected and tested for 8 major respiratory viruses (RSV, Influenza A/B, PIV 1-3, Adenovirus and hMPV) using direct fluorescence assay, RT-PCR, and shell vial culture. According to their study, at least one respiratory virus was detected in 59.9% of cases, and co-infection with multiple (two or more) viruses occurred in 10.8% of the participants. [126]

Regarding the clinical characteristics of hMPV infection, van den Hoogen et al. (2003) reported the mean age of hMPV-infected children to be 15 months [47], whereas Williams et al. (2004) reported a mean age of 11.6 months. [52] Taking into account that all participating children in this study were less than 1 year of age, it was found that 37.5% of the hMPV-positive patients were younger than 3 months, 25% were in the age between 3 to less than 6 months, and 37.5% were older than 6 months.

In current study, the mean age for all hMPV-positive patient's was 4.50 months (range, 1 to 10 months). The mean age of the 8 hMPV-infected children was found to be greater than that of the total enrolled 50 patients whose mean age was 3.76 months. According to these results, hMPV tend to infect slightly older children when compared with hRSV and other respiratory viruses. This results are consistent with those of Bouscambert-Duchamp M. et al. (2005) who reported the median age of children infected with hMPV to be 4.5 months (range, 2 to 6 months) [71], and those of Qaisy LM el al. study (2012) in Jordon, in which hMPV was detected in 12.7% of hospitalized children whom median age was of 7 months (range 1.3 to 24 months). [127]

Yahia S. el al. (2012) in Al-Mansoura University have also shown that the rate of hMPV infection among hospitalized children with ARTIs was significantly higher among children aged 2-24 months compared to other age groups (11.9% vs. 3.7% and 4.0% for 2-24, 25-60, 61-108 mo respectively).[117] Unlike the present study in which we found the majority of hMPV-positive patients to be males (75%) (Probably because most of the 50 enrolled cases in the study were male participants), Yahia S. el al. has found that the rate of hMPV infection among children with acute respiratory infections was significantly higher among females than males (12.6% vs. 6.6%). [117] However, Ebihara et al. (2004) [128] and Hara et al. (2008) [129] reported no sex differences in detection of hMPV.

Epidemiological findings suggest that hMPV may circulate worldwide and may have a seasonal distribution in winter months for temperate and spring/summer for tropical countries. [130] According to Bouscambert-Duchamp M. et al. (2005), hMPV was detected in children from November 2001 to March 2002, with a peak in January. [71] Their study reported that hMPV infections were mainly detected during the RSV epidemic period. In this study, 50% of the eight hMPV-positive samples were detected in January (peak month of infection), 37.5% were detected in February, and only 12.5% were detected in March.

In the study of hMPV-associated LRTIs in Egyptian adults in 2009, hMPV was commonly found in the period from November to January, the winter months in Egypt. [125] In their study, distribution of hMPV during different months of the year in Egypt was as following: 50% of hMPV PCR-positive cases were detected from November to January, 32% were detected from February to March, 10% were detected from July to October and only 9% were detected from April to June. This seasonal pattern is consistent with

previous studies performed in North America, where the highest numbers of hMPV cases were detected between January and April. [131]

In the present study, cough, wheezing, fever, and hyper-inflated chest were observed in all of the eight hMPV-positive patients. Feeding difficulties, tachypnea, and rhinorrhea were also observed but at relatively lower frequencies (75%, 62.5% and 50% respectively). According to the results, no statistically significant differences in any of these clinical features were observed when children with hMPV infections were compared to children infected with other respiratory viruses or children in whom no viral agent was identified.

According to van den Hoogen el al. study (2003), clinical symptoms observed for hMPV-infected children included cough (72%), rhinitis (80%), fever (61%), dyspnea (28%), wheezing (24%), feeding difficulties (36%), retractions (60%), hyperventilation (42%), tachycardia (23%) and cyanosis (8%). [47]

Clinical manifestations produced by hMPV are usually indistinguishable from those caused by other viral pathogens. However, according to some studies, hMPV infection tended to be somewhat less severe causing milder clinical courses than those of patient's with hRSV. Other studies have noted no differences or have reported longer hospitalizations in children with hMPV infections compared to those infected with hRSV. (46, 84, 132)

In this study, 6 out of the 8 hMPV-positive patient's (75%) didn't require hospital admission. Only 2 cases (25%) were hospitalized and admitted as in-patient. One of these two hMPV positive inpatients had a mixed hMPV viral infection (hMPV plus one of the most common 7 respiratory viruses), while the other had only hMPV detected.

Regarding the clinical impact of hMPV coinfection with other respiratory viruses, clinical and radiological findings found in the two patients with only hMPV detected were compared with those of the six patients coinfected with hMPV and at least one more type of respiratory virus. Cough, fever and wheezing were found in all cases of both types of patients. Rhinorrhea was found too in the same frequency in both types of patients (50% of cases for each group). However, patients with only hMPV detected had lower frequencies for the other observed symptoms when compared with patients having the mixed hMPV infection.

In some reports, none of the children infected with hMPV required admission to the intensive care unit, others indicate that 15-25 % of children required intensive care unit admission. [43, 48, 73]

In the current study, however, no statistically significant difference was observed when the severity of bronchiolitis in patients with only hMPV detected was compared to that of patients having hMPV coinfection with any of the seven most common respiratory viruses screened by IF. Similar to this result, van Woensel et al. (2006) reported the absence of hMPV coinfection in any of the 30 mechanically ventilated children with RSV lower respiratory tract infection, suggesting that hMPV co-infection is not very common in severe RSV respiratory tract infection. [88]

Semple MG el al. (2005) reported that dual infection of infants by hMPV and RSV is strongly associated with severe bronchiolitis. (87) In a report by Greensill et al. (2003), a high frequency of hMPV and RSV coinfection was found in patients with severe bronchiolitis requiringmechanicalventilation.(86)

SUMMARY

Respiratory tract infections (RTIs) are a leading cause of morbidity and mortality world-wide. For children younger than five years, RTIs are ranked as the second leading cause of death. In pediatric populations, acute bronchiolitis is the most frequent lower RTI affecting approximately 10% of all infants in their first year of life. Clinically, acute bronchiolitis is characterized by respiratory distress with wheezing, fever, coryza or cough. Infants with pre-existing conditions such as prematurity, immunosuppression and/or cardiorespiratory disorders are known to be at risk for more severe illness and higher morbidity. Acute bronchiolitis is predominately a viral illness caused mostly by RSV, parainfluenza virus (mainly type 3), influenza type A, adenovirus and rhinovirus. In about third of the viral bronchiolitis cases, however, an infectious agent cannot be identified. These observations suggested that previously unknown pathogens may be circulating and may be responsible for a substantial proportion of respiratory tract disease. Recent advances in molecular biology have allowed virologists to detect many of these previously undetected viral pathogens. One of these was the human metapneumovirus (hMPV) discovered in 2001.

Human metapneumovirus is an enveloped, single-stranded negative-sense RNA virus that has been recently identified as a new cause of upper and lowers RTIs in children and adults, precisely in patients in whom screening tests for other viral pathogens had been negative. The virus has been considered to be second to hRSV as a cause of lower RTIs requiring hospitalization. Several studies suggested that it may be responsible for about 10% of viral respiratory infections in which common respiratory viruses are not diagnosed. Infants and young children are the most commonly affected by hMPV, but the virus has also been documented in adults and the elderly. Infection with the virus causes a broad spectrum of respiratory illness, ranging from mild symptoms to severe cough, bronchiolitis, and pneumonia. Globally, hMPV infections account for at least 5-7% of the RTIs in hospitalized children, but in the general community hMPV infections account for at least 3% of patient's infections who visit a general practitioner for RTIs. The virus has a worldwide distribution and it's epidemiology and seasonality is similar to that of RSV, with most episodes occurring during the winter months. Seroepidemiologic studies revealed that by the age of five, nearly all individuals had evidence of hMPV infection. Taxonomically, hMPV is classified as the first non-avian member of the *Metapneumovirus* genus.

In the present study, we aimed to identify the role of hMPV that contribute to bronchiolitis in infants and young children. Fifty Egyptian infants, clinically diagnosed with acute bronchiolitis at the Alexandria University Children's Hospital of El-Shatby during the period from January to April 2013, were enrolled in the study. All of the studied patients were less than one year in age; 44% of them were in the age between 1 to less than 3 months (peak age of infection). A real-time RT-PCR assay was used in our study to detect hMPV genomic sequences in all patient's respiratory secretions. According to several reports, hMPV has been estimated to account for 3% to 19% of bronchiolitis cases. In our study, the virus was detected in only 8 cases (16%) out of the 50 studied cases. A similar Egyptian study carried out in Al-Mansoura University in 2012 found a virus prevalence of 8% among children hospitalized with acute respiratory infections.

Using an indirect immunofluorescence antigen detection assay, respiratory samples were also tested for the presence of any of the seven most common respiratory viruses (RSV, influenza virus A and B, parainfluenza virus types 1-3 and adenovirus) that might be involved in infection. In our study, 76% of the cases were reported to be positive at least to one or more of those seven mentioned viruses, whereas no viral etiology was detected in 20% of the study cases. The most significant risk factors for acute bronchiolitis that have been observed among the studied patients were young age, exposure to tobacco smoke and living in an overcrowded environment. The mean age of hMPV-infected children was found to be higher than that of RSV-infected children, indicating that hMPV tend to infect slightly older children when compared to RSV or other respiratory viruses. Clinical symptoms of hMPV infections were similar to those seen with other respiratory viral infections, and also include fever, rhinorrhea, dyspnea, tachypnea, and wheezing. In our study, only two patients (4%) had hMPV as the sole respiratory pathogen detected, whilst six cases (12%) had a co-infection of hMPV with other respiratory viruses. According to our results, no significant difference was observed when the severity of bronchiolitis in patients with only hMPV detected was compared to that of patients having hMPV coinfection with any of the 7 respiratory viruses screened and detected by immunofluorescence.

CONCLUSION AND RECOMMENDATIONS

- Human metapneumovirus (hMPV) is a newly emerging respiratory viral pathogen that has been found to be a common cause of acute bronchiolitis among pediatric patients in Alexandria, Egypt with a prevalence of 16%.
- It has a significant clinical impact particularly on infants and children younger than two years and thus must be considered in etiological diagnosis.
- In patients with acute bronchiolitis, hMPV could be found as a sole pathogen or may be present associated with hRSV or other common respiratory viruses. A considerable percentage of viral respiratory infections still, however, have an etiologic agent that remains unidentified.
- Future advances in molecular and diagnostic techniques in the field of Microbiology would help in identifying new respiratory viruses that have been previously unidentified before.