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ORIGINAL ARTICLE

Detection of the Human Metapneumovirus and Its Comparison with the Respiratory Syncytial Virus in malignant pediatric patients with Acute Respiratory Tract Infections

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ABSTRACT

Background: *Acute viral respiratory tract infections are a leading cause of illness in both children and adults. RSV is the most common cause of respiratory tract disease in children also hMPV was first isolated from children suffering from acute respiratory tract disease. The disease is more severe (sometimes lethal) in immunocompromised hosts* ***Objective:*** *The aim of the present study was to study the frequency of hMPV and RSV infections in children suffering from different types of malignancy with acute respiratory tract infection and to compare their occurrence in the studied patients.* ***Methodology:*** *This is a cross sectional study carried out on 50 malignant patients diagnosed as having acute respiratory infections. The included patients were children up to 6 years of age selected from hospitalized patients in National Cancer Institute. Throat swabs were obtained. The collected specimens were tested for the presence of hMPV and RSV by real time PCR.* ***Results:*** *RSV was detected in 6 out of 50 (12%) of the studied cases, while hMPV virus was not detected among our studied patients. All detected cases were less than two years old (median age 16 months) with a highly statistical significant association between detection of the RSV and age of patients (P<0.01). There was a statistically significant association between patients with pneumonia and detection of RSV in the studied cases.* ***Conclusion:*** *RSV was detected in 12% of pediatric malignant patients aged less than two years with ARTI, pneumonia was the most common presentation of ARTI in RSV infected cases.*

INTRODUCTION

Acute respiratory tract infections (ARTIs) represent a major global health burden. Globally, about 4.2 million ALRI deaths are estimated to occur among all age groups; of these 1.8 million are estimated to occur among children below 5 years [1] .Viral etiology plays a significant role in ARTIs in infants and children. RSV & hMPV infections have been diagnosed in all age groups [2], nevertheless, children 5 years old and younger are usually at a higher risk of experiencing severe acute respiratory infection (SARI) and hospitalization [3,4].

The hMPV was first isolated from children suffering from acute respiratory tract disease. It appears to be responsible for about 7 to 10% of cases of acute respiratory tract infections in infants. The clinical symptoms observed most frequently in hMPV-positive children are cough, wheezing and dyspnea [5].

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Co-infection with hMPV and RSV has been reported to occur in 5-10% of children with acute respiratory tract diseases; most probably due to the overlap of the seasonal distribution between these two viruses [6].

METHDOLOGY

1. Study Design and subjects

The current study was carried out on 50 patients in the period from May 2012 and May 2013. The included patients were children of both sexes under 6 years of age presenting with symptoms and signs of acute respiratory tract infection and suffering from different types of malignancy. The selected patients were children patients admitted to Inpatient Ward of National Cancer Institute, Cairo University receiving chemotherapy for different types of malignancies. The study was approved by the ethical committee of The National Cancer Institute, Cairo University and all patients' parents granted their consent to share in the study. 80% of the studied patients were in the age group of more than 2 years up to 6 years of age followed by

the age group of 1-2 years representing 14% of total number of patients with equal percentage of male and females.

2. Sample collection and processing

Swabbing of both the tonsils and the posterior pharynx was done using a dry sterile dacron throat swab. Throat swabs were collected from children with recent onset of symptoms suggestive of respiratory tract infection, such as cough, coryza, repeated sneezing and/or difficulty in breathing.

Swabs were labeled with the patient's ID and then suspended in a sterile tube containing 2ml phosphate buffered saline (PBS) (Immco Diagnostics, USA). The swabs inside the tubes were agitated vigorously for 10 seconds using a vortex mixer to free cells from the swab tip, then the swab were removed from the tubes and discarded followed by storing the samples immediately at -70^{o}C until further analysis.

3. Molecular detection of RSV and hMPV

Extraction of viral RNA was done using QIAamp viral RNA mini kit (QIAGEN, Germany) according to the manufacturer's protocol, The QIAamp Viral RNA spin protocols can be fully automated on the QIAcube. The sample was first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions are then adjusted to provide optimum binding of the RNA to the QIAamp membrane, and the sample was loaded onto the QIAamp Mini spin column. 140 µl of thawed sample was used for extraction in a fully automated process on the QIAcube machine (QIAGEN, Germany). Automated extraction was performed using the innovative QIA cube machine which performs the same steps as the manual procedure (lyse, bind, wash, and elute) enabling the use of the QIAamp Viral RNA Mini Kit for purification of high-quality viral RNA. The extracted RNA was checked for concentration and quality using the NanoDrop 2000/2000c spectrophotometer. Using Primer design, genesig advanced kit for Respiratory Syncytial Virus (all species) / Human Metapneumoviruses genomes (United Kingdom) for quantitative real time PCR of RSV and hMPV, the kits targeting the amplification of the nucleoprotein gene of both viruses. PCR was done according to the manufacturer's protocol in a one-step approach using Applied Biosystem 7500 RT-PCR machine, USA.

4. Statistical analysis

Data were statistically described in terms of frequencies (number of cases) and percentages. IBM SPSS statistics (V. 22.0, IBM Corp., USA, 2013) was used for data analysis. Data were expressed as median and percentiles for quantitative non-parametric measures in addition to both number and percentage for categorized data. The P value at 0.05 was considered significant, while at 0.01 and 0.001 were considered highly significant.

RESULTS

Among the studied patients, the most common malignancy was acute lymphoblastic leukemia representing 46%, followed by acute myeloid leukemia (30%) and then non- Hodgkin's lymphoma (10%) (table1). RSV was detected in 12% of all studied patients while hMPV was not detected in any of the patients. The RSV positive cases were detected in winter months.

There was a high statistical significant association between the age of the patients and the detection of RSV but there was no statistically significant relation between sex of patients and detection of the virus with a median age of 16 months in the detected cases of RSV (table 2).

The most common infection was bronchopneumonia (46%) followed by pneumonia (44%) and then acute pharyngitis (10%) (table 3). A statistically significant association was detected between patients suffering from pneumonia and infection with RSV (table 4). Also there was high statistically significant association between patients with acute myeloid leukemia and detection of RSV (table 5).

Table 1: Types of malignant tumors in the studied patients

Type of malignancy	*Number*	*Percent*
a) Hematological malignancies		
Acute lymphoblastic leukemia	23	46%
Acute myeloid leukemia	15	30%
Biphenotypic leukemia	1	2%
Non Hodgkin's lymphoma	5	10%
b) Solid tumors		
Neuroblastoma	4	8%
Wilm's tumor	1	2%
Ewing sarcoma	1	2%
Total	50	100%

Table 2: Relation between age, sex of patients and detection of RSV

Variable	*RSV detection*		*P value*
	Detected	**Not detected**	
Age			
1-3 mon	0	0	
>3-6 mon	0	0	**<0.01**
>6-9 mon	2(33.3%)	0	
>9-12 mon	1(16.7%)	0	
> 1year-2years	3(50%)	4(9.1%)	
>2 years-6 years	0	40(90.9%)	
Sex			
Female	5(83.3%)	20(45.5%)	>0.05
Male	1(16.7%)	24(54.5%)	

Highly significant = P value < 0.01

Table 3: Types of chest infection among the studied cases

Type of infection	*Number*	*Percent*
Bronchopneumonia	23	46%
Pneumonia	22	44%
Acute bronchitis	0	-
Acute pharyngitis	5	10%
Bronchopneumonia & RD	0	-
Acute bronchiolitis & RD	0	-
Total	50	100%

Table 4: Relation between type of chest infection and the detection of RSV

Type of infection	*Detected RSV no.(percent to total detected) n=6*	*Undetected RSV (percent to total undetected) n=44*	*P value*
Bronchopneumonia	1(16.67%)	22(50%)	> 0.05
Pneumonia	5(83.33%)	17(38.64%)	**< 0.05**
Acute pharyngitis	0	5(11.36%)	> 0.05
Acute bronchitis	0	0	-
Bronchopneumonia & RD	0	0	-
Acute bronchiolitis & RD	0	0	-
Total	6(100%)	44(100%)	

Significant = P value < 0.05

Table 5: Relation between the type of malignancy and the detection of RSV

Type of malignancy	*Detected RSV no. (percent to total detected) n=6*	*Undetected RSV (percent to total undetected) n=44*	*P value*
Hematological malignancies			
Acute myeloid leukemia	5(83.33%)	10(22.73%)	**< 0.01**
Acute lymphoblastic leukemia	1(16.67%)	22(50%)	> 0.05
Biphenotypic leukemia	0	1(2.27%)	> 0.05
Non Hodgkin's lymphoma	0	5(11.3%)	> 0.05
Solid tumors			
Neuroblastoma	0	4(9%)	> 0.05
Wilm's tumor	0	1(2.27%)	> 0.05
Ewing sarcoma	0	1(2.27%)	> 0.05
Total	6(100%)	44(100%)	

Highly significant = P value < 0.01

DISCUSSION

Few studies have reported that respiratory viral infections are important complications in pediatric malignant patients representing a potential cause of severe pneumonia with high morbidity and mortality [7,8,9,10]. Therefore, the present study involved cancer patients with ARI admitted to the National Cancer Institute, Cairo University. In the current study, we used real time PCR for detection of our target viruses as it is the most sensitive and specific method for detection of respiratory viruses including new emerging ones [11]. Using molecular techniques, viruses were identified in approximately twice as many RTIs as previously reported in a daycare cohort by viral culture methods [12]. Also, other study showed that virus culture yielded positive results in 35.4% of samples compared to 76% of positive samples by RT-PCR [13]. In another PCR-based study on children below 2 years old with acute bronchiolitis, the viral isolation rate rose from 48% to 90% after applying PCR technique in addition to the traditional virus culture [14].Moreover, another study compared multiplex polymerase chain reaction (PCR) to viral culture methods in the diagnosis of RSV and influenza viruses, multiplex PCR showed more positivity than the viral culture and it was recommended as an effective primary test [15].

Human metapneumovirus was not detected as a cause of ARI in the studied cancer patients. This was in agreement with a study in Upper Egypt in a period from 2005 till 2008, where hMPV was not detected among 300 of their patients out of total 520 patients in the first two years of the study[16]. Also other study carried out from 2000 till 2010 on 797 pediatric patients aged up to 4 years old, found that the frequency of hMPV ranged from 0% in 2000 and 2001 to 32.8% in 2009 and 2010 with an overall prevalence rate of 11.9% [17]. As a result of that yearly variation, the incidence of hMPV was negatively correlated with the average monthly temperature and rainfalls, so climatic factors may affect the prevalence of hMPV within a certain geographical location [18]. Moreover, a study stated that the prevalence of hMPV is known to vary from year to year and depends on the study period whether it is a mild or an intense cold winter [19]. Our results are in partial agreement with other study showed that hMPV was positive in a single case only out of a total of 402 samples of immunocompromised children who underwent hematopoietic stem cell transplantation [8]. On the other hand, a slightly higher detection rates were reported in other study which detected hMPV in 2.6% from a total number of 1294 specimens [20]. Also, a detection rate of 6 % was reported in another study on 3490 hospitalized children [21].

In the current study, RSV detection rate was 12% which is comparable with a study reported that a detection rate of RSV was 11% among 51 pediatric patients with leukemia [22]. However, our results show a higher detection rate than other studies which reported that RSV was detected in 7.1% out of 820 pediatric patients with hematologic malignancies [23]. Also, another study reported a detection rate of 8.7 % of RSV among a total of 48 cancer patients with a median age of 12 ± 5.2 years [7]. In the present study, the positive RSV specimens were collected in the winter season. This is coinciding with a study detected all RSV positive cases during the winter months mainly in December and January [19]. Also, two other studies detected most of their RSV positive cases from patients during cold months[24,25].

All positive RSV cases were less than 2 years old with a median age of 16 months in the detected cases of RSV. This was in agreement with a study found that the RSV positive cases were significantly younger than other non-RSV cases [25], also other study reported that all of the RSV positive patients were less than 24 months of age [26].

Among studied patients, RSV was mostly detected among patients with acute myeloid leukemia (5/6; 83.3%) which was highly significant. Whereas, other study reported that RSV was mostly detected among patients with lymphoma with a detection rate of 38.9% [27]. Also, acute lymphoblastic leukemia was the most common malignancy representing 44% of RSV infected malignant cases in another study [28].

REFERENCES

1. WHO, (2009): Pandemic (H1N1) 2009: Available at http://www.who.int/csr/disease/swineflu/en/index.html.
2. CDC. (2010) Centers for Disease Control and Prevention. Respiratory syncytial virus/clinical description and diagnosis Available at http://www.cdc.gov/rsv/clinical/description.htmlKahn J.: Epidemiology of Human Metapneumovirus .Clinical microbiology reviews.2006; 19:546-557
3. Ahmed JA, Katz MA, Auko E, Njenga MK, Weinberg M, Kapella BK, Burke H, Nyoka R, Gichangi A, Waiboci LW, Mahamud A, Qassim M, Swai B, Wagacha B, Mutonga D, Nguhi M, Breiman RF and Eidex RB: Epidemiology of respiratory viral infections in two long-term refugee camps in Kenya, 2007-2010. BMC Infect Dis.2012;12:7.
4. Nair H, Nokes DJ, Gessner BD, et al: Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet.2010; 375: 1545-1555.
5. Chano F, Rousseau C, Laferriere C, Couillard M and Charest H: Epidemiological survey of human metapneumovirus infection in a large pediatric tertiary care center. J Clin Microbiol.2005; 43: 5520-5525.
6. Maertzdorf J, Wang C, Brown J, Quinto J, Chu M, Graaf M, Hoogen B, Spaete R, Osterhaus A and Fouchier R: Real-time reverse transcriptase PCR

assay for detection of human metapneumoviruses from all known genetic lineages. J Clin Microbiol.2004; 42: 981-98.
7. Benites EC, Cabrini DP, Silva AC, Silva JC, Catalan DT, Berezin EN, Cardoso MR and Passos SD : Acute respiratory viral infections in pediatric cancer patients undergoing chemotherapy. J Pediatr (Rio J). 2014; 90:370-6.
8. Choi JH, Choi EH, Kang HJ, Park KD, Park SS, Shin HY, Lee HJ and Ahn HS : Respiratory viral infections after hematopoietic stem cell transplantation in children. J Korean Med Sci.2013; 28: 36-41.
9. Kroll J and Weinberg A: Human metapneumovirus. Semin Respir Crit Care Med.2011; 32:447-53
10. Renaud C and Campbell A: Changing epidemiology of respiratory viral infections in hematopoietic cell transplant recipients and solid organ transplant recipients. Curr Opin Infect Dis.2011; 24: 333-343.
11. Broor S, Bharaj P and Chahar H: Human metapneumovirus: a new respiratory pathogen. J Biosci.2008; 33: 483-493
12. Fairchok MP, Martin ET, Chambers S, Kuypers J, Behrens M, Braun LE and Englund J: Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. J Clin Virol.2010;49:16-20.
13. Lim G, Park TS, Suh JT and Lee HJ: Comparison of R-mix virus culture and multiplex reverse transcriptase-PCR for the rapid detection of respiratory viruses. Korean J Lab Med.2010; 30:289-94.
14. Miron D, Srugo I, Kra OZ, Keness Y, Wolf D, Amirav I and Kassis I: Sole pathogen in acute bronchiolitis: is there a role for other organisms apart from respiratory syncytial virus? Pediatr Infect Dis J.2010; 29:e7-e10.
15. Lee V, Yap J, Cook AR, Chen M, Tay J, Barr I, Kelso A, Tan B, Loh JP, Lin R, Cui L, Kelly PM, Leo Y, Chia K, Kang WL, Tambyah P and Seet B: Effectiveness of public health measures in mitigating pandemic influenza spread: a prospective sero-epidemiological cohort study. J Infect Dis.2010; 202:1319-26.
16. Embarek MS, Reiche J, Jacobsen S, Thabit AG, Badary MS, Brune W, Schweiger B, and Osmann AH: Molecular Analysis of Human Metapneumovirus Detected in Patients with Lower Respiratory Tract Infection in Upper Egypt. International Journal of Microbiology. 2014;2014:1-11.
17. Reiche J, Jacobsen S, Neubauer K, Hafemann S, Nitsche A, Milde J, Wolff T and Schweiger B :Human Metapneumovirus: insights from a ten-year molecular and epidemiological analysis in Germany. PLoS ONE.2014 ;(9): e88342.
18. Zaki WK, Fathi MS and Ismail R : Detection of human metapneumovirus among infants with bronchiolitis. International Journal of Virology.2014; 10:37-45.
19. Jokela P, Piiparinen H, Luiro K and Lappalainen M: Detection of human metapneumovirus and respiratory syncytial virus by duplex real-time RT-PCR assay in comparison with direct fluorescent assay. Clin Microbiol Infect.2010; 16: 1568-1573.
20. Gray GC, Capuano AW, Setterquista SF, Erdman DD, Nobbs ND, Abed Y, Doern GV, Starks SE and Boivin G: Multi-year study of human metapneumovirus infection at a large US Midwestern Medical Referral Center. J Clin Virol.2006 ;37: 269-276.
21. Edwards KM, Zhu Y, Griffin MR, Weinberg GA, Hall CB, Szilagyi PG, Staat MA, Iwane M, Prill MM and Williams JV : Burden of Human Metapneumovirus Infection in Young Children. N Engl J Med.2013; 368: 633-643.
22. Koskenvuo M, Möttönen M, Rahiala J, Saarinen-Pihkala UM, Riikonen P, Waris M, Ziegler T, Uhari M, Salmi TT and Ruuskanen O: Respiratory viral infections in children with leukemia. Pediatr Infect Dis J.2008; 27:974-80.
23. Srinivasan A, Wang C, Yang J, Inaba H, Shenep JL, Leung WH and Hayden RT: Parainfluenza Virus Infections in Children with Hematologic Malignancies. Pediatr Infect Dis J.2011; 30: 855-859.
24. Pavlova S, Hadzhiolova T, Abadjieva P and Kotseva R: Application of RT-PCR for diagnosis of respiratory syncytial virus and human metapneumovirus infections in Bulgaria, 2006-7 and 2007-8. Euro Surveill.2009;14:19233.
25. Fattouh AM, Mansi YA, El-anany MG, El-kholy AA and El-karaksy HM: Acute lower respiratory tract infection due to respiratory syncytial virus in a group of Egyptian children under 5 years of age. Italian Journal of Pediatrics.2011; 37:1-6.
26. Nikfar R, Shamsizadeh A, Makvandi M and Khoshghalb A: Detection of respiratory syncytial virus in hospitalized children with acute lower respiratory tract infections, using RT PCR in Ahvaz, Iran. Arch Pediatr Infect Dis.2013;1: 118-21.
27. Moreira LP , Watanabe AS, Carraro E, da Silva ER, Guatura SB, Granato C and Bellei NJ: A survey strategy for human respiratory syncytial virus detection among haematopoietic stem cell transplant patients: epidemiological and methodological analysis. Mem Inst Oswaldo Cruz.2013; 108:119-22.
28. Simon A, Schildgen O, Panning M, Bode U and Huebinger A: Respiratory syncytial virus infection in patients with cancer: still more question s than answers. Clin Infect dis.2008;46:1933-1934.