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RESEARCH PAPER

Molecular Detection of Par influenza Virus 1, 2, 3 and 4 Serotypes and Immunological Response in Otitis Media Patients in Babylon-Iraq

Ayam M. Salih[1*], Jawad Kadim T. Al-Khafaji[2], Ghanim A. J.AL-Mola[3]

[1]*University of Babylon, College of Basic education, Department of Biology*

[2]*University of Babylon, College of medicine, Department of Microbiology*

[3]*University of Babylon, College of Science for Women, Department of Biology*

***Corresponding author: Ayam M. Salih**

Abstract

(AOM) occur as a case of viral upper respiratory tract infections in young children. Respiratory viruses and AOM both exhibited seasonal discrepancy. Respiratory paramyxo viruses consist of human par influenza viruses are highly distribution, cause the common of childhood croup, bronchiolitis, and pneumonia. The study was start from6March 2016 to 20May 2016 fifty cases of otitis media infection were detect inpatient's admitted to AL- Hilla teaching hospital in Babylon - Iraq. Ear swabs and nasopharynx secretion specimens were collected for studies the viral infection detection. The results display the percentage of parainfluenza virus in AMO 22(44%) was positive from fifty cases. The results also show percentage of parainfluenza virus one; two, three, and four types were 27.2%, 22.7%, 31.8% and 18.1% respectively the study include measurement of IL-6 cytokine by using ELISA assay. Results show elevation level of IL-6 in sera of otitis media patients comparative with control group. The highest level of IL-6 in male occur in (>5-6 year) age group it was 218.39 Pg/ml .The result also shows elevation of IL-6 level in female The highest level of IL-6 in female occur in (>6-7 year) age group it was 209 Pg/ml. Result shows significant differences comparative with control group ($P \leq 0.05$).

Keywords: *Otitis media, Ear swabs, Nasopharyngeal secretion, Parainfluenza virus, Cytokines.*

Introduction

Otitis media (OM) is infection of the middle ear which may occur as either acute otitis media or otitis media with discharge. AOM revealed rapid onset, middle ear secretion and symptoms of middle ear inflammation including fever, headache, vomiting, and irritability, whereas OME is middle ear effusion in the absence of symptoms of acute infection. (AOM) is the important cause of bacterial pediatric infections correlation with viral upper respiratory infections [1].

Bacterial cause AOM correlate with viral respiratory infection that the increased of bacteria adherence and speed inflammation in the nasopharynx and Eustachian tube, that aid bacterial infusion into the middle ear space[2].The main risk factor for AOM is a Viral URI however, the tendency to cause disease differ depend on the specific viruses [3]. More than sixty percentage of upper respiratory tract infection series lead to acute otitis media (AOM); a common reason for outpatient hospital admission and antibiotic use in children[4]. Four distinct serotypes of Human par influenza viruses have envelop and single-stranded RNA belonging to the paramyxovirus family, They are about 150–250 nm in size and include of negative sense RNA with a genome compose ~15,000 nucleotides [5].

HPIVs are pleomorphic viruses whose envelope is derived from the host cell they last infected. These viruses are 150-300 nm in diameter and possess a single-stranded, no segmented, negative-sense RNA genome with nucleoprotein P and L proteins. AOM is associated with viruses that cause upper respiratory tract infection (URTI) alone in up

to 30% of cases[6-7] The extent of this association has been recently strengthened by two, 12 month prospective research studies of healthy children, incorporating comprehensive clinical examination and improved viral detection techniques[8-9]. Chonmaitree and co-workers [9] reported that 97% of the children experienced one or more URTI's per year, for children 6 months to three years of age at an average of 5.4 infections over the 12 month period.

Nearly 10% of the children less than three years of age suffered more than 10 URTIs per year. Otitis media was identified in 61% of URTIs reported, with AOM being present in almost one-third of these children and OME present in almost a quarter of URTI's reported. Children in the study experienced an average of 1.7 episodes of AOM per child per year and child age was the strongest predictor of AOM development after URTI, after controlling for sex, race and ethnicity.

The immune response in the middle ear to infection is characterized predominantly by an inflammatory, results in clearance of microorganisms from the middle ear cavity [10-11].OM development is often preceded by viral URT which may predispose to secondary bacterial infections through mucosal epithelial damage, impaired mucociliary function and up regulated inflammatory cytokine response [12]PIV-infected cells significantly released a number of cytokines namely proinflammatory cytokines include IL-6[13].

Material and Methods

Fifty ear swab collected from 50 patients who admission to AL- Hilla teaching hospital in Babylon – Iraq for viral diagnosis, after collection Ear swab and nasal-wash specimen placed in 1-mL viral transport medium and transmitted immediately to the laboratory on ice box within 2 hour of collection and add 3 to 5 ml of phosphate buffered saline (PBS) to the specimen prior to centrifugation to reduce the viscosity and dilute the mucus. Centrifuge the mucus extractor at room temperature (25C°) for 10 minutes at 380rpm. After the remove of the supernatant resuspend the cell deposit in 2mL PBS and vortex gently, until the mucus is broken up and cellular material is released the sample were kept frozen at -87 C° for r RT-PCR assay. Detection of par influenza virus 1, 2, 3 and 4 serotypes antigen was performed according to the manufacturer's instructions (Sacace, Italy).

Fifty blood samples of 5ml volume each were collected aseptically using sterile syringes from patients admitted to AL-Hilla Teaching Hospital. Each blood sample was collected in sterile gel tube, label then all samples were incubated at room temperature at 30 minutes till clotted, sera sample were collected seperoly after centrifugation at 3000 rpm for 5 min .Sera sample were distributed in 0.5 ml aliqutes in sterile eppendorf tubes and stored at –20C°[14]until used for ELISA assay.

Result and Discussion

Role of viruses in the otitis media has been obtained by determine the presence of nucleic acids of par influenza virus in ear swab and nasopharyngeal secretion in children with supportive otitis media. Our result showed by user RT-PCR assay that out of 50 samples of otitis media patients, about 22(44%) from total cases were positive for par influenza virus include 9 of them from ear swab ,7 from nasopharyngeal secretion, and other 6 from ear swab and nasopharyngeal secretion) while 28(56%)had no par influenza virus

The result by use PCR assay in ear swab showed HPIV-3 type was the more predominant 7(31.8%), followed by HPIV-1 type 6 (27.3%), and HPIV-2 5(22.7%) ,and then HPIV-4 type 4(18.2%). In nasopharyngeal secretion the result show HPIV-1 type was more predominant 5(22.7%), followed by HPIV-3 type 3(13.6%), and HPIV-2 2 (9.1%) , and then HPIV-4 type 1(4.5%)

In mix the result show HPIV-3 type was the more predominant 5(22.7%), followed by HPIV-1 type 4 (18.2%), and HPIV-2 1(4.5%), and then HPIV-4 type 1(4.5%).No significant difference ($P \geq 0.05$) as illustrated in figure (1) and table 1) .There is a mix infection between types HPIV-1, HPIV-2, HPIV-3, and HPIV-4 in ear swab sample and in nasopharyngeal secretion sample, in addition there is a mix infection between ear swab and nasopharyngeal secretion swab when detected . The r RT-PCR techniques offer a rapid, more sensitive, specific and accurate diagnostic method for detection of HPIV which enables early detection and control [15].

The results agree with researcher [16].The Ct-value represent the value of cutting with threshold that relation with viral titer, as show in figure (1 A, B, C, D, E and F), that revealed the Ct- value was less than 35 that represent the high titer of the
par influenza virus

Determination about 22(44%) from total collection samples were positive for par influenza virus and this percentage divided according to aged. Found about 2(28.5%) from total positive cases in aged group (<1-2year) type I par influenza virus and 1 (14.2%) was type II par influenza virus, 3(42.8%) type III par influenza virus but type IIII par influenza virus was 1(14.2%) . In the aged group (>2-3 year) found only type I, type III and type IIII were 2(33.3%), 4(50%), and1 (16.6%) respectively. While the aged group (>3-4year) the percentage of type I, type II and type III were 1(25%), 2(50%) and 1(25%), respectively and not found type IIII. Aged group (>4-5 year) found only type II and type IIII par influenza virus were 1(50%) and 1(50%). Aged group (> 5-6year) found only type II par influenza virus were 1(100%). Finally aged group (>6-7 year) the percentage of type II and type IIII were 1(50%). There was significant association between par influenza virus serotypes among aged groups in otitis media patients. ($P \leq 0.05$), as shown in table (1). The study includes measurement of IL-6 cytokine by using ELISA assay. Results show elevation level of IL-6 in sera of otitis media patients comparative with control group. The highest level of IL-6 in male occur in (>5-6 year) age group it was 218.39 Pg/ml while the lowest level occur in (1-2 year) age group it was 80Pg/ml. The result also shows elevation of IL-6 level in female The highest level of IL-6 in female occur in (>6-7 year) age group it was 209 Pg/ml while the lowest level occur in (1-2 year) age group it was 64Pg/ml. Result shows significant differences comparative with control group ($P \leq 0.05$). Table (2).

Human par influenza virus infection cause increase level of cytokines such as proinflammatory cytokines (interleukins-6 and tumor necrosis factor- α), anti-inflammatory cytokines, th2 cytokines (interieukine-4, IL-5) [17- 18].serumIL-6 level were found to be increase in serum of children with par influenza virus infection [19].

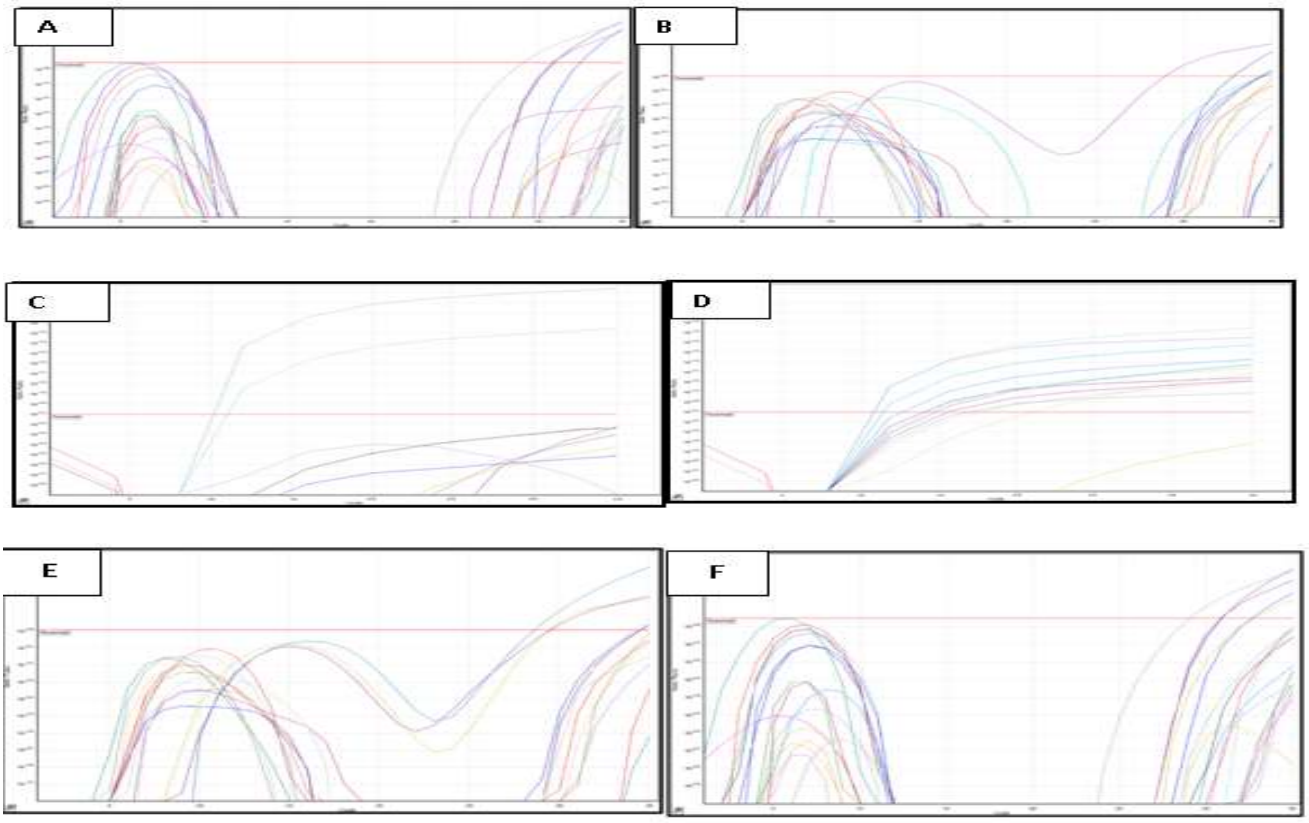

Figure 1: A Positive specimen for par influenza virus Type 1 using rRT-PCR techniques, B Positive specimen for par influenza virus Type 2 using rRT-PCR techniques, C Positive specimen for par influenza virus Type 3 using rRT-PCR techniques, D Positive specimen for par influenza virus Type 4 using rRT-PCR techniques, E Positive control for par influenza virus using rRT-PCR techniques, F Negative control for par influenza virus using rRT-PCR techniques as show in yellow color.

Table 1: Prevalence of par influenza virus serotypes in the middle ear swabs and nasopharyngeal secretion in children with otitis midair-PCR techniques

Age group/year	Positive specimens	HPIV types by PCR			
		HPIV1	HPIV2	HPIV3	HPIV4
1-2	7	2(28.5%)	1(14.2%)	3(42.8%)	1(14.2%)
>2-3	6	2(33.3%)	0	3(50%)	1(16.6%)
>3-4	4	1(25%)	2(100%)	1(25%)	0
>4-5	2	1(50%)	0	0	1(50%)
>5-6	1	0	1(100%)	0	0
>6-7	2	0	1(50%)	0	1(50%)
Total	22	6(27.2%)	5(22.7%)	7(31.8%)	4(18.1%)

χ^2=72.382

P_{value}=0.00001

Table 2: Concentration of IL-6(Pg/ml) cytokine in sera of otitis media patients

Age (Year)	treatment	IL-6 (Pg/ml) (Mean ±S.D)	
		Male	Female
1-2	Control	14.25±3.77	14±2.94
	Patient	*80.09±12.59	*64.61±9.26
>2-3	Control	12.75±1.71	17.5±2.86
	Patient	*100.26±30.73	*100.69±7.88
>3-4	Control	19±6.68	18.67±8.38
	Patient	*112.57±34.33	*132.67±28.32
>4-5	Control	16±4.55	15.66±2.49
	Patient	*133.19±22.38	*88.79±3.24
>5-6	Control	11.5±0.41	19.0±0.82
	Patient	*218.39±37.85	*183.87±25.14
>6-7	Control	18.5±2.04	11.0±0.82
	Patient	*190.95±14.57	*209.07±2.08
$LSD_{(0.05)}$	**7.76**		

***significance difference ($P \leq 0.05$)**

Conclusion

The percentage of AOM cases during infection with par influenza virus is mainly dependent on age, AOM occur in high number in children. Para influenza virus serotype 3 and 1 highly distribution .m RT-PCR assay could be used for the accurate diagnosis and detection of HPIV.

Further, expanded surveillance throughout the country will help in better epidemiological analysis, for implementation of better public health programmers in controlling virus induced respiratory in fections reported. Par influenza virus infection can induce different local cytokine responses.

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