

## RESPIRATORY VIRAL INFECTION IN NEONATES CLINICALLY DIAGNOSED AS BACTERIAL SEPSIS

By

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### ABSTRACT

**Background & Objectives:** *Respiratory viral infections in neonatal intensive care units (NICUs) can cause significant morbidity and mortality. Testing for viral pathogens is not routinely done in many NICUs. Their clinical presentations are unpredictable and on infant sepsis evaluation, bacterial cultures are frequently sterile. The study aimed to estimate the prevalence of respiratory viral infections among neonates clinically diagnosed with sepsis using multiplex polymerase chain reaction (PCR).*

**Patients and Methods:** *A prospective study on 150 neonates (gestational age >28 weeks) without major malformation and central causes for respiratory distress admitted with neonatal sepsis clinically to Children hospital NICU, Ain Shams University, from January 2015 to December 2016. Neonates were selected by simple random method. PCR on nasopharyngeal secretions was done for 40 neonates whose blood cultures were negative.*

**Results:** *Sixteen neonates (40%) were PCR positive and 24 neonates (60%) were negative. Herpes simplex virus was the most frequently detected (25%) followed by adenovirus, rhinovirus, and enterovirus. No significant correlations were found between viral detection and any of clinical or laboratory data except the liver enzymes which were significantly higher in positive viral cases.*

**Conclusion:** *Prevalence of respiratory viruses was 40% of cases presented with clinical signs of neonatal sepsis. PCR is recommended for diagnosis of respiratory viral infections to improve management of sepsis cases and limit the unnecessary use of antibiotics.*

**Key words:** *Neonate, sepsis, respiratory, viruses, infections, multiplex PCR*

## INTRODUCTION

Respiratory viral infections taking place in the neonatal intensive care units (NICUs) can cause significant morbidity and mortality. Testing for viral pathogens is not routinely done in many NICUs, hence, data about their occurrence is limited. Moreover, most outbreaks or prospective surveillance of clinically stable newborns, consequently, widening the knowledge gaps (**Bennett et al., 2012**). The clinical presentations of respiratory viral infection among newborns in the NICU are not entirely expected. On evaluating these newborns for sepsis, their bacterial cultures are often sterile. Due to lack of confidence in culture results, newborns may have extended antibiotic therapy (**Cantey and Sanchez, 2011**). Treatment with antibiotics evolves development of antibiotic resistance in bacteria with no effect on most of acute viral respiratory infections (**Spurling et al., 2011**). Owing to lack of clinically valid diagnostic tests verifying a viral etiology, antibiotics are often prescribed (**Byington et al., 2002**). Sensitive methods, such as quantitative real-time polymerase chain reaction (qPCR) analyses on nasopharyngeal samples, for a number of viruses have been

launched in hospitals as a sensitive diagnostic tool among infants with respiratory tract infection (**Jartti et al., 2013**). Many comparative studies concluded that real-time reverse-transcription polymerase chain reaction (rRt-PCR) assays are much more sensitive than conventional methods, such as viral culture and immunofluorescence assays (**Lassaumene et al., 2010**). Furthermore, comparing multiplex rRT-PCR to conventional PCR and other real-time methods revealed its apparent advantage as it permits simultaneous amplification of many viruses in single reaction. This makes cost effective diagnosis easier, allowing multiple viruses detection in a single specimen, with high sensitivity (91%) and high specificity (100%) (**Paranhos et al., 2008**).

## AIM OF THE WORK

To estimate the prevalence of respiratory viral infections among neonates who were primary diagnosed as sepsis cases using multiplex PCR technique.

## Ethical consideration:

1. Written consent was taken from the parents/care-givers before participating in the study.
2. This study was approved by the ethics committee of

Faculty of Medicine of Ain Shams University. With approval number: 3117, 24 12 2014 The parents of all patients provided written consent after explaining to them the nature of the study.

3. The patient has the right to withdraw from the study.
4. The results of the study were confidential.
5. Authors declared no conflict of interest nor fund was granted for this study.
6. No financial support regarding the study or publications.

#### **Sample size:**

Epi info program was used for sample size that was 150 neonates. Calculation guided by the following data: power after test was (80%), confidence level (95%), Alfa Score (5%), standard deviation (SD10)

#### **PATIENTS AND METHODS**

This prospective study was conducted on 150 neonates (gestational age more than 28 weeks) from January 2015 to December 2016 in NICU of Children hospital, Ain Shams university hospitals, Cairo, Egypt. They were selected by simple random method.

#### **Inclusion Criteria:**

1. Admission at any time from birth up to 28 days postnatal.
2. Admission with clinical picture of sepsis.
3. Multiplex viral PCR was done only for neonates with negative blood culture.

#### **Exclusion Criteria:**

1. APGAR score < 6 at 5 minutes.
2. Major congenital malformation and chromosomal aberrations.
3. Respiratory manifestations secondary to central causes.

Careful history was taken from all neonates including maternal, obstetric, and perinatal history. Respiratory data and need for respiratory support were recorded. Oxygenation index OI was calculated:  $\text{MAP} \times \text{FiO}_2 \times 100 / \text{PaO}_2$  (MAP: mean airway pressure, FiO<sub>2</sub>: fractional of inspired oxygen, PaO<sub>2</sub>: partial pressure of oxygen (**Ortiz et al., 1987**).

Birth weight, crown-heel length and head circumference was also recorded. Neonatal clinical signs of sepsis were divided into five categories:

1. Temperature instability (<37.0°C or >38.5°C).

2. Respiratory distress (dyspnea, tachypnea, apnea, ventilation support, oxygen requirement).
3. Cardiovascular dysfunction (tachycardia, bradycardia, decreased peripheral circulation, hypotension, need for vasopressor support or inotropic medication).
4. Neurological irregularities (hypotonia, lethargy, irritability).
5. Gastrointestinal problems (feeding intolerance, vomiting, abdominal distension, suspicion of necrotizing enterocolitis (Frakking et al., 2007)).

**Blood culture:** one mL blood from peripheral venipuncture + BD BACTEC Peds Plus/F Medium; BD, Heidelberg, Germany) was obtained from every patient on admission before starting antibiotics to perform blood culture. Blood cultures were withdrawn from the 150 neonates with clinical picture of sepsis, at time of admission, which divided them into two groups; group of neonates with positive blood cultures (n: 110), group of neonates with negative blood culture (n: 40). For these 40 neonates (with negative blood culture results), multiplex viral PCR was done on their nasopharyngeal secretions,

dividing them into two groups; group 1: neonates with negative viral multiplex PCR and group 2: neonates with positive viral multiplex PCR. The sample was withdrawn during suction in ultramed mucous extractor supplied with ULTRA company, the sample was stored at -4°C for at least 24 hours before transfer to the laboratory. Multiplex PCR for eight respiratory viruses (hMPV), (RSV), Influenza virus A and B, Herpes simplex viruses 1 & 2, Rhinovirus, Adenovirus, Coronavirus, and Enterovirus were performed according to (Marchall et al., 2007).

#### **Multiplex polymerase chain reaction (mPCR):**

Nasopharyngeal and tracheal aspirates were stored at -4°C for at maximum 24 hours before transfer to the laboratory. Nucleic acid extraction was done using HotStarTaq DNA&RNA extraction kit, Qiagen RNA extraction and Qiagen DNA extraction kits as described by the manufacturer Qiagen, Germany. Protocol viral RNA was operated using 140 µL from the sample to result in an eluted volume of 60 µL. DNA extraction 200 ul of the sample was used to give elution volume of 100 ul per sample. Synthesis of cDNA reverse transcription was done using the cDNA synthesis kit for converting

RNA to cDNA Synthesis (Omniscript cDNA synthesis kit Qiagen Germany. Conventional multiplex PCR using multiplex hot start master mix supplied by Qiagen, Germany and operated by PXE 0.2 thermal cycler (Thermo USA), followed by gel electrophoresis for specific amplified product detection, which simultaneously detects 8 respiratory viruses [adenovirus (AdV), influenza A virus (Flu A), influenza B virus (Flu B), rhinovirus (HRV), respiratory syncytial virus (RSV), metapneumovirus (MPV), coronavirus and enterovirus (HEV)].

The optimized cycling conditions were: 94°C for 15 min followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min. Final extension was done at 72°C for 10 min. The Amplified products were

subjected to electrophoresis in 1% agarose gels (Electrophoresis grade, Invitrogen) in 50x TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.2) followed by ethidium bromide staining and visualized under UV light as shown in **figures (1) &(2)**.

### **Statistical Methods:**

Data was tabulated using MS Excel 2013 and analyzed using R v.3.3.2 statistical software. Mean and standard deviation were used to summarize normally distributed quantitative variables. While median and interquartile range (IQR) were used to summarize quantitative variables, which does not follow the normal distribution. Student's t-test was used to compare normally distributed variables between two groups.. All tests were two tailed, p-values less than 0.05 were considered statistically significant.

**RESULTS**

Multiplex PCR for respiratory viruses was performed to 40 neonates who were clinically diagnosed as neonatal sepsis, with negative bacterial blood

culture. Sixteen neonates (40%) were positive for viral multiplex PCR (group 2) and 24 (60%) were negative (group 1).

**Table (1): Demographic and clinical characteristics for the studied neonates (n=40)**

	<b>Group 1 (n=24)</b>	<b>Group 2 (n=16)</b>	<b>p-value</b>
Gestational age (weeks)	37 (36 - 37) <sup>^</sup>	37 (37- 37) <sup>^</sup>	0.208 <sup>a</sup>
Postnatal age on NICU admission (days)	5 (3 - 7.25) <sup>^</sup>	5.5 (3.75 - 16) <sup>^</sup>	0.345 <sup>a</sup>
Birth-weight (grams) (mean $\pm$ SD)	2930 $\pm$ 700#	2930 $\pm$ 438#	0.989 <sup>b</sup>
Cesarean section, n (%)	14 (58.3%)	6 (37.5%)	0.197 <sup>c</sup>
Mortality, n (%)	11 (45.8%)	5 (31.25%)	0.356 <sup>b</sup>
Maternal DM	1 (4.2%)	1 (6.3%)	1.000 <sup>d</sup>
Maternal Pre-eclampsia	0 (0%)	2 (12.5%)	0.154 <sup>d</sup>
PROM	3 (12.5%)	2 (12.5%)	1.000 <sup>d</sup>
Resuscitation, n (%)	4 (16.7%)	2 (12.5%)	1.000 <sup>d</sup>
Exposure to contacts with respiratory viral symptoms, n (%)	9 (37.5%)	9 (56.25%)	0.243 <sup>c</sup>

PROM: premature rupture of membranes

aWilcoxon rank sum test (df = 39). bStudent's t-test (df = 39). cPearson's chi square test (df=1). dFisher's exact test.

<sup>^</sup>: Data are expressed as median & interquartile range, #: Data are expressed as mean $\pm$ SD

**Table (1)** showed insignificant difference regarding

demographic and clinical data between the two groups.

**Table (2): Clinical signs of the studied neonates (n=40)**

	Group 1 (n=24) (%)	Group 2 (n=16) (%)	p-value
Temperature Instability	15 (62.5%)	10 (62.5%)	1.000 <sup>c</sup>
Gastrointestinal signs	22 (91.7%)	16 (100%)	0.508 <sup>d</sup>
Neurological signs	19 (79.2%)	12 (75%)	1.000 <sup>d</sup>
Chest findings	20 (83.3%)	10 (62.5%)	0.159 <sup>d</sup>
Hypotension	15 (62.5%)	9 (56.3%)	0.693 <sup>c</sup>
Inotropes	19 (79.2%)	9 (56.3%)	0.166 <sup>d</sup>
Ventilation need, n (%)	22(91.7%)	11(68.75%)	0.094 <sup>a</sup>
-CPAP	8(33.33%)	9(56.25%)	0.151 <sup>c</sup>
-Mechanical ventilation	14 (58.33%)	9 (56.25%)	0.896 <sup>c</sup>
-High Frequency ventilation (%)	9 (37.50%)	5 (31.25%)	0.685 <sup>c</sup>
Duration of ventilation (days), Median(IQR)	15(3-40)	15(4-38)	0.637 <sup>c</sup>

aWilcoxon rank sum test (df = 39). cPearson's chi square test (df=1). dFisher's exact test.

**Table (2)** showed presentation, need and duration of mechanical ventilation in both groups. It showed no significant difference regarding the clinical signs.

**Table (3): Laboratory data of the included neonates (n=40)**

	Group 1 (n=24)	Group 2 (n=16)	p-value
Leukopenia (<4X10/L) n (%)	6(25%)	0 (0.0%)	0.064 <sup>d</sup>
Platelets count	320±177#	320±166#	0.998 <sup>b</sup>
Elevated liver enzymes, n (%)	8 (33.3%)	11 (68.75%)	0.028 <sup>c*</sup>
ALT (U/L)	32.5 (22 - 52.75) <sup>^</sup>	81 (38.25 - 123.25) <sup>^</sup>	0.012 <sup>a*</sup>

ALT: Alanin aminotransferase. a Wilcoxon rank sum test (df = 39). bStudent's t-test (df = 39). c Pearson's chi square test (df=1). dFisher's exact test.

**Table (3)** showed that laboratory investigations were insignificant between the two groups except for the liver enzymes concentrations were significantly higher in patients with positive viral multiplex PCR (group 2) than those with negative viral multiplex PCR (group 1).

**Table (4): Frequency and types of the viruses in the study (n=16)**

Viruses	No.	%
Adenovirus	3	18.8%
Enterovirus	3	18.8%
Herpes virus	4	25.0%
Influenza virus	2	12.5%
Rhinovirus	3	18.8%
Rhinovirus and influenza virus	1	6.3%

**Table (4)** recorded that herpes simplex virus was the most frequently detected

followed by adenovirus, rhinovirus, and enterovirus.

### **DISCUSSION**

Data on respiratory viral infections occurrence in many NICUs was not well known because testing for viral pathogens was not performed routinely. (Bennett et al., 2012). Clinically, there was often little difference between sepsis that was caused by an identified pathogen and sepsis that was caused by unknown pathogen especially viral pathogens. Multiplex-RT-PCR assays had been developed to detect respiratory viruses as a method of culture independent technique (Raymond et al., 2009).

In the current study, multiplex PCR was done to detect the respiratory viruses in 40 neonates with clinical sepsis and negative blood culture results. Sixteen neonates (40%) showed positive results for presence of respiratory viral infection. Similarly, previous

studies reported respiratory viruses as an etiological agent of neonatal sepsis to be 41%, 52% 10.2%, 10%, 8% , 21% proved by multiplex PCR technique (Van Piggelen et al., 2010, Bennett et al., 2012, Smit et al., 2013, Kidszun et al., 2014, Ronchi et al., 2014 and Kujari et al., 2014) respectively. The demographic and clinical data of the 40 included newborns were comparable which was similar to earlier studies (Farzin et al., 2015).

Moreover, in this study 56.25% of positive viral group had a history of exposure to a contact with respiratory viral symptoms. However, previous study reported that all infants who had a respiratory virus detected during the sepsis evaluation were more likely to be older, and to be exposed to individuals with respiratory viral symptoms

**(Ronchi et al., 2014)**. On the other hand, previous study found that only 7% of infants with confirmed respiratory viral infection by multiplex PCR had a history of exposure to contact with respiratory viral symptoms **(Kujari et al., 2014)**.

Additionally, the present results showed insignificant difference between positive and negative viral groups as regards oxygen ventilation need and duration. In contrast, previous study reported that infants with positive PCR multiplex viral test had higher and prolonged use of ventilator support with greater number of clinical deteriorations than negative one **(Bennett et al., 2012)**.

Moreover, in this study, infants with positive and negative viral respiratory infection had comparable laboratory investigations, except for elevated liver enzymes in positive viral group. Furthermore, elevated liver enzymes were the only predictors of viral infection by regression analysis. This disagreed with other studies, in which there was no difference between two groups regarding liver enzymes **(Vieira et al., 2003)**, and the only predictor of viral infection was the care givers' clinical suspicion of viral infection **(Ronchi et al., 2014)**.

The multiplex PCR for respiratory viruses was positive in 16 neonates (40%); three were caused by adenovirus, three by enterovirus, four neonates with herpes-simplex virus (HSV), two were influenza virus positive, three rhinovirus and one was positive to both-rhinovirus and influenza virus, the most frequent viral pathogens detected in this study were HSV and rhinovirus (four cases for each one). Similarly, previous study showed that rhinovirus was the most frequent viral pathogen in 50% of cases detected. They detected co-infection with rhinovirus and enterovirus in 25% of their cases **(Ronchi et al., 2014)**. These results were similar to previous studies in which the most frequent viral pathogen was rhinovirus in 40%, 50%, 94% and 50% of cases **(Vieira et al., 2003, Van Piggelen et al., 2010, Steiner et al., 2012, and Kujari et al., 2014)** respectively.

On the contrary, other studies showed that parainfluenza virus-1 was the most frequent viral pathogen detected **(Bennett et al., 2012 and Smit et al., 2013)** others reported that the most frequent viral pathogens were picornavirus in 8.4% of cases **(Kidszun et al., 2014)**, or respiratory syncytial virus in 91% of pneumonia cases and enteroviruses in two third of

sepsis cases (**Farzin et al., 2015**). Difference in prevalence of respiratory viruses might be due to difference in geographical environments and climate. On comparing multiplex PCR assays and conventional techniques for diagnosing respiratory virus infection in children admitted to the hospital with acute respiratory illness, PCR was more sensitive and had the advantages of a shorter delay in specific diagnosis and a lower cost than immunofluorescence or culture (**Freymuth et al., 2006**). Although, other studies showed a limitation of the use of PCR assay showing that asymptomatic carriage of a respiratory virus occurred frequently in young children (**Jansen et al., 2011**) and the presence of viral-nucleic acids might not always reflect an association with infectious virus production (**Wright et al., 2007**) but carrier state would not be the case in neonates.

### **CONCLUSION**

Respiratory tract infections in neonates can be of viral origin, as recorded in the current study 40% of studied patients were positive for viral detection by multiplex PCR technique. Viral infection was significantly correlated to elevated liver enzymes so; the presence of elevated liver enzymes in a patient with sepsis like picture

may help us to suspect viral etiology. The identification of specific viral pathogens will limit over use of antibiotics and will facilitate giving specific antiviral therapy when indicated.

### **RECOMMENDATIONS**

Respiratory viral pathogens screening should be done for any neonate with clinical sepsis picture, negative blood culture & elevated liver enzymes, as their identification is important to limit unnecessary use of antibiotics & give specific antiviral therapy.

### **LIMITATION OF THE STUDY**

The limitation of our study was that multiplex PCR was not performed to the all 150 neonates limiting the overall viral pathogen prevalence data if co-exist with other bacterial pathogens. Studying the respiratory viral infection prevalence among neonates that was held in our tertiary NICU was the first study in Egypt & Middle East Countries.

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**Authors contributions:** Tarek El-Gammasy & Maha H Mohamed thought of the research idea. Yasmin A. Farid & Tayseer Gad collected the data. Wafaa Zaki performed the viral PCR. Zeinab

El-Kabbany, Tarek El-Gammasy, Maha Mohamed & Yasmin A. Farid interpreted & tabulated the data. Maha Mohamed, Wafaa Zaki & Yasmin A. Farid were major contributors in writing the manuscript. All authors read & approved the final manuscript.

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