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Respiratory Syncytial Virus Infection among Children in Lagos, Nigeria

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Abstract

Background Information: Acute Lower Respiratory Tract Infections (ALRTIs) are among the major cause of mortality and morbidity worldwide. Respiratory Syncytial Virus (RSV) is recognized as the most important viral cause of ALRTIs in children under the age of five years.

Objectives: This study determined molecular prevalence of RSV among under five children admitted with ALRTIs in a tertiary hospital, Lagos State University Teaching Hospital (LASUTH), Ikeja, Lagos State. It also highlighted the clinical presentation and identifies the risk factors associated with the acquisition of RSV-ALRTIs.

Methods and Materials: A hospital based cross-sectional study conducted among children under the age of five years diagnosed and admitted with ALRTIs in the paediatric department of LASUTH. Nasopharyngeal swabs were obtained from the participants for the determination of RSV using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Data was analyzed by both descriptive and inferential statistics using SPSS.

Results: A total of 200 participants were recruited over a period of eight month. Forty five (22.5%) had detectable RSV RNA. The prevalence of RSV was highest among the age group

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2-6 month (32.4%). The main clinical symptoms of RSV-ALRTIs were fever, cough, tachypnoea and chest wall in drawing.

Conclusion: This study showed that RSV is an important viral cause of ALRTIs necessitating hospitalization in children under the age of five and the imperative for rapid, easy and less expensive method of diagnosis of RSV infection to avoid unjustifiable use of antibiotic.

Keywords

Respiratory Syncytial Virus; Infectious Diseases; Respiratory Tract Infection

Abbreviations

RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; ALRTI: Acute Lower Respiratory Tract; WHO: World Health Organization; RSV: Respiratory Syncytial Virus; LASUTH: Lagos State University Teaching Hospital; PCR: Polymerase Chain Reaction; RT-PCR: Reverse Transcription PCR; OR: Odd Ratio; ICU: Intensive Care Unit

Introduction

Acute Lower Respiratory Tract Infections (ALRTIs) are important cause of morbidity and mortality in infant and young children in developed and developing countries [1-3]. The World Health Organization (WHO) in 2015 reported that about four million children aged less than five years die annually and 1.9 million of these deaths result from complications of ALRTIs, mainly pneumonia. About 70% of all ALRTI deaths occur in Sub Sahara Africa and Southeast Asia [3-5].

In developed countries, most ALRTIs have been reported to be due to viral pathogens. Respiratory Syncytial Virus (RSV) is one of the most important viral agents, while other viral aetiological agents includes influenzae A and B; Parainfluenza virus, adenovirus, rhinovirus and newer viral aetiological agents of ALRTI like human metapneumovirus, human coronavirus type and human bocavirus [6-9].

Respiratory syncytial virus associated ALRTI account for a major cause of hospitalization into the pediatric ward [2,10,11]. Almost all children before the age of 2 years would have had RSV infection at least once and about 1-2% of otherwise healthy children will require hospitalization for RSV associated ALRTI [2,11-13]. The groups at risk for severe RSV infection include children born with prematurity children with chronic lung disease, congenital heart disease and immunodeficiency [2,3,5,10,14,15].

There is paucity of data in Nigeria and other Africa countries on the viral etiology of ALRTI and the prevalence of RSV infection among under five children, probably due to lack of modern diagnostic techniques [4,16]. In addition, there are also no guidelines for its management resulting in mismanagement and inappropriate use of antibiotic [17].

WHO recognize that there was scant evidence on the role of RSV in causing ALRTI among children in developing countries therefore recommend that new studies be carried out in developing countries to develop standardized protocol for the management of RSV [18,19]. The objectives of the protocol are to determine the age-specific incidence of RSV-associated respiratory infections in children less than five years of age, to assess the severity of acute respiratory infections due to RSV, and determine the seasonal variation of infection [16,18].

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Methodology

Study Site

The study was conducted at the children ward of the Lagos State University Teaching Hospital (LASUTH).

Study Population

The study population comprise children admitted to the Lagos State University Teaching Hospital with ALRTI. All patients admitted to a general ward or to the Intensive Care Unit (ICU) were be recruited in the study [18].

Study Design

The study was a cross-sectional study to determine the prevalence of respiratory syncytial virus detected in children admitted with Acute Lower Respiratory Tract Infections (ALRTIs) in LASUTH.

Ethical Consideration

Approval for the study was obtained from the Ethics and Research Committee of the Lagos State University Teaching Hospital. A written informed consent was also obtained from the parent/care givers of the study participants. The informed consent form contained the following

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information: names and affiliation of investigator, a plain language description of the study, the duration of the study, the right to withdraw at any time, the ethics committee approval and the privacy guarantee.

Data Collection

Structured interviewer-administered questionnaire which has three sections was used. Section A was designed to collect socio-demographic data of participants (age, sex, birth weight etc.). Section B consist of attributes that might directly or indirectly put them at risks for the acquisition of RSV infection such as history of congenital heart disease, nasal instrumentation, and family history of atopy among others. While, section C consist of presenting symptoms among participants.

Laboratory Procedures

RNA Extraction

The samples were brought to stand at room temperature. RSV- RNA was extracted from 1 ml of each sample by the use of transzol reagent (Trans GenBiotech, Beijing) genomic RNA extraction mini kit according to the manufacturer instructions.

Extraction Procedure

The samples were brought to room temperature for few minutes to thaw and then vortexed to homogenize and the supernatant decanted. Two hundred (200) μ L of chloroform was pipetted into the sample and centrifuged at 10,000 x g at 40°C for 15 minutes. The supernatants were pipetted into a new RNase free eppendroff tube. Five hundred (500) μ L of Isopropanol was added into the tube and incubated at room temperature for 10 minutes. Samples were centrifuged at 10,000 x g at 40°C for 10 minutes, thereafter supernatant decanted. 1 ml of 75% Ethanol was added to the sediment and vortexed for 5 minutes, thereafter samples were centrifuged at 7,500 x g for 5 minutes at 40°C. The supernatants were discarded and the sediment (RNA pellets) was air dried. One hundred (100) μ L of dissolving solution was added into the RNA pellets and incubated at 600°C for 10 minutes. This is thereafter stored at -700°C for PCR processing.

Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction (PCR) is a very sensitive and powerful technique for the exponential amplification of specific DNA sequences in vitro by using sequence-specific synthetic oligonucleotides and a thermo-stable DNA-polymerase. The method was used for the amplification of genomic DNA for genotyping and in combination with reverse transcription of RNA for determination of mRNA expression levels of stretch-inducible gene products.

Reverse Transcription PCR (RT-PCR)

To generate a representative cDNA pool from RNA templates, the following were mixed in a single tube to make a total volume of 20 µl.

Sample RNA- 7 µl; Rmix- 10 µl; Emix- 0.4 µl; Primer F- 0.4 µl; Primer R- 0.4 µl; RNase free water 1.8 µl.

The content of the tube was gently mixed and incubated at 42°C for 50 minutes for first strand cDNA synthesis. Then, the reaction was inactivated by incubating the mixture at 70°C for 10 minutes and 180 µl of RNase free water was added to dilute the resulting cDNA. These samples were used for the PCR reactions. The PCR reaction was performed in an automatic thermocycler (Biometra) programmed as shown in below.

	Temperature (°C)	Time	
Reverse Transcriptase	45	30 minute	Step 1
Initial Denaturation	94	5 minutes	Step 2
Denaturation	94	0.3 seconds	Step 3*
Annealing	45	0.3 seconds	Step 4*
Extension	72	0.3 seconds	Step 5*
Final Extension	72	10 minutes	Step 6
* Step 3- 5 are repeated 50 times.			

Table 1: The steps performed for PCR reaction.

Agarose Gel Electrophoresis

From the negative control, sample and the ladder mixes, twenty (20) µl of each was set on 2% agarose gel (2% w/v in 1x TAE buffer) and electrophoresed in 1x TAE buffer for 45 minutes at 100V. The bands were visualized under gel documentation system (BioRad Gel Doc-XR, USA) and screenshots captured. The size of the separated bands (DNA fragments) was compared with GeneRuler™ 100bp+ DNA ladder (MBI Fermentas, Life Sciences, Canada).

Data Management

Data collected from each participant was subjected to descriptive and inferential statistical analysis using the software Statistical Package for Social Sciences (SPSS) version 20. Results are presented in tables and charts. Statistical significance between variables was determined using chi-square and level of significance was considered at $P < 0.05$.

Results

Prevalence of Respiratory Syncytial Virus (RSV) Infection

Table 2 below shows that out of 200 participants in this study, Respiratory Syncytial Virus (RSV) was detected in 45(22.5%) of the children.

Prevalence of RSV Infection in Different Age Group

The prevalence of RSV among children age group 2-6 month (32.4%) and age group 25-60 months (27.1%) was higher than other age groups. There was statistical significant difference in the prevalence among the age groups $p = 0.04$. This is shown in Table 3 and Figure 1.

Symptoms and Sign of Participants with RSV Infection

All participants diagnosed with RSV-ALRTIs have fever and cough while 2/3rd of the participants have wheeze, tachypnoea and chest in drawing, only about 1/5th have cyanosis. This is demonstrated in Table 4.

Logistic Regression Showing Independent Risk Factors for RSV- ALRT Infection

Children that are not exclusively breastfed are 2.55(25%) more likely to have RSV-ALRTIs compare to exclusively breastfed children (CI 1.3-5.0, $p < 0.001$). Also, children without history of nasal instrumentation are 22% less likely to have RSV-ALRTIs (CI 0.074- 0.641, $p = 0.006$) while children without family history of atopy are 25% less likely to have RSV-ALRTIs (CI 0.048- 0.929, $p = 0.039$). Table 5 illustrate this further.

RSV	Frequency	Percentage (%)
Positive	45	22.5
Negative	155	77.5
Total	200	100

Table 2: Prevalence of RSV infection.

Age Group (Month)	RSV Positive n (%)	RSV Negative n (%)	Total	P-value
<2 month	–	9 (100)	9 (100)	
6-Feb	12 (32.4)	25 (67.6)	37 (100)	
12-Jul	2(6.7)	28 (93.3)	30 (100)	
13-24	12(22.2)	42 (77.8)	54 (100)	
25-60	19(27.1)	51 (72.9)	70 (100)	
Total	45 (22.5)	155 (77.5)	200	0.04

Table 3: Prevalence of RSV infection in different age group.

Clinical features	Number (n)	Percentage (%)
Fever	45	100
Cough	45	100
Wheezing	27	60
Chest in drawing	30	66.7
Tachypnoeacyle/min	36	80
Creepitation	25	55.6
Cyanosis SPO ₂	10	22.2

Table 4: Symptoms and sign in subject with RSV infection.

Variables	Odd Ratio (OR)	95% Confidence Interval	P-value
Crèche attendance • No* • Yes	1.447	0.443-1.673	0.659
Exclusive breastfeeding • No* • Yes	2.559	1.300-5.040	0.000
Nasal instrumentation • No* • Yes	0.217	0.074-0.641	0.006
Family history of atopy • No* • Yes	0.211	0.048- 0.923	0.039

*Indicates reference group

Table 5: Logistic regressions showing independent risk factor for RSV infection.

Figure 1: The prevalence of RSV in different age group.

Discussion

This study evaluates the burden of Respiratory Syncytial Virus (RSV) among children under the age of five years with clinical diagnosis of ALRTIs admitted in Lagos State University teaching Hospital, Ikeja. A prevalence of 22.5% was found by RSV-RNA detection. This prevalence obtained is close to 23.5% reported in a sero-epidemiological study of respiratory syncytial virus among children in Ibadan, South West, Nigeria [19]. However, this finding is lower than 34.6% reported in another study in Ibadan among children attending secondary health facility for ALRTIs [20]. In Abeokuta a prevalence of 5.4% was reported among children under the age of five years attending paediatric outpatient for ALRTI [21]. The disparity in prevalence reported within the same geographic region may be related to the duration of the studies. The studies in Ibadan were carried out over a period of one year, while the study in Abeokuta was carried out within 4 month, mostly in the dry season of the year. Studies from other African countries among children under the age of five years hospitalized for ALRTIs reported prevalence of 14.1% for Ghana and 16.1% for Egypt respectively [5,17]. This is however lower than what was reported in other part of the world like Italy 40.6% [22].

The distribution of RSV according to age group indicates that age group 2-6 month has the highest prevalence (32.4%) compared to other age groups and this is statistically significant. This finding is in agreement with studies conducted in Ibadan, Zaria, and Benin Nigeria [23]. In studies conducted in Ghana and Kenya among hospitalized children, the highest prevalence was reported in children aged 0-6 months [17]. A slight deviation from this is a study conducted

in Egypt among children under the age of five years hospitalized for ALRTIs. The highest prevalence was reported among children aged 0-12 months [22]. In contrast to findings in this study, some studies reported highest prevalence among other age groups. Age group 7-12 month age group 25-60 month, age group 13-60 months and age group 37-60 months [13,20,22,23]. The reasons attributable to varied prevalence among different age groups may be due to disparity in number of children that were recruited in the various studies. Children start to attend crèche/school at age 18 months and older, where they have daily contact with other children. Even though some may have antibodies, these may not be enough to protect them against re-infections from different strains of the RSV [24].

In this study cough and fever were clinical features seen in the entire patient with ALRTIs caused by RSV. This is in tandem with studies from Nigeria and other part of the world where fever and cough were reported as the commonest symptoms seen in ALRTIs caused by RSV [22]. In Ibadan, fever was seen in 78% while cough was seen in 82% of ALRTIs caused by RSV [20]. Similarly, a study conducted in Zaria found that fever and cough were seen in 82% and 79% of children with RSV infection respectively [25]. Difficulty in breathing was reported in 80% of children with RSV infection in this study. This is similar to 85.0% in Benin [24]. This finding is higher than in Sokoto [19]. This disparity is likely due to the variation in inclusion criteria. All the studies mentioned above recruited children from paediatric emergency and paediatric outpatient units, while this study recruited from paediatric emergency unit only. Other symptoms found in children with RSV infection include wheezing (60%) and crepitation (55.6%). These findings were also reported in similar studies [25].

This study also found an increased risk of RSV infection among children who had nasal instrumentation in the past. This agrees with a study carried out in Denmark aiming at identifying risk factors for Severe RSV infection. The study reported a 32% risk of RSV infection in children who had nasal procedure done in the past [26].

Environmental smoke exposure did not show a significant association with RSV infection in this study. This finding did not agree with findings from other studies, that reported that children who are exposed to environmental smoke especially those whose parent smoked has a higher risk of RSV infection [27]. The reason for the findings in this study is likely due to the fact that most mothers will not volunteer the information that their husband smoke cigarette, steaming from the fact that cigarette smoking is considered as a social menace in our society.

Conclusion

This study reveals that RSV is an important viral cause of ALRTIs necessitating hospital admission in children under the age of five years. Age distribution showed that RSV-ALRTI occurred significantly in children ages 2-6 months in this study.

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