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EVALUATION OF **IgG** ANTIBODIES AGAINST RESPIRATORY SYNCYTIAL VIRUS (RSV), AND ASSOCIATED RISK FACTORS FOR SEVERE RESPIRATORY TRACT INFECTIONS IN PRE- SCHOOL CHILDREN IN NORTH-CENTRAL, NIGERIA.

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Abstract

Background: Childhood mortality and morbidity due to RSV is increasing. Our current study was aimed at determining the sero-prevalence rate of RSV IgG antibodies and investigates certain known risk factors for RSV disease severity in infants and pre-school children presenting with various forms of respiratory tract infections in Ilorin, Nigeria.
Materials and Methods: About 280, children and 30, aged matched controls were enrolled into the study at the specialist hospital Ilorin. Blood testing for anti RSV IgG was done using a commercial ELISA kit by IVD Research Inc® Carlsbad. California U.S.A. Information regarding Nutritional status, socio-economic status and other demographic variables were collected.
Results: A prevalence rate of 85.7% was recorded among tested children and 23.3%, in controls, across age groups and gender. A statistically significant difference in age groups were recorded among patients with LRTI, ($p < 0.05$), age <1 41%, age 1 <5, 27.6%. This was also the case for children with SRTI (Pneumonia and Bronchiolitis), with age < 1yr, 9%, and 1 <5yr, 19.8%. Analysed risk factors for disease severity showed thatnutritional status of children were statistically significant for disease severity, p-value, 0.039 (Chi square test).
Conclusions: We report a high level of exposure to RSV in infancy and early childhood among children from a representative population in a major central Nigerian City, further studies into neutralising antibody levels and subtype distribution of RSV are advocated.

Key Words: RSV, Respiratory tract infection, Seroprevalence, Ilorin.

Introduction

Respiratory tract infections are serious medical conditions in both man and animals. Majority of the agents responsible for respiratory tract infections are Viruses, these infections are more common and severe among children and infants, although the elderly patients are also severely afflicted. There are about 200, human respiratory viruses failing mainly within six families, with Paramyxoviridea and Orthomyxoviridea being the most important. Together viruses falling within these 2, families **are** responsible for the over 1 million annual infections globally (White and Fenner 2007). Respiratory syncytial virus (RSV), is a member of the family Paramyxoviridea, it is an enveloped virus with a single stranded positive sense RNA genome (Collins and Crowe, 2007). RSV was first isolated in 1959, from a chimpanzee and was subsequently shown to be of human origin and the cause of serious paediatric respiratory tract disease (Collins and Crowe, 2007). Approximately two-thirds of infants are infected with RSV during their first year of life and 90% of these infants will be re-infected at least once by age 2 (Hull, 2007). Disease severity with RSV is very variable, for instance of 50% infected infants >1yr only 3% are Hospitalised, of the RSV hospitalised children only 10% require mechanical ventilation (DeVincenzoo 2007, Fhoda et al., 2007).

In a 13, year prospective study of Infants and children in the U.S.A, RSV was detected in 43%, 25% 11% of hospitalisations for Bronchiolitis, Pneumonia, and Bronchitis (McNamara 2002). Risk factors for infection with RSV in children and young infants include low birth weight, young age of less than 6months, congenital heart disease, and Immunodeficiency/immuno-supression (Sommer et al., 2011). Previous studies have also indicated that seasonal changes can also contribute to increased incidence of RSV infections in temperate regions, although in tropical countries there is no specific seasonal pattern in RSV incidence (Stenabale et al., 2009). In Nigeria previous studies have highlighted the Medical importance of RSV in the general population (Akinloye et al., 2011, Gbadero et al., 1995). There is however insufficient information on the level of RSV infection and disease severity in our various hospitals and Medical institutions, although a recent study has revealed the presence of RSV in children with severe RTI in South west Nigeria (Akinloye et al., 2011). No report has evaluated RSV infection in the Northern part of the country where environmental conditions are harsher and could promote the spread of air-borne infections. We have therefore conducted this study to determine the prevalence of previous RSV infection and associated risk factors for infection in children with various RTI in Ilorin Northern, Nigeria.

Materials and Methods

Study site and Study design

The study is a prospective evaluation of RTI, conducted at the Paediatrics department of the Specialist hospital Ilorin, Kwara State, Nigeria, from November 2010 to June 2011. The Hospital is centrally located and serves as a referral centre for the general populace of Kwara State, and border towns of neighbouring States of Niger and Kogi states. The study is a prospective study of fewer than 5 children presenting with various form of mild to severe respiratory infections.

Patients and case definitions

Children were enrolled in this study based on the following criteria; less than 5 years as at the time of study enrolment. Infants between the ages 6 months, and below were excluded because of the possibility of the presence of maternally derived antibodies against RSV, which might serve as a confounding factor in this study. Thirty healthy children without any documented history of lower respiratory tract infection or disease presentation consistent with RSV infection were selected as control subjects. For the study population, enrolment was based on presentation with acute respiratory tract infection in line with case definitions determined by the physicians of the Paediatrics department of the Specialist hospital Ilorin, absence of any congenital disease including low birth weight. Children born to HIV sero-positive mothers were also excluded from the study. Case definitions for each respiratory tract infection were determined by clinical charts drawn by specialist paediatricians at the study site. However for this study, the various clinical conditions were stratified into groups, namely Upper respiratory tract infection

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(URTI), which constituted infections such as dry cough, Rhinitis and sore throat and Pharyngitis. Lower respiratory tract infection (LRTI) which included, Laryngitis, Bronchitis and Laryngo-tracheobronchitis (Croup), and lastly Bronchiolitis, and Pneumonia which were classified as severe Lower respiratory tract infection (SLRTI).

Sample collection and processing

Enrolled children were bled aseptically for about 3 mls of venous blood which was drawn into anticoagulant bottles. Samples were separated by low speed centrifugation and plasma was all quoted and stored at -20^{O}C until further testing. Plasma samples were tested for the presence of anti-RSV specific IgG antibodies by ELISA, with a commercial ELISA kit by IVD Research Inc® Carlsbad, California U.S.A. Assay for IgG antibodies was done following manufacturer's instructions with all the washing steps done using a tween-80/PBS based washing buffer, IgG was bound by a horse radish peroxidise anti-human IgG conjugate. After colour development with the substrate the Micro-titre plates were read at 450nm wave length with a Micro-plate reader (SEAC®, Radium Inc). Controls were read first before the test results and results were interpreted according to manufacturer's instructions.

Statistical analysis

Data generated was sorted averaged and organised into tables using descriptive statistical tools, statistical associations were done using, chi square test for parametric variables and a p-value of less 0.05 ($p< 0.05$) was set as significant.

Ethical issues

The study was approved by the Nigerian Institute of Medical research Institutional Review board (N.I.M.R. - IRB). Approval was also given by the study site Institution, written or oral informed consent was taken from the wards of the children before enrolment into the study.

Results

A total of 310, children were enrolled during the study period, consisting 280, subjects and 30 control subjects, age range 6, months to 5, years, median age 2, years. RSV IgG antibody test revealed that all the children tested positive 240 (85.7%), while 7(23.3%) of controls tested positive. Age and gender distribution of antibody positive children are shown in table 1. Clinical presentations of tested children were stratified according to the various case definitions for each RTI condition and grouped into the various sub groups as shown in table 2, with children with URTI showing the highest occurrence rate.

Seroprevalence and demography

Prevalence of anti RSV IgG antibodies was tested and 85.7% positivity was recorded for all tested children in both genders and age groups the details of which are highlighted in Table 1, below.

Table 1: Gender and age range distribution of RSV IgG antibodies among under 5 children in Ilorin

Gender	**Subjects**		**Controls**		**P value**
	No tested	RSV pos (%)	No tested	RSV pos (%)	
Male	178	163(91.6)	15	5(33.3)	P <0.05
Female	102	77(75.5)	15	2(13.3)	
Age					
>1yr	88	74(84.1)	4	0(0)	P <0.05
1<5 yrs	192	166(86.4)	26	7(27)	
Total	**280**	**240(85.7)**	**30**	**7(23.3)**	**P <0.05**

Disease severity in relation to age of presentation

The relationship between type of RTI disease presentation and age range was investigated in our current study. For the sake of convenience and ease of analysis of data, we have stratified the various clinical forms of RTI presentation into 3 major sub-groups. Results show that URTI consisting of, Rhinitis, nasal congestion, Tonsillitis, and Pharyngitis, recorded the highest distribution in both age ranges, with <1yr having 50 %(n=88), and 1 <5, 52.6 %(n=152). A statistically significant difference was recorded among patients presenting with LRTI, (p <0.05) age <1 41% (n=88) and 1 <5, 27.6 %(n=152). This was also the case for patients who presented with SRTI (Pneumonia and Bronchiolitis), with < 1, 9%, and 1 <5, 19.8%, as shown in Table 2.

Table 2: Distribution of various cases of RTI in relation to age range among RSV seropositive under 5 children

Disease case	**Age range (< 1)**	**Age range (1 <5)**
URTI	44 (50%)	80 (52.6%)
LRTI	36 (41%)	42 (27.6%)
SLRTI	8 (9%)	30 (19.8%)
Total	**88 (100%)**	**152 (100%)**

Results of various risk factors for RSV infection and RTI disease severity in less than 5 children in Ilorin, Nigeria.

Analysis of selected risk factors for RSV disease severity revealed that gender was not a statistically significant risk factor for RSV disease severity with a P value of 0.712 ($P < 0.05$ X^2) males recorded a distribution of 61.2% disease severity. Nutritional status of enrolled children revealed that RSV associated disease severity was statistically significant in malnourished children with a distribution of 57% and P value of

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0.039 ($P < 0.05$ X^2), economic status and duration of breast feeding were not statistically significant risk factors for RSV related RTI disease severity, P values of 0.142 and 0.334 respectively.

Table 3: Table showing associations between some risk factors and RSV related RTI disease severity in seropositive pre-school children in Ilorin, Nigeria.

Risk factor	Disease severity		P value X^2
	Non severe(n=124)	Severe(n=116)	
Male	73(59%)	75(61.2%)	0.712
Female	51(41%)	41(38.8%)	
Malnourished	54(43.5%)	66(57%)	0.039
Nourished	68(56.5%)	50(43%)	
Mod/High income	52(41.9%)	38(32.8%)	0.142
Low income	72(58.1%)	78(67.2%)	
Breast feeding > 6mths	59(47.6%)	48(41.4%)	0.334
Breast feeding < 6mths	65(52.4%)	68(58.6%)	

N.B: Severe disease includes all lower respiratory tract infections, including pneumonia and Tonsillitis. While non severe constitutes of all other upper respiratory tract infections.

Discussion

Respiratory syncytial virus is the commonest cause of severe respiratory tract infection in infants and children with 76% of children being re-infected by the second year of life (Bhattarakosol et al., 2003, Groothius et al., 1993). In Africa previous studies on RSV infection have demonstrated a significant and a high seroprevailence of IgG antibodies in healthy children (Rocca et al., 2003). In our study we reported a 85.7% seroprevailence rate of anti RSV antibodies among the study population as compared to 23.7% in age matched controls; this is in concordance to previous reports (Bhattarakosol et al., 2003). Previous studies have also highlighted the protective role of maternally acquired antibodies in primary RSV infection (Bhattarakosol et al., 2003). Presence of RSV antibodies alone is not sufficient to protect against re-infection although high antibody titres have been shown to significantly decrease the rate of RSV hospitalisations and disease severity (Doroudchi eta al., 2004, Legg et al., 2002). Both humoral and cellular immunity are however required for efficient protection from RSV infection. Our results highlight a high level of RSV infection even among young infants who otherwise are likely to be less exposed to respiratory tract infections. This indicates a high level of RSV circulation and transmission among inhabitants of Ilorin; although this was not independently investigated it should be further investigated. Age range distribution and RTI disease severity revealed that age 1<5 yrs recorded a higher occurrence rate of RSV related SRTI as compared to <1 yr old children (Table 2), this observed trend in disease severity is not agreement with previously reported studies majority recording a higher rate of hospitalisation and disease severity in children less than six months (Fodha et al., 2002, Lanari et al.,2002).Infection with RSV before 3 months of age has been associated with higher severity of Low respiratory tract disease (Nielsen et al., 2003), although our study did not reveal actual RSV infections, clinical diagnosis which was the basis for our disease stratification indicates a higher probability of occurrence of RSV infections in children 1>5 yrs in our study setting. This might be attributed to the fact that there was efficient transfer of maternally derived antibodies which was protective to initial RSV infections in enrolled infant of between 6 months and 12 months of age which constituted a significant number in the total number of enrolled children. It has been hypothesised that antibody levels above 9 log 2 are protective against RSV infections (Glezen et al., 1996, Suara et al., 1996). One limitation to our study was our inability to demonstrate geometric mean titre of RSV IgG antibodies among our tested participants, this is however regretted and further study into representative levels of neutralising antibodies against RSV is hereby recommended.

We analysed selected risk factors that have been previously reported by several workers for RSV associated disease severity (Blomers, 2007, Suara et al., 1996). Risk factors analysed includes; male gender, socioeconomic class, and Nutritional status of children as at time of disease onset, and duration of breastfeeding during infancy. From the generated data, Male risk factor was not statistically significant in relation to RTI disease severity P-value 0.712 (Chi square). Males recorded a distribution of 61.2% of SRTI table 3, this result not in agreement to various other reports indicating a higher degree of hospitalisations and attack of RSV infection in male children, (Sommer et al., 2011), this observation has been attributed to the anatomical nature of the male trachea, which has been suggested to be shorter and narrower than that of the females and was substantiated in the Canadian PICNIC study (Law et al., 2004), this hypothesis is however not universally accepted (Figueras-Aloy et al., 2004). Results on nutritional status and RSV related disease severity revealed that, Malnourished children suffered most from SLRTI and disease severity, with a distribution of 66/57% (n=119) and P-value 0.039 (Chi square test) this observation is again also expected as nutritional status directly influences immune status and protein energy malnutrition is directly linked to a lack of sufficient protein in diet (Salimonu 2004). Socioeconomic status was also investigated and found to deviate from expected values as P-value was not statistically significant (0.142 [Chi square]), although there was a higher rate of disease severity in the Lower economic class it was not statistically significant, this might be due to improved personal hygiene or reduction in birth rate and average family size in Nigeria due to public enlightenment and social factors. Duration of breast feeding of enrolled children in relation to disease severity RSV IgG positive children was also investigated and results revealed a non statically significant relationship P-value (0.334) Chi square test. This is not in agreement to past reports that investigated this risk factor in RSV infected children, for instance the Spanish FLIP study indicated breastfeeding > 2months a significant protective factor against RSV infection (Figueras-Aloy et al., 2004) although breastfeeding alone is not sufficient to protect against Primary RSV infection in neonates and young infants (Simores 2003, Sommer et al., 2011).

Conclusion

In conclusion, we report a high level of exposure to RSV in infancy and early childhood among children from a representative population in central Nigeria, there is also evidence of substantive Herd immunity in these children, characterised by 85.7% seroprevailence to anti-RSV IgG antibodies with a statistically significant difference in controls ($p<0.05$). Our inability to determine the titre of neutralising

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antibodies is however regretted; further study into the geometric mean titre of neutralizing antibodies to RSV in both hospitalised and healthy children is hereby advocated. Risk factors for disease severity revealed that nutritional status was a significant factor for disease severity. Further research into RSV subtype distribution and LRTI disease is also advocated in response to global advancement in vaccine development.

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