SEROEPIDEMIOLOGIC STUDY OF HUMAN PARAINFLUENZA VIRUSES (HPIVS) AMONG CHILDREN IN ILORIN, NORTH CENTRAL NIGERIA

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Abstract: Respiratory infection is an important public health problem owing to their high incidence and ease of spread in the community and they impact significantly on health worldwide. This study was conducted to determine the sero-prevalence rate of HPIVs 1, 2 and 3 IgG antibodies and investigate certain risk factors for acquisition of infection in children between the ages of 1 – 10 years presenting with various forms of respiratory tract infections in Ilorin, Nigeria. 200 children were enrolled into the study at University of Ilorin Teaching Hospital. Blood testing for anti HPIVs IgG was done using the commercial ELISA Diagnostic kit by R-Biopharm AG, 62927 Darmstadt Germany. A prevalence rate of 72.5% (140/200) was recorded among the tested children. Relevant demographic data and risk factors were. Age, educational level, day care attendance, residential location, presence family member with respiratory tract infection and presence of catarrh were significantly associated with the infection ($p<0.05$). Observation from the study showed a high level of exposure to HPIVs in infancy and early childhood among children from a representative population in major central Nigerian City. This indicates the importance of HPIVs as an agent of respiratory tract infection in children. Further studies into quantifying the antibody and distribution of subtypes are being advocated for. Also, as vaccines and drugs against HPIVs are not available in the country as of present, preventive measures should be encouraged and strictly adhered to in the control of the infection.

Keywords: Human Parainfluenza virus, children, antiviral, Nigeria

Introduction

Infections of the respiratory tract exert a major impact on health globally and most of which have viruses as their etiological agents. Viral respiratory infections may provoke a severe illness in individual with chronic medical conditions especially the very young and older individuals (Hayden *et al*., 2006); children predominantly suffer from acute otitis, sinusitis or pneumonia. Among viruses implicated in most respiratory tract infections are influenza A and B viruses, Enteroviruses, Parainfluenza viruses, Respiratory Syncytial viruses, Adenovirus and Rhinovirus (Allander *et al.*, 2007). In Nigeria, acute respiratory infections are the major cause of hospital admission and death in children. A report from a prospective cohort study conducted in Ilorin, Nigeria, it was shown that per year, the rate of acute respiratory infection per child was at least three episodes (Fabule *et al.,* 1994). As observed in most countries, seasonal variation exists in respiratory tract infections with more occurrences seen during the rainy season (Johnson *et al.,* 1996).

Of the aforementioned viruses, Human Parainfluenza Virus (HPIV) is the second leading causes of acute lower respiratory tracts infection (Karron *et al.,* 1993). Changes in airway immunity and integrity are important factors that play a role in the pathogenesis of this infection, especially in vulnerable groups such as neonates, premature infants, people with congenital or acquired immunodeficiencies and people with cardiac or respiratory condition (Meissner *et al.,* 2004). Effective preventive and therapeutic options are not available for respiratory infection caused by HPIV. Worldwide, HPIV types 1, 2 and 3 are responsible for majority of childhood cases of croup, bronchiolitis, and pneumonia (Collins *et al.,* 1996). HPIV type 3 alone is accountable for 11% of respiratory hospitalization in the United States Paediatrics (Chanock *et al.,* 1990). Also, according to Weinberg *et al.* (2009), in children less than 5 years, HPIV is responsible for 6.8% of all hospital admissions as a result of fever, acute respiratory illnesses or both.

Diagnosis of acute respiratory tract infection however has been made difficult owing to the overlapping of its symptoms (cough and difficult/fast breathing) with that of malaria. In Nigeria for example, minors with these symptoms are often ignored by home management strategy focused on treating fevers such as malaria (Ukwaja *et al.,* 2010). Although respiratory viral infections and their impact on health care in developed countries are well understood, there is a dearth of information on the burden of respiratory viral infections in developing countries. From the public health perspective, it would be of immense value to know the viral causative agents, symptoms, frequency and prevention of these infections (Rudan and Conseus, 2005). No report has listed HPIV infection in Ilorin, a north central region of Nigeria despite the existence of harsh conditions that could promote the spread of air borne infections. It is therefore the objective of this study to determine the prevalence of previous HPIVs infection and associated risk factors for infection in among children with various RTI in Ilorin, North Central Nigeria.

Materials and Methods

Study site and study design

The study is a cross -sectional evaluation of RTI, conducted at the Peadiatrics department of the University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria from January 2015 to December 2016. The Hospital 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 children between the ages of 2 months and 10 years presenting with various form of mild to severe respiratory infection. A well-structured questionnaire was administered and patient's demographic data was collected. The questionnaire was used to gather socio-demographic and risk factors for infection. The sample size was determined using Fischer's formula (Araoye, 2003).

Sample collection and processing

Enrolled children were bled aseptically for about 5 mls of venous blood which was drawn into labelled sterile screw cap sample bottles and allowed to clot. Samples were separated by centrifugation and sera were separated. Sera storage was done at -20^{o}C until enough samples were collected for assay.

HPIV IgG assay procedures

This was achieved by the use of Enzyme-Linked Immunosorbent Assay (ELISA) kit by Cortez Diagnostics,

Inc., 21250, Woodlands Hills, California 91367, USA. This is a glycoprotein G-based enzyme-linked immunosorbent assay technique and test result was qualitative. All specimens and kit reagents were allowed to assume 25^0C (room temperature) and gently mixed before use. The laboratory procedure was performed following the manufacturer's instructions with all washing steps done using tween-80/PBS based washing buffer, IgG was bound by a horse radish-peroxidase anti-human IgG conjugate. After colour development with the substrate the Micro-titre plates were read at wave length of 450 nm with a Micro-plate reader (Sigma Diagnostics). Controls were read first before the test results and results were interpreted according to manufacturer's instructions.

Ethical issues

Ethical clearance for the study was obtained from the Ethical Review Committee (ERC) of University of Ilorin Teaching Hospital, and the children's caregivers gave their consent.

Data analysis

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

Results and Discussion

The ELISA result showed that 140 (70%) samples tested positive to Human Parainfluenza Virus IgG antibody while 60 (30%) samples tested negative to HPIV IgG antibody. Hence, the total seroprevalence of 70% was observed among the tested children. One hundred and twenty four (124) males were tested and 90 (64.3%) were observed to be HPIV seropositive, while 50 (35.7%) females were HPIV seropositive out of the 76 that were tested. No statistical association was observed ($p>0.05$). A statistical significant was recorded with respect to age of the subjects ($p= 0.002$). The mean age of the study subjects was 3.5 years, and age group 37-48 months had the highest seroprevalence of 32/35 (91.4%), followed closely by the age group 49-60 months with seroprevalence of 19/21 (90.5%). Those in the nursery school had the highest seroprevalence of 77.3% and a significant statistical association was observed ($p= 0.032$) (Table 1).

It was observed that there was significant association ($p = 0.009$) between residential area and the HPIV infection, with those residing in the rural settlements having the highest seroprevalence of 70.8%. Though there was no significant association ($\chi2 = 2.489$, $p = 0.115$), children whose residential home is very close to a livestock settlement had a higher prevalence rate of infection (74.8%) as compared with those whose house are not close. Household size was not significantly associated ($\chi2 = 6.716$, $p = 0.082$) but households with above 6 persons had higher (90.5%) infection rate compared to those with 1-2 persons where the infection rate was 63.8% (Table 2).

Other risk factors assessed include attendance of day care, duration of breastfeeding, family member with symptoms of respiratory tract infection, whether or not parents smoke. There was no significant association between exclusive breastfeeding and human parainfluenza virus infection in children (Table 2). Table 2 further shows significant association ($p = 0.017$) between day care attendance and the infection with HPIV. Children whose parent smoke had 100% infection rate when compared to those of non-smoking parents (68.6%) and a statistical significance was observed ($p= 0.044$). Also, subjects with family members with respiratory tract infection had a higher positivity rate and a statistical association was recorded (Table 2).

Of the total number of 200 children tested, 168 (84%) had symptoms of catarrh/running nose and 32 (16%) had no symptoms of catarrh/running nose. Sero-positivity was observed in 155 (92.3%) of those with catarrh/running nose and 25 (78.1%) of those without catarrh/running nose. This was statistically significant ($X^2 = 7.398$; $P=0.025$) (Table 4). A total of 192 (96%) had cough while 8 (4%) had no cough. Sero-positivity was observed in 135 (70.3%), this was however not statistically significant ($X^2 =0.223$; $P>0.05$) (Table 3).

Table 1: Association of demographic data with human parainfluenza virus infection in children

Age group (Months)	No. of Children screened	No. of sero-positive (%)	% sero-positive per group	% sero-positive per total	$\chi2$ (P value)
0-12	33	20	60.61	10	24.632 (0.002*)
13-24	44	25	58.81	12.5	
25-36	44	28	63.40	14	
37-48	35	32	91.40	16	
49-60	21	19	90.47	9.5	
61-72	15	13	86.70	6.5	
73-84	8	3	37.50	1.5	
85-96	0	0	0	0	
97-108	0	0	0	0	
109-120	0	0	0	0	
Gender					
Female	76	50	67.79	25	1.035 (0.309)
Male	124	90	72.58	45	
Educational level					
Pre nursery	83	50	60.2	25	8.798 (0.032*)
Nursery	88	68	77.3	34	
Primary	29	22	75.9	11	
Secondary	0	0	0	0	
Total	**200**	**140**		**70.0**	

$\chi2$ = chi-square; p = level of significance; * = significant association exists at $p < 0.05$

Table 2: Associations between some risk factors and human parainfluenza virus infection

Parameter	No. Tested	No. of seropositive	% sero-positive per group	% sero-positive per total	χ2 (P value)
Residence					
Urban	176	123	69.9	61.5	0.924(0.009*)
Rural	24	17	70.8	8.5	
Proximity to livestock settlement					
Close	107	80	74.8	40	2.489 (0.115)
Not close	93	60	64.5	30	
Household size					
1-2	69	44	63.8	22	6.716 (0.082)
3-4	75	55	73.3	27.5	
5-6	35	22	62.9	11	
Above 6	21	19	90.5	9.5	
Attendance of daycare					
Yes	72	43	59.7	21.5	5.569(0.017*)
No	128	97	75.8	48.5	
Exclusive breast feeding					
Yes	161	117	72.7	58.5	2.805 (0.094)
No	39	23	59.0	11.5	
Family member with symptoms of RTI					
Present	138	92	66.7	46	8.254(0.016*)
Absent	60	48	80	24	
Parental smoking					
Yes	9	9	100	4.5	4.039 (0.044)
No	191	131	68.6	65.5	
Total	**200**	**140**		**70.0**	

χ2 = chi square; p = level of significance; * = significant association exists at p < 0.05

Table 3: Distribution of HPIV antibody based on the symptom of catarrh/running nose and cough

Symptoms	No. of Children screened	No. of seropositive (%)	% sero-positive per group	% sero-positive per total	χ2 (P value)
Catarrh/Running Nose					
Present	168	115	68.5	57.5	7.398 (0.025*)
Absent	32	25	78.1	12.5	
Cough					
Present	192	135	70.3	67.5	0.223 (0.637)
Absent	8	5	62.5	2.5	
Total	**200**	**140**		**70.0**	

χ2 = chi square; p = level of significance; * = significant association exists at p < 0.05

HPIV causes several serious respiratory diseases in children for whom there is no effective prevention or therapy. In addition to the common association of HPIV with croup and bronchiolitis, HPIV is associated with uncomplicated upper respiratory tract infections in adults, serious lower respiratory tract infections in infants, and non-respiratory tract infections in children and adults. HPIV types 1, 2, and 3, cause the majority of childhood cases of croup, bronchiolitis, and worldwide. PIV3 alone is responsible for approximately 11% of pediatric respiratory hospitalizations in the US and is the predominant cause of croup in young infants. In our study we recorded a 70% seroprevalence rate of anti HPVs antibodies among the study population, this is agreement with previous reports of Bonire *et al*. (2015) conducted in Kaduna Nigeria where a slightly higher prevalence of 76.6% was recorded, Glezen and Denny (1997) which showed that 75% of children aged 5 years had antibodies to HPIV-1 and 2 as well as that of Akinloye *et al.* (2011) in Ibadan who observed that 80% of children have antibodies to the virus by ten years of age. However, the prevalence recorded in this study is in discordance with the results of Sale *et al*. (2010) which showed a prevalence of 46.4% among children aged 1 – 5 years in Zaria, Kaduna and also with the 11.8% prevalence rate reported by Calvo *et al*. (2011) in Spain with prevalence 11.8%. This recorded prevalence can be due to the fact study was carried out during the Harmattan (equivalent to the winter season) which usually is the peak period of the infection in children especially (Ray, 2004). The results highlight a high level of HPV infection even among young infants who otherwise are less exposed to respiratory tract infections; this is an indication of a high level of HPIV circulation and transmission among Ilorin inhabitants. The distribution of HPIV according to age indicates that age group 37-48 months (91.40%) had the highest seroprevalence and was closely followed by 49-60 months age group (90.47%) (P=0.002). This finding show that age had a significant association with HPIV infection, and this is in agreement with the submission of Bonire *et al*. (2015).

The study also revealed that there was increase in seropositivity with age which could be as a result of reinfection that occurs in the presence of antibodies elicited by an earlier infection. As there in no permanent immunity to HPIV infection, the earlier elicited antibodies only modify the disease, hence reinfection usually present simply as nonfebrile

upper respiratory infection. This in consonance with the findings of Brooks *et al.* (2007) as well as that of Sale *et al.* (2010) and Bonire *et al*. (2015). Infection with the human paramyxoviruses such as the HPIVs can occur throughout life Hall, however subsequent infections are usually mild or sub clinical unlike primary infection in the very young (Hall, 2001). The mechanism behind the reinfection with these viruses has been attributed to the incomplete and diminishing immunity that develops after primary infection (Okamoto *et al*., 2010).

From the data generated in this study with respect to gender, more male subjects were enrolled and a higher seropositivity was observed as compared to the female subject although no statistical association was observed. This observation is supported by the earlier observations by researchers stating that a higher degree of hospitalizations and attack of respiratory infection in male as a result of the shorter and narrower trachea in male as compared with female (Sommer *et al*., 2011). With respect to the educational level of the tested children, a statistical association was observed, with higher seropositivity among those in the nursery and primary level. This may possibly be explained by the fact that some of these children may have come in contact with other children in their schools who could have been actively secreting the virus and so infection can easily be transmitted amongst the subjects since HPIV infection is transmitted via contact and air particles (Burke *et al*., 2013).

Selected risk factors that have been previously reported by several workers for HPIV infection was analysed. These include; residential location, exclusive breast feeding, household size, proximity to livestock settlement, family member with respiratory tract infection, attendance of daycare, parental smoking. A statistical association was observed between the distribution HPIV with respect to residence in rural setting, daycare attendance, parental smoking and presence of family member with respiratory tract infection. Children who reside in the rural settings had a higher infection rate when compared to those in the urban setting. This agreed with earlier reported findings that geographic and socioeconomic difference impacts on rates of infection in various populations (Burke *et al*., 2013). The test of association of family member with respiratory tract infection with the infection showed there was a significant association, with children with family member recently diagnosed with respiratory tract infection having a higher prevalence. This might be so as most homes are not mindful of methods of controlling the transmission of the virus and as such, disinfection, proper hygienic practices and environmental control of short-range transmission are not strictly adhered to.

Although, household size was not significantly associated, households with more than 6 persons had higher (90.5%) infection rate compared to those with 1-6 persons where the infection rate was lower. This is suggestive of the fact that overcrowding results in indoor air pollution. This result agrees with that of Rudan *et al.* (2008) and Bonire *et al*. (2015). Attendance of daycare and the infection was statistically significant. This result shows that the virus is easily transmitted among younger children who freely mix symptomatic children and in the process get infected as well. Exclusive breastfeeding had no statistical association with infection. This could be due to the fact that a larger number of the children involved in the study were exclusively breast fed. Also this shows that the action of maternal antibodies in children for the first six months of life does not have enough protective immunity against the acquisition of HPIV and also the protectiveness diminishes as they get older (Schomacker *et al*., 2012). Children of parents that smoke had higher infection with human parainfluenza virus than those of non-smoking parents though smoking and a statistically significant association with the infection was observed. This is in consonance with the findings of Laurichesses *et al* (1999) who identified environmental smoke as a predisposing factor to HPIV infection. A significant association was observed between symptoms of catarrh and Parainfluenza infection with those with catarrh having a higher prevalence. The result agrees with that of Brooks *et al.* (2007) and Bonire *et al*. (2015). This is however in contrast with the observation of Sale *et al.* (2010) whose findings showed that respiratory symptom is not statistically significant to HPIV infection. Parainfluenza infection is a respiratory tract infection and as such patients usually show respiratory symptoms such as cold, cough and catarrh. Also a large proportion of the children enrolled in the study had respiratory symptoms because most of the samples were gotten in the Harmattan season which resulted to the high seropositivity. Test of association revealed that there was no significant association between cough and HPIV infection. This is in agreement with the work of Sale *et al*. (2010). The symptom of cough could be as a result of other microbial agents.

Conclusion

The study we report a high level of exposure to Human Parainfluenza Virus in infancy and early childhood among children from a representative population in North Central Nigeria, there is also evidence of substantive Herd immunity in these children characterised by 70% seroprevalence to anti-HPV IgG antibodies. Age is an important demographic and risk factor of Human Parainfluenza Infection in children. This study suggests the need for prompt and easily methods of diagnosis. Also there is need for mass surveillance in order to device public intervention strategies to reduce the rate of HPIV infection among children.

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