Prevalence of Human Rhinovirus Infection in Children with Acute Respiratory Symptoms in Ilorin, Nigeria

Type of Article: Original

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ABSTRACT

BACKGROUND

Human rhinoviruses are positive-strand RNA, non-enveloped virus detected mostly in the early phase of infection showing symptoms in children experiencing mild upper respiratory tract infections.

METHOD

In this study, 200 patients were screened for rhinovirus infection using Human Rhinovirus antigen (RhV-Ag) Elisa Kit (MBS269914).

RESULTS

Demographic characteristics revealed that the prevalence of rhinovirus infection in children showed 38% positivity of which 20 (10.0%) were males while 17 (8.5%) were females. Children between the ages of 0 – 24 months have the highest prevalence of 45.9% while those older than 96 months have the least prevalence of 5.4%. No significant difference was observed between the genders and rhinovirus infection (p = 0.622). A total of 54.0%, 2.7%, 29.7% and 13.5% of the children attend daycare, crèche, nursery and primary school respectively. A total of 140 (70%) in the urban recorded a positivity value of 11.0% and 59.0% negativity as against 60 (30%) who lived in the rural area with a value of 7.5% positivity and 22.5% negativity. Forty (20.0%) of the tested subjects had genotype AA out of which 6 (3.0%) was positive for rhinovirus infection, the remaining 34 (17.0%) were negative for the rhinovirus infection.

CONCLUSION

This study established the detection of rhinovirus infection in children attending the pediatrics clinic in Ilorin. This may become useful for diagnosing respiratory illness in high-risk populations with immune compromised individuals.

KEYWORDS

Rhinovirus, ELISA, prevalence, risk factors, Human RhV-Ag, Ilorin

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INTRODUCTION

Human rhinoviruses are positive-strand RNA, non-enveloped virus detected mostly in the early phase of infection showing symptoms in children experiencing mild upper respiratory tract infections. Rhinoviruses cause approximately two-thirds of cases of the common cold and are probably responsible for more human infections than any other agent[1]. Rhinoviruses have been associated with asthma exacerbations and decompensation in chronic lung disease, sinusitis, otitis media, LRTIs and wheezing in young children, adults[2-6] and immune compromised individuals[7].

Rhinovirus viremia has been detected by RT-PCR in 11.4% of young children and 25% of children with rhinovirus-associated asthma

exacerbation[8,9]. According to Chung et al.,[10], Rhinoviruses are often the more prevalent virus detected in children with acute respiratory disease.

Miller et al.,[8] and Winther et al., [11]reported the detection of rhinovirus as a major respiratory virus in hospitalized children with an average number of 6 (20%) per year being asymptomatic. Another study in Belgium reported that NASBA and RT-PCR produced comparable results than culture specimens from hospitalized children[12,13].

In Africa, Heidi et al.,[14] reported wheezy illness as an important cause of morbidity in children. Investigations revealed that RV-A subtype have been frequently identified mainly in children with bronchitis and Community Acquired Pneumonia in Burundi, Central Africa[15].

Oluwabukola et al.,[16] found all major groups of viruses in association with respiratory illnesses in young children of West Africa. Several other studies have also implicated respiratory tract viruses in Nigerian patients using both traditional diagnostic techniques and modern molecular techniques[17,18,19].

This study was carried out using molecular method to determine the prevalence of Human Rhinovirus infection in Children attending the Pediatrics Clinics. In addition, we sought to determine the contribution of such molecular tests, especially PCR and ELISA, to substantiate the roles of Human Rhinovirus as a viral agent in acute respiratory disease in children. Early detection may become useful for diagnosing respiratory illness in high-risk populations such as immuno compromised individuals. For now, the relevant statistics are not available on the Prevalence of Human Rhinovirus Infection in Children attending the Pediatrics Clinic in Nigeria and especially in Ilorin, Kwara State. Therefore, this study provides a reliable epidemiological data for future researchers in the field of Epidemiology and Community Health.

METHODOLOGY

Nasal swab samples were obtained for rhinovirus screening from 200 children aged below 14 years old attending the Pediatrics Unit of University of Ilorin Teaching Hospital Ilorin, Nigeria with acute respiratory symptoms such as runny nose, mild fever, nasal congestion, cough, sore throat and sneezing. Children were excluded if they are above 14years, or if the parent/guardian did not give their consent. Children with critical health conditions placed under strict observations and those known to have underlying cardiac or chronic pulmonary diseases and daily treatment of oral corticosteroids for more than 2 days prior were also excluded. Demographic and clinical information, including age, sex and clinical symptoms, were documented with the aid of a structured proforma. The study protocol was approved by the Ethical Review Committee (ERC) of the University of Ilorin Teaching Hospital after meeting all the necessary requirement of the Committee. Written informed consent was received from participants; parents provided consent on behalf of children who were unable to respond after clear explanation of the objective and logistics of the study.

Sampling Techniques

Nasal swabs were obtained by inserting a commercially purchased sterile swab into the nostril to a depth of 2-4cm and retracting it in a slow rotating motion, in order to trap epithelial cells in the swab. The nasal swabs were then stored in 1ml of transport medium (Hanks' balanced salt solution containing gelatin, lactalbumin, yeast, and antibiotics) and transported to the laboratory after collection. The Hematology Department, ELISA Unit of the University of Ilorin Teaching Hospital, Ilorin, Kwara State was used for analyses of the samples. The samples were adequately stored and transported in dry ice packs.

Virological analyses

Human Rhinovirus antigen (RhV-Ag) ELISA Kit (MBS269914) was sourced from MY

BIOSOURCE Company (California). The concentrated washing solution and other reagents were strictly prepared based on manufacturer's instruction.

The test strips were removed from zip lock bag and allowed to balance to room temperature. Blank wells were ignored because the dual-wavelength reading plate was used. When color for high concentration of standard curve become darker and color gradient appeared, the hatching was then stopped. The optical density (OD) of the plate was read at 450nm using Automated BIO-RAD microplate Reader within 10mins of reaction.

Results were expressed as an optical density (OD) value (range, 0.06 to 2.5). The smallest value (OD = 0.06) is the same as that for the substrate blank in antibody-coated plates. The cut-off value of positivity was defined as the mean for the negative controls plus five times the standard deviation of the mean. The OD values which were smaller than the mean for the negative controls plus three times the SD value were defined as negative. The OD value of each sample was minus that of blank well and a standard curve was drawn manually. OD reading of samples was used to determine the positivity and negativity value for rhinovirus infection.

Data analysis

Data were analyzed using Statistical package for Social Sciences (SPSS) version 15 (2006). Descriptive statistics such as mean, percentage and proportions were generated. The relationship between continuous variable and outcomes was determined using the Chi square when assumptions are met. Non-parametric test was applied when assumptions are not met.

RESULTS

Two hundred (200) patients were recruited for this study, 92 (46%) were males while 108 (54%) were females. Out of the entire 200 samples collected, 37 (18.5%) samples were positive for rhinovirus while the rest 163 (81.5%) tested negative.

The distribution of rhinovirus infection in the 37 positive samples revealed that 20 (10.0%) of the patients are males while the remaining 17 (8.5%) are females. Out of the 163 negative samples, 72 (36.0%) and 91 (45.5%) were males and females respectively. A p-value of 0.622 revealed that there was no significant difference between the genders and rhinovirus infection (Table 1).

Distribution of the various age groups of children revealed that for both the positive and negative samples, children between the age group 0–24 months have the highest prevalence of 45.9% and 45.5% respectively while those older than 96 months had the least prevalence of 5.4% and 4.0% positivity and negativity respectively. However, there was no significant difference between these ages and rhinovirus infection (p = 0.794).

Eleven percent (11.0%) of those positive for rhinovirus infection resided in the urban area while 7.5% lived in the rural area. 59.0% of those in the urban area and 22.5% of those in the rural area tested negative. However, there was significant difference between the prevalence rate of rhinovirus infection and the residential area of children (p = 0.000).

Table 1: Prevalence of rhinovirus infection in relation to the demographic characteristics of children.

	No. Tested (%)	No. Positive (%)	No. Negative (%)	X	^{2}df	p-value
Sex				0.243	1	0.622
Male	92 (46.0)	20 (10.0)	72 (36.0)			
Female	108 (54.0)	17 (8.5)	91 (45.5)			
Age groups				11.24316		0.794
<=24.0	91 (45.5)	17 (8.5)	74 (37.0)			
24.5 – 48.0	43 (21.5)	6 (3.0)	37 (18.5)			
48.5-72.0	40 (20.0)	8 (4.0)	32 (16.0)			
72.5-96.0	18 (9.0)	4 (2.0)	14 (7.0)			
> 96.5	8 (4.0)	2 (1.0)	6 (3.0)			
Residential Area				22.135	3	0.000
Rural	60 (30.0)	15 (7.5)	118 (59.0)			
Urban	140 (70.0)	22 (11.0)	45 (22.5)			

p<0.05 is significant, p<0.05 is not significant

Figure 1revealed the distribution of the various educational backgrounds of children

in relation to prevalence of rhinovirus infection. A total of 54.0% of the children attends the daycare. As shown on the chart, 2.7%, 29.7% and 13.5% attend the crèche, nursery and primary school respectively.

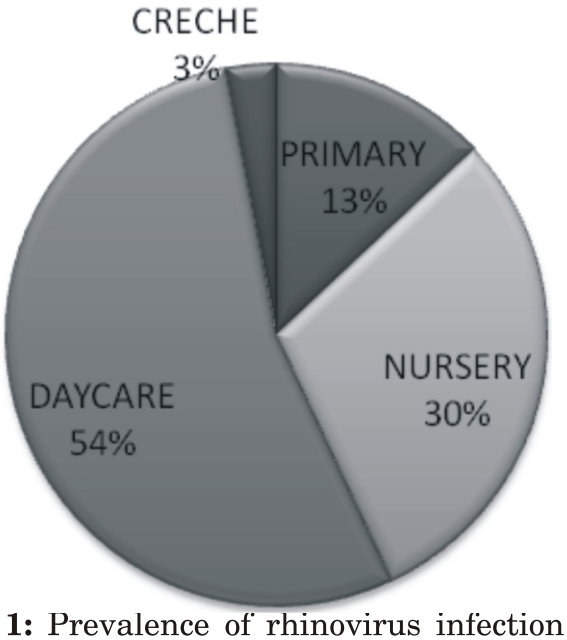

Fig 1: Prevalence of rhinovirus infection in relation to the educational level of children. The prevalence rates of rhinovirus infection in children as it relates to the occupation of parents revealed that out of the 37 positive samples, 48.7% of the fathers were traders as against 64.9% of the mothers (Figure 2).

Children with the highest prevalence of rhinovirus had both parents as traders while those with least prevalence had both parents as artisans.

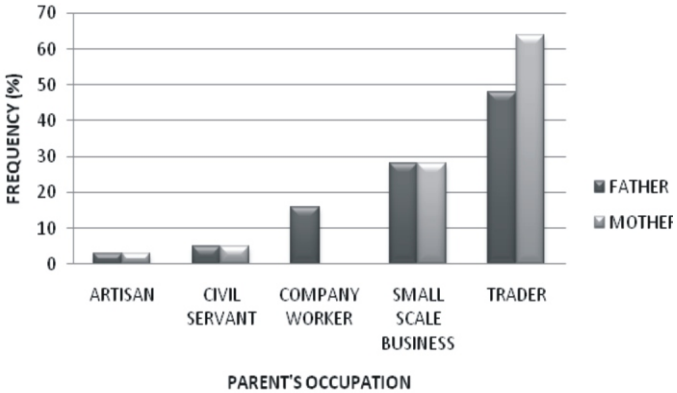

Fig. 2: Prevalence of rhinovirus infection in relation to the occupational distribution of parents.

Only 40 (20.0%) of the tested subjects were AA out of which 6 (3.0%) were positive for rhinovirus infection, the remaining 34 (17.0%) were negative for the rhinovirus infection. Out of those with genotype 'AS' 10 (5.0%)and 'SS' 6 (3.0%)none tested positive to rhinovirus infection. A total of 144 (72.0%) of the participants did not know their genotype out of which 31 (15.5%) tested positive to rhinovirus infection (Table 2). Significance difference was observed between the prevalence rate of rhinovirus infection and genotype of the children (p=0.000). However, only subjects with AA genotype are positive for rhinovirus infection while none of the other genotype AS and SS showed positivity.

Table 2: Genotype of children in relation to the prevalence of rhinovirus infection.

	No. Tested (%)	No. Positive (%)	No. Negative (%)
Genotype			
AA	40 (20.0)	6 (3.0)	34 (17.0)
AS	10 (5.0)	NIL	10 (5.0)
SS	6 (3.0)	NIL	6 (3.0)
UNKNOWN	144 (72.0)	31 (15.5)	113 (56.5)
TOTAL	200	37 (18.5)	163 (81.5)

$p<0.05$ is significant, $p<0.05$ is not significant

DISCUSSION

Much emphasis has been placed on detection of Rhinovirus infection by serological and molecular methods in recent time. In this study nasal swab specimen from 200 patients were collected out of which 37 (18.5%) samples were positive for rhinovirus while the rest 163 (81.5%) tested negative. According to previous reports by Peltola et a[20], about six Rhinovirus-positive index children (75%) and nine Rhinovirus-negative index children (56%) had symptoms of respiratory infection at the point of hospitalization. This report negates the findings from that study[20] and this may be due to the fact that it was not conducted during the high epidemic episodes of Rhinoviruses. As further observed by Peltola et al. [20]rhinoviruses were detected most efficiently during the early phase of infection and the highest amounts of Rhinovirus in nasal washings was obtained during the first 3 days of symptoms, after which, the presence of

Rhinoviruses was no longer correlated with the occurrence of respiratory symptoms. This is further corroborated by Jartti et al.,[21] who disclosed that nasal samples can be positive for Rhinovirus for up to 5 weeks after a symptomatic infection. In this study the observed difference between our findings and those from earlier researches may be because infections with mild symptoms are not readily noticeable in patients. Also, most patients often visit the clinic after taking self-prescribed medications. As observed in this study the positive samples still tested positive to rhinovirus even after two weeks of symptomatic infection.

As revealed in Table 1, there was no significant difference between the genders and Rhinovirus infection. This result showed that whereas more females (108, 54%) than males (92, 46%) children participated in the study, a higher percentage of males showed positivity (10.0%) as against the females (8.5%). This result is in consonance with those of Peltola et al.,[20] who reported a positivity of 10 and 7 in male and female children respectively. He also stated that 7 of the males were symptomatic while only 1 of the female was symptomatic. To the best of our knowledge, no single research has been carried out to determine the relative risk factors responsible for this observation of reported prevalence in genders.

Our study reports the distribution of various age groups of children in relation to prevalence of Rhinovirus infection. Results showed that children in the age group 0-24 months have the highest prevalence of 45.9% while children older than 96 months recorded the least prevalence of 5.4%. However there was no significant difference between these ages and Rhinovirus infection (p=0.794). This findings is in agreement with the report of Heidi et al., [14] who stated that Human Rhinovirus was detected in children, most (72%) of whom were under 2 years of age. Also, Peltolaet al.,[20] and Miller et al.,[8] reported that Rhinovirus-associated hospitalization was more in children 0-5 months old and 6-23 months old respectively. However they reported a significant difference between these ages and Rhinovirus infection at a value of p=0.01. The result obtained may be connected to the impact of environmental condition on this age group of children. Also, the fact that children in this age group may not be well protected from aerosols of respiratory droplets and from direct person-to-person or self-inoculation of the eye or nose may have contributed immensely to their high prevalence.

The prevalence rate for Rhinovirus infection that was observed among the children residing in the urban area (Table 1) may be due to the fact that most patients that visited the hospital during the period of study reside in the urban area and most sub-urban cases reported are not usually documented. However significant difference was observed between the prevalence rate of Rhinovirus infection and the residential area of children (p=0.000). This is in agreement with a US research which showed that urban infants have a different pattern of viral respiratory illness from sub-urban infants; this could help explain why they are more likely to develop asthma[22]. Several researchers have been able to reveal that social economic status played a significant role in the positivity of Rhinovirus infection among patients especially in adults[23,24,25].

In Figure 1, the distribution of the various educational backgrounds of children in relation to prevalence of Rhinovirus infection revealed that 54.0% of the children attended the daycare of which 2.7%, 29.7% and 13.5% attended the crèche, nursery and primary school respectively. This can also be supported by the fact that the children belonging to this educational level falls within the age of enrolment for the respective level. This result is supported by the findings of Peltola et al.,[20] who disclosed that Rhinovirus infections are most frequent in children and that the infection was usually transmitted from school-aged children to other family members.

This study further revealed that parents that are traders have children with highest

prevalence rate of 48.7% and 64.9% for the fathers and mothers respectively. As revealed in Figure 2, 16.2% of fathers were company workers while none of the mothers' worked in a company. Children that had both parents as artisans had the least prevalent rate of 2.7%. The high prevalent rate observed among children that had parents who are traders may be due to the fact that majority of parents seen during the period of recruitment for this study were traders. However, several literatures exist that support social economic status as a risk factor affecting transmission of infections[8,26,27].

Considering the result of the genotype of children recruited for the study, the prevalence of Rhinovirus infection revealed that only 3.0% of the 'AA' genotype children tested positive (Table 2). Among the children with genotype 'AS' and 'SS' it was observed that none tested positive for Rhinovirus infection. However, 31 (15.5%) of those who did not know their genotype tested positive to Rhinovirus infection. No single literature has compared genotype as a risk factor in the Rhinovirus infection cycle. Majority of researchers only focused on the immunogenic response to the infection. According to Peltola et al.,[20]adults are often protected from symptomatic infection, probably by acquired immunity, because most have serum antibodies against many Rhinovirus types. As such the clinical significance of these findings is presently unknown. However, other studies would be needed to corroborate these findings.

CONCLUSION

This study established that detection of Rhinovirus infection in children attending the Pediatrics clinic can be carried out by ELISA techniques using nasal swabs. Prevalence of Human Rhinovirus infection was demonstrated to be directly associated with mild or moderate clinical disease. Demographic characteristics revealed that Rhinovirus infection may be the commonest virus in young children with respiratory symptoms, since they play a central role in the spread of virus. Further studies to determine more serotypes and develop suitable vaccines should be adequately encouraged. Extensive studies to include symptomatic and asymptomatic children could also be considered.

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