HUMAN RHINOVIRUS INFECTIONS IN SYMPTOMATIC AND ASYMPTOMATIC SUBJECTS

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ABSTRACT

The role of rhinovirus asymptomatic infections in the transmission among close contacts subjects is unknown. We tested health care workers, a pair of one child and a family member and immunocompromised patients (n =191). HRV were detected on 22.9% symptomatic and 3.6% asymptomatic cases suggesting lower transmission among contacts.

Key words: symptomatic, asymptomatic, rhinovirus infection

Rhinovirus infections are among the most frequent causes of the common colds (18). It is the most common etiology of viral respiratory infections among diverse populations, including adults, children and more recent studies have linked HRVs to more severe lower respiratory illnesses in otherwise healthy young children (11-12), immunocompromised (4,6), and elderly patients. (5,13, 23). In Brazil, there are few HRV studies, Arruda et.al. (1991), realized the first Brazilian study detecting a 45.5% rate on symptomatic children in Fortaleza – CE. Another study realized in Salvador – BA also showed a high prevalence (48.5%) (Souza ,2003). The only study describing adults was realized by Bellei and colleagues (2007) detecting 37.7%.

The dynamic of rhinovirus transmission is relevant to address for the epidemiology characteristics of infection within families, schools and nosocomial outbreaks. Rhinovirus can be easily transmitted from person to person mainly through hand contact with infected respiratory secretions (15). Studies of asymptomatic infected individuals pointed to 15 – 30% rates (9,19, 14, 20, 7, 19) of HRV infection but the role of infected subjects as reservoirs for secondary cases infections is unknown.

Many studies have investigated the occurrence of rhinovirus among community cases but there is a lack of information about the frequency of rhinovirus asymptomatic cases. We investigated HRV infections rates on selected populations of a pair of one child and one family member, health care workers (HCW), and immunocompromised patients with and without respiratory symptoms from June to September.

In this study, a total of 191 nasal swab (NS) specimens were collected from three groups. One hundred and eleven health care workers (HCW) from São Paulo Hospital, 36 pairs of one child and one family member and 8 blood marrow transplanted hospitalized patients (BMT). They were considered eligible symptomatic patients if possible viral etiology within 7 days of symptoms onset. The clinical criterion was presentation of at least one respiratory symptom (cough, sore throat, or nasal congestion) and one constitutional symptom (headache, malaise, myalgia, chills). For

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asymptomatic patients the criteria was the absence of respiratory symptoms up to one week before sampling. All subjects were interviewed by a research team after evaluation by a physician. Written informed consent was obtained from all adult participants; parents provided consent on behalf of children participants and a questionnaire was applied including demographic data, place of work, their clinical presentation and household children contact.

The symptomatic group included subjects with acute respiratory infections (ARI): children, BMT patients and health care workers. The asymptomatic group included one parent of each symptomatic children, health care workers caring for BMT symptomatic patients or others referring a close contact with symptomatic patient in the hospital.

The nasal swab was obtained from the single nostril from a depth of 2 - 3 cm by using a sterile swab that was then inserted into a vial containing 2.0 ml of viral transport medium (Cultilab, Brazil). The samples were immediately transported to the Clinical Virology Laboratory for routine respiratory viruses testing. All samples were stored at -70ºC until analyzed.

For each sample the viral RNA was extracted using QIAamp Viral RNA extraction Kit (QIAGEN, Germany), according manufacturer´s instructions. Amplification of 5'NCR and VP4/VP2 genes of HRV was done by RT-PCR assay described elsewhere (17-18), with minor modifications. The eluted RNA was transcribed into cDNA with Moloney Murine Reverse Transcriptase (MMLV-RT; Invitrogen, USA) and virus specific oligonucleotide primer, for 1 h at 37°C. After, MMLV-RT denaturation at 70°C, virus-specific oligonucleotide primer (0.6μM), 2.5U Platinum Taq DNA Polymerase (Invitrogen, USA), 1x PCR Buffer, 0.2mM each dNTP, 3.5mM $MgCl_2$ and nuclease-free water were added. The amplification condition was performed in a thermo cycler under the following settings: initial denaturation at 95° for 10 min, followed by 40 cycles consisting of denaturation (45 sec at 95°), annealing (45 sec at 61°C), and DNA extension (1 min at 72°C). The presence of PCR products were visualized on an

1,5% agarose gel electrophoresis according to their 549bp molecular weight. Positive (HRV-39) and negative controls (water) were tested in all reactions.

Descriptive statistics consisted of the characterization of the studied individuals and the assessment of symptomatology and rhinovirus infection through calculation of the respective percentages, median value and range. Bivariate analysis consisted of Fisher's Exact Test for the comparison of categorical values, with a significance level of $p < 0.05$. In multivariate analysis, non-conditional logistic regression was used to identify associations between presence of symptomatology, groups of individuals and rhinovirus infection status. All reported values are two-tailed. The dependent variable was presence of rhinovirus infection and the independent variables were presence of symptomatology and groups of individuals. The results were presented as odds ratio (OR) with the respective 95% confidence interval (CI) and p value. All data were entered into and analyzed by using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

Epidemiological and clinical characterization of patients is shown in Table 1.

A total of 191 samples from both symptomatic (81) and asymptomatic cases (110) were tested for the presence of rhinovirus and 23 samples (12%) were positive. HRV was detected in 23.5% (19/81) of symptomatic subjects, compared with 3.6% (4/110) of asymptomatic subjects. The HRV infection was associated with the presence of symptoms [p <0.0001, OR 8.1 (95% CI 2.6 – 25.0)].

Table 2 shows the HRV infection rates among symptomatic and asymptomatic individuals, divided by groups. Six isolated cases of symptomatic parents negative for HRV were excluded from this analysis. There were no significant associations.

The symptoms reported by symptomatic group were fever (38.5%), coryza (86.7%), cough (74.7%), headache (21.7%), sore throat (35%), myalgia (12%). Wheezing was observed in 39% of children, but none of them were HRV positive. The symptoms among positive children cases were coryza (100%),

cough (77.8%) and fever and sore throat (22%).

The rate of rhinovirus infection among a pair of asymptomatic parent of a rhinovirus symptomatic child was 2.8% (1/36). This was the only case with a close contact with a laboreatory confirmed patient. The others three asymptomatic infection had no close contact with positive symptomatic studied patients. Two symptomatic patients reported close contact a BMT patient and his nurse.

Rhinovirus infections occurred all over the year in Brazil (2). There is a lack of studies about asymptomatic rhinovirus infections. The majority of reported data regarding asymptomatic rhinovirus infections have been conducted in hospitalized children elsewhere and high rates were reported - 12% to 45% (16,14,7,3). Jartti et al. (2008) reviewed many studies describing asymptomatic subjects with high respiratory virus detection rates using PCR techniques. Van Kraajj and colleagues (2005) identified etiology in 63% and 9% of symptomatic and asymptomatic adult stem cell transplants recipients respectively, and rhinovirus was the predominant pathogen detected.

In our study, HRV was detected more frequently in symptomatic than asymptomatic individuals as previous reported (21,22,10) and a low rate as identified by Johnson et al. (1993) in their study among in immunocompetent adults (4%). Discrepancies among different studies may be explained by the fact that most of them included hospitalized children instead of community population. Indeed nosocomial transmission may occur without clinical expression.

Health care workers group had a 25.8 % rate. Bellei and colleagues (2007) reported the detection of HRV in 37.7% of symptomatic health care workers samples. Professional profile is an important transmission pathway in hospitals. Long-term clinical studies can clarify the impact of this reservoir in the transmission of the virus for patients (10).

We found a high frequency of rhinovirus infection in parents than health care works. The study from Bellei and colleagues (2008) also reported that 39% of those HCW had exposure to children up to 5 years old and rhinoviruses were detected in half of the personnel from pediatric wards.

Despite of the small number of subjects included, our study showed lower detection in selected asymptomatic individuals in contrast to previous studies that found higher frequencies on epidemiological surveys. Further studies would contribute to better understand the dynamic of rhinoviruses infections.

Table 1. Epidemiological and clinical characterization of patients

Characteristics	No .Cases (%)			
	child	parent	HCW	BMT Patients
Median age , years (range)	6.2 (0.5 - 12)	38.5(27-66)	25.5 (21-44)	51.3 (19-80)
Gender				
male	17(47.2)	2 (5.5)	36 (31.8)	6 (75)
Female	19(52.8)	34(94.5)	75 (68.2)	2(25)
Smoker	0	6(16.7)	2 (1.8)	
exposure to children (≤ 5 years)	-	17 (47.2)	23 (20.3)	2(25)
Patient contact	-	31(86.1)	94 (83.2)	1 (12.5)
Health Status				
no comorbidities	-	26(72.2)	110 (97.4)	-
Asthma	-	3 (8.3)	1 (0.8)	-
Others lung diseases	-	2 (5.5)	-	-
diabetes	-	1 (2.8)	-	-
Hypertension	-	3 (8.3)	2 (1.8)	-
Cardiovascular diseases	-	1 (2.8)	-	-
Total	36	36	111	8

HCW – health care workers, BMT – bone marrow transplanted hospitalized patients

Table 2. Rhinovirus infection among symptomatic and asymptomatic subjects

	Symptomatic			
	Positive	**Negative**	**p**	**OR(95%CI)**
Child	9 (25%)	27 (75%)	-	1
Health care worker	8 (25.8%)	23 (74.2%)	0.94	1.04 (0.35-3.14)
BMT Patient	2 (25%)	6 (75%)	1.00	1.00 (0.17-5.87)
	Asymptomatic			
	Positive	**Negative**	**p**	**OR(95%CI)**
Parent	2 (6.7%)	28 (93.3%)	0.30	2.79 (0.37-20.73)
Health care worker	2 (2.5%)	78 (97.5%)	-	1
BMT Patient	-	-	-	-

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