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The Prevalence of Human Metapneumovirus among Children with Acute Respiratory Tract Infection in North Kordofan State, Sudan

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Abstract

Objective: To determine the prevalence of human metapneumovirus (hMPV) among children with acute respiratory tract infection and the correlation between (hMPV) and age group. **Methods:** This prospective, descriptive cross-sectional hospital-based study is carried out from January to May 2019 among children from under five years old who were admitted to El-Obeid pediatric teaching Hospital, North kordofan, Sudan with acute respiratory tract infection. **Results:** Fifty hospitalized children with acute respiratory tract infection were enrolled in the study. Two of these (4%) were tested positive for (hMPV) in the age group (7 months - 2 years) old. Moreover, the study showed no significant correlation between infection with (hMPV) and age.

Keywords

Human Metapneumovirus, Respiratory Tract Infection, Real Time, Sudan

1. Introduction

Human metapneumovirus (hMPV) is a negative-sense single-stranded RNA virus of the family Pneumoviridae [1]. It was isolated for the first time in 2001 in the Netherlands [2]. It is the second most common cause after human respiratory syncytial virus (RSV) of upper and lower respiratory infection in young children [2].

Since the discovery of human metapneumovirus (hMPV) the virus has been identified worldwide, the major challenges faced by the medical and scientific communities are the understanding of the pathogenesis of hMPV disease and

the development of a safe and effective vaccine to protect against infection and disease caused by this newly recognized respiratory virus [2].

Worldwide, HMPV prevalence in hospital inpatient or community studies, in children or elderly adults, varies widely from as low as 1.7% to as high as 17%, with generally higher prevalence in outpatients compared to inpatients and, also, more in children younger than 5 years compared to older age groups [3].

Human metapneumovirus is most likely spread from an infected person to others through secretions from coughing and sneezing, close personal contact, such as touching or shaking hands in addition to touching objects or surfaces that have the viruses on them then touching the mouth, nose, or eyes [4].

Upon entering the body, hMPV infects the cells in your respiratory tract. The infection of these cells by the hMPV leads to the release of local chemicals and hormones that can trigger the body's immune response. This response leads to the classic symptoms of a "cold". In some individuals, the disease can spread to the main airways, or bronchi, causing atypical pneumonia [4].

In term of detection, the efforts cultivating hMPV virus were faced by difficulties due to its poor growth inconventional cell culture and have no characteristic cytopathic effects. Sensitive (RT-PCR) Molecular techniques, in addition to touch-down genomic target sequences, enzyme-linked immunosorbent assay and immunofluorescence serological test are used in detection of the virus from clinical specimen [5].

Documented information regarding the prevalence of hMPV disease in Sudan is scarce. Hence, the aim of this study was the generation of preliminary information about hMPV infection among children patients attending El-Obeid pediatric teaching hospital with acute respiratory tract infections, where no such study has previously been conducted.

2. Methods

2.1. Time and Duration of the Study

This was a descriptive cross-sectional hospital-based study, carried out from January to May 2019 among children from zero to five years old whom were admitted to El-Obeid teaching Hospitals, North Kordofan State, Sudan. The hospital is a 250-bed tertiary care facility, which serves as a referral Centre for North Kordofan State. The average patient turnover at the hospital is 50 to 150 patients per day.

2.2. Study Population

Fifty children co-patients at the pediatric ward El-Obeid teaching Hospitals during the study period were asked to answer a structured questionnaire consisting of socio-demographic data (age, gender and residence). The informed consent related volunteers were recruited randomly, any child, with acute respiratory tract infection admitted to the pediatric ward has a chance to be selected a once. Fifty children parents have consented for their children to participate in

this study with a moderate rate of refusal.

2.3. Sample Collection

Throat swab samples were obtained from patients by inserting sterile nylon swab (Regular Flocked swab, Cat. No. 520CS01, Copan Diagnostics Inc., Murrieta, Calif, USA) and rubbing the tonsils and the posterior wall of the pharynx. Samples collected were transported in an ice pack on the same day of collection to the Virology lab, Central Laboratory and stored at −80˚C until tested.

2.4. RNA Extraction

Total RNA was extracted by using the QIAamp Viral RNA Mini spin according to the protocol of the manufacturer (Qiagen, Germany). Briefly, 140 µl of throat swab sample was added to 560 µl buffer AVL containing carrier RNA, and then incubated at room temperature for 10 minutes. Subsequently, 560 µl of ethanol (96% - 100%) was added to the sample after which 630 µl of the resulting solution was applied to a column. A volume of 500 µl of AW1 and AW2 was added for washing and the nucleic acids were eluted with 60 µl AVE buffer and stored at −80˚C until used.

2.5. Real-Time RT-PCR

One-step RT-PCR was done to detect viral RNA by using a commercial kit following the manufacturer's instructions (AgPath-ID™ One-step RT-OCR Kit Ambion USA) and primer/probe for hMPV.

The real-time PCR master mix for one reaction was prepared as follows: 12.5 µl of 2× RT-PCR buffer reaction mix (consisting of a proprietary buffer system, $MgSO_4$, dNTPs), 2 µl of primer/0.5 probe, 1 µl enzyme mix, 1 µl enhancer, 3 µl of molecular grade water, and 5 µl of total RNA. The final volume was 25 µl for a single reaction. The reaction was performed in Rotor-gene Q real-time PCR (Germany). The thermal cycling conditions were 15 minutes at 45˚C for reverse transcription, 2 minutes at 95˚C for initial denaturation and 45 cycles of 15 seconds at 95˚C for denaturation and 45 seconds at 60˚C for annealing and extension [**Figure 1**].

2.6. Ethical Review

The Ethical Review Committee (ERC) of the Ministry of Health North Kordofan State, Sudan approved the study.

2.7. Statistical Analysis

Data were analyzed using the statistical package (SPSS) version 22.

3. Results

A total of fifty pediatric patients (less than five years old), were enrolled in this study; 17 were male and 33 were females. The prevalence of hMPV was 4%, 2 (50) by Real-time RT-PCR (**Table 1**).

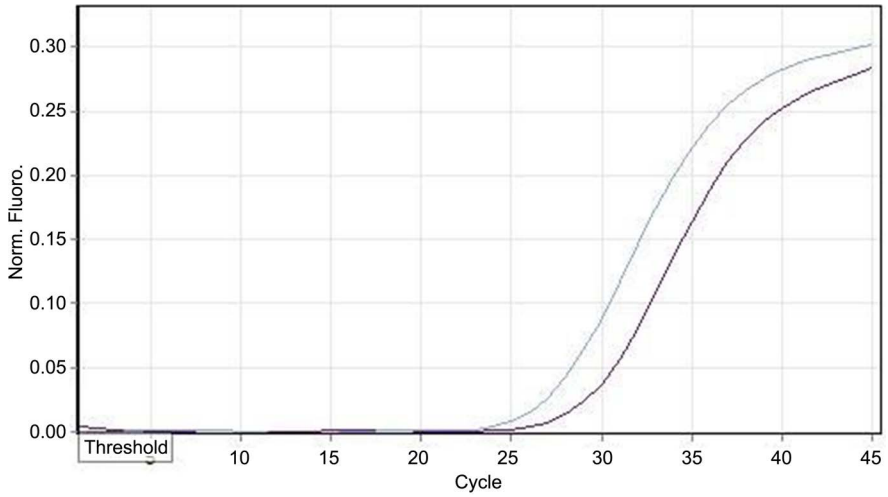

Figure 1. hMPV real-time PCR results. The horizontal axis represents the number the cycles for amplification of a targeted DNA molecule during the PCR, while the vertical axis represents the amount of the fluorescence produced in real time PCR.

Table 1. Frequency of hMPV among acute respiratory infection patients.

	RT PCR Test		
Gender	Frequency (%)	Positive	Negative
Male	17 (34%)	0	17
Female	33 (66%)	2	31
Total	50 (100%)	2 (4%)	48 (96%)

The males to females ratio was (0:0.1) in patients who are (hMPV) positive.

Human MPV positive patients restricted among 7 months to 2 years age groups (Table 2). Statistically there is no significant association between infection with hMPV and age group.

4. Discussion

There is a paucity of information regarding the prevalence of hMPV in sub-Saharan Africa despite a scarce studies documented in Sudan, Kenya and South Africa. This is a first study was conducted in El-Obeid pediatric teaching Hospital North Kordofan state, to determine the prevalence of Human metapneumovirus (HMPV) in children complaining of acute respiratory infection, which was considered upon main causes of morbidity and mortality in children, their behavior tends to be seasonal and vary by geographical locations.

The present study revealed the prevalence of hMPV infection was 4% out of 50 patients, which was lower compared to study done by Attar and his colleagues in Sudan, whom reported the prevalence of hMPV was 26.5% this difference may be due to seasonal distribution of the virus. A high incidence of hMPV infection observed during the winter-spring season [6].

A similar results obtained by Mona *et al.*, Al-Turab *et al.*, and Ramirez *et al.*,

Table 2. Distribution of hMPV positive patients age groups.

Age group	Frequency	Positive	Negative
0 - 6 months	2	0	2
7 months - 2 year	27	2	25
2 year - 5 years	21	0	21

in Egypt, Kuwait and Colombia, showed that incidence of (hMPV) was (4%), (5%) and (5.2%) respectively [7] [8] [9].

Moreover, in Saudi Arabiaa study performed by Alsuheel *et al.*, found that the prevalence of human metapneumovirus was 9.9%, which higher than the prevalence documented by this study [10].

Furthermore, a study concluded in China, reported 2.0% for the prevalence of hMPV infections among (RTIs)hospitalized children, which is considered lower than the determine by our study, and the age group of the positive hMPV were 1 to 2 years, while in our study was (7 months - 2 years), which is relatively close to our study findings [11].

5. Conclusion

This study revealed the existence of hMPV among children patients attending El-Obeid teaching Hospitals, Northern Kordofan State, Sudan that was not previously established in this hospital. These findings are useful for future studies since there is a paucity of information regarding hMPV infection in Sudan.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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