Common Microbial Agents in Asthmatic Children With Respiratory Infection and Productive Cough

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Abstract

Background

Microbes can influence on the development and somehow on triggering of asthma attacks. Therefore, in this study the more significant microbial infections which trigger the attacks in children were evaluated.

Methods

A total of 41 nasopharyngeal and oro-pharyngeal swabs were obtained from the Pediatric Allergy Clinic of two educational hospitals, and sent to a Molecular Laboratory for evaluation of 21 bacterial and viral respiratory pathogens using qPCR-TaqMan method.

Results

The main bacterial infections were *S.aur* 18/41 (43.9%), *S.pneu* 16/41 (39%), *C.pneu* 12 /41 (29.3%), and HIB 17/41 (41.5%) while the most viral infections were and HRSV 3/41 (7.3%) and FluB, HRV, HMPVA.B, HPIV-2,3,4, HcoV-63 and HcoV-229 in 2 cases (4,9%), in asthmatic children. Although, bacterial infections were more common in both gender, the frequency of those agents were statistically difference between girls and boys population ($P=0.02$). There were positive correlations between *S. pneu* infection with asthma attack and bronchitis ($P= 0.02$ and $P= 0.001$, respectively). Furthermore, a positive correlation was found between AV and RSVA.B infections with allergic rhinitis ($P= 0.02$ and $P= 0.001$, respectively).

Conclusion

In conclusion, it is more likely that in this region with a population of 6.2 million and more than 25 million tourists, the bacterial respiratory infections, particularly, HIB, *S.aur* and *S. pneu* were more common, however, and consistence with other studies HRSV and Flu.B have been dominant viral infections in asthmatic attacks.

Background

Asthma is classified as an inflammatory airway disorder. The triggering factors, such as aeroallergens, air pollutants, and viral infections promote asthma symptoms (1-3). Despite the progress made in the prevention and management of the disease, unfortunately, the number of morbidities is increasing every year (4-6).

Asthma is the most common persistent disease in childhood. The prevalence of asthma has been increased by 12.6% from 1990 to 2020 (7, 8). Currently, asthma has stricken more than 300 million individuals worldwide. Despite the fact that the physicians are able to improve their life span by used of new therapeutic regimes, around 250,000 patients still expire annually (5, 8). Moreover, allergic anaphylaxis and asthma attacks are the major life threatening events in asthmatic patients. Despite

endurable attempts of researchers to discover the exact pathogenesis of asthma attack, the precise mechanism is not fully understood.

In general, microbial agents in pulmonary diseases are the main inducing and progressive factors in their clinical exacerbation, which asthma is not an exception. More than eighty percent of asthma exacerbations conditions accrued in children mainly are caused by respiratory viruses (9-11).

In the context of asthma, any colonized respiratory microbial infection in the airways can induce inflammatory reactions in which the mediators and chemokines recruit neutrophils and eosinophils and consequently trigger asthmatic reactions (12, 13).

In vitro studies suggested that the airway epithelium of subjects with asthma is defective in the production of the first line of viral defense, IFN- α and λ , increasing the risk of severe attacks (14). In the school age-children the main pathogen identified in symptomatic asthma subjects is *Human Rhinovirus* (HRV) (15). However, many other respiratory viruses including *Adenovirus* (AdV), *Bocavirus* (BoV), *Coronavirus* (CoV), *Cytomegalovirus* (CMV), *Enterovirus* (EnV), *Herpes Simplex Virus* (HSV), *Influenza Virus* (IFV), *Metapneumovirus* (MPV), *Parainfluenza Virus* (PIV), and *Respiratory Syncytial Virus* (RSV) have been identified for triggering the asthma exacerbation (16, 17).

For example, a study of 175 children with 2–15 years of age showed that the HRV (73%), followed by IFV A (27%) and RSV (7.7%) were the most frequently viruses associated with asthma exacerbations. Despite the frequency of the viral infections in asthma promotion, many other infections by bacteria and fungi, including *M. pneumoniae* (M.p), *S. pneumonia* (S.p), *C.pneumoniae* (C.p), *H.influenza* (Hin) and *Aspergillus spp* can induce the asthma crisis (18, 19). However, bacterial infections may sometimes occur due to the post-viral infection and exacerbate the asthma reactions (19).

Management of asthma exacerbations, which have been consistently linked to upper and/or lower airway infections, is vital. Prompt and accurate diagnosis is the initial attempt at infection monitoring and surveillance because it leads to appropriate treatment choices and prevention of overusing antibiotics. The environmental and seasonal conditions are also important in the presence of bacterial and viral spores, and infectious disease outbreaks (20). Our information about the microbial cause of asthma exacerbation in Khorasan Razavi Province with a population of around 6 million (Census 2011, <https://www.amar.org.ir/Portals/1/Iran/census-2.pdf>), as a pilgrimage and tourist region in the northeast of Iran, is very limited. In the current study, the frequency and the implication of respiratory viruses and bacteria in pediatric asthma exacerbations were evaluated for 21 of the mostly reported microbial agents in the literature.

Methods

Sample preparation

A total of 41 nasopharyngeal and oro-pharyngeal swabs were obtained from the Pediatric Allergy Clinic of the educational hospitals, the affiliates of the Medical School, Mashhad University of Medical Sciences (MUMS) and in a transfer medium were sent to Navid Molecular Medical Laboratory for evaluation during January 2016 to December 2016. The inclusion criteria were the pediatric asthma subjects with 4-16 years of age with productive cough and children who had not received antibiotics during the previous month. The inclusion criteria included lack of any other autoimmunity or other immunopathologic disorders, asthmatic children without any serious asthma attack, who attended for periodic clinical assessment, without any underlying disease such as cardiac disorder or bronchiectasis and duration of recent illness 5 days or longer. The demographic and clinical assessments were collected from their clinical records. The exclusion criteria consisted of patients with any underlying disease such as cardiac disorder, bronchiectasis, duration of recent illness for 5 days or longer before visiting the clinic and antibiotic consumption. The study was approved by the Ethics Committee for Biomedical Research with approval code of MUMS: 931307. Furthermore, an informed consent was obtained from the participants and their parents.

DNA/RNA extraction

DNA/RNA was extracted using a highly pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Penzberg, Germany) according to the manufacturer's instructions. The extracted DNA was recovered in 50 µL of the elution buffer and stored at -20 °C.

Quantitative multiplex real time PCR

The FTD Respiratory Pathogens 21-plus kit (Fast Track Diagnostics, Luxembourg) six was used for the detection of the following pathogens causing respiratory infections: *Influenza A*, *Influenza A* (H1N1) swl, *Influenza B*, *Coronaviruses* NL63, 229E, OC43 and HKU1, *Parainfluenza* 1, 2, 3 and 4, *human Metapneumovirus A and B*, *Rhinovirus*, *Respiratory syncytial viruses A and B*, *Adenovirus*, *Enterovirus*, *Parechovirus*, *Bocavirus*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus influenzae B* and *Staphylococcus aureus*. All the real-time polymerase chain reactions (PCRs) were performed using a Rotor-Gene 6000 instrument (Qiagene, Germany). The real-time PCRs were performed as follows: 50°C for 15 min; 95°C for 10 min; 40 cycles of 95°C for 8 s, 60°C for 34 s. Simplification of the two-step method resulted in increased repeatability of analysis and efficiency of reaction. Negative control (sterile water) and positive control (DNA from the bacterial strain) were included in each run.

Statistical Analysis

The Chi-square tests were used to analyze the categorical variables. Statistical analyses were performed by using the SPSS software package, version 11.5 (SPSS Inc., Chicago, IL, U.S.A.). The P-values ≤ 0.05 were considered significant.

Results

The mean age (mean±SEM) of the participants, consisting of 29 males and 12 females, indicated 6.6 ± 0.46 years (with the range of 2-16 years). The main microbial infections among these asthmatic children were 41.5%, 17/41 and 7.3%), i.e., 3/41 for H1B and HRSV, respectively. In the case of the infection by influenza viruses, only flu-B infection was observed in 2 cases (4,9%), while there were no positive cases of flu-A, HRV, HMPVA. B, HPIV-2,3,4, HCoV-63 and HCoV-229 observed in these asthmatic children. The actual incidence of 21 microbial infections among children with asthma symptoms is presented in Table 1.

According to non-parametric Chi-square and Pearson correlation analysis, a positive correlation was found between H1N1 infection with asthma, bronchitis and food allergic ($P= 0.001$, $P= 0.002$ and $P= 0.02$, respectively). There were also positive correlations between S.pneu positivity with asthma attack and bronchitis ($P= 0.02$ and $P= 0.001$, respectively). Moreover, positive correlation was found between EV infection and atopic dermatitis ($P=0.001$). Furthermore, a positive correlation was found between AV infection and RSVA, B with allergic rhinitis ($P= 0.02$ and $P= 0.001$, respectively).

The frequency of the studied infections according to gender is presented in Table 2. Of the total girl's population, only 6 cases (50%) had contamination with HIB, S.aur and S.pneu, while, in the boy's population, 11 (37.9%) were infected with HIB, 12 (41.4%) with S.aur and 10 (34.5%) with S.pneu. Of the total number of the studied boys, 3 (10.3%) were infected with HRSV- A, B, while this infection was not observed in the girl's population.

Fifty-nine percent of children suffered from chronic cough, while the start age of coughing in the affiliated children was 2-4-year-old (70.6%) (table 3). Other risk factors including cold weather, the season of symptom crisis and family history were observed in 5.9%, 76.5% and 23% of children, respectively. Rhinitis, pneumonia and asthma attack were also observed in 94.1%, 5.9% and 5.8%, respectively. Almost 59% of the children experienced common cold frequently in winter.

Results from the Chi-Square test showed that there was a close relationship between the results and the clinical symptoms ($p=0.003$). Viruses H1B, S.aur, S.pneu and C.pneu were the most microbial agents in the clinical symptom exacerbations. Also, the results of Kruskal-Wallis test showed a meaningful relationship between the clinical signs and HCoV-43 ($p=0.0001$).

Discussion

In this study, respiratory pathogens were assessed in asthmatic children with 2-16 years of age in the pilgrimage and tourist region of the Khorasan Razavi Province in the northeast of Iran. The results were consistent with those observed by several authors around the world, accompanied by various respiratory pathogens (21, 22). The key findings of this study were a) H1B following by HRSV-A,B, PV, EV, HCoV-43, HCoV-HKU and flu-B are the most frequent pathogens in children associated with asthma symptoms, b) male children were more susceptible to the respiratory infections than the female children, and c) compared to the adults, the infants and children were more vulnerable to the respiratory viruses (7, 23-25). The results in the current study showed that exposure to viruses in children with asthma could

increase the airway obstruction, inflammation and lung dysfunctions, causing asthma exacerbation. The data clarify the vital function of viral respiratory infections in the asthma exacerbations and confirm the close connection of viral infection and asthma deterioration. Viral infections are generally recognized as important contributors to acute asthma exacerbations (50–85%) (26-28). However, prospective monitoring of nasopharyngeal samples during the peak season for viral infections and asthma exacerbations provides evidence of a close relationship between viral, bacterial, and respiratory symptoms. Although it has been shown that allergen exposure, gender, age, environmental and inherited factors are associated with asthma exacerbations; previous studies proposed the high rate of viral respiratory infections and their inflammatory responses as the major cause of morbidity and mortality in asthmatic patients (29-31). Our data is consistent with prospective studies demonstrating that the asthma exacerbations coincide with the prevalence of respiratory viruses, particularly in autumn and winter (32, 33). In individuals who were monitored regularly, respiratory viruses are discovered in ~ 40% of asthma worsening (10, 34, 35). According to the results of the analysis, some specific infections such as H1N1, EV and AV have a close connection with asthma attack, bronchitis, food allergy, atopic dermatitis and rhinitis, respectively (36). Also, our findings showed that it is more likely that after colonization of the viral respiratory infections, *S.pneu* infection worsened the asthma attacks because of the weakness of the immune system.

A positive correlation between RSV-A, B with allergy and rhinitis symptoms was also notified. Former clinical surveys approved the role of RSV, and to some extent, that of HMPV in induction of asthma in the respiratory tract. Our collected data is in line with other surveys showing that RSV and *picornaviruses* infections were mostly associated with acute expiratory wheezing in hospitalized infants (17, 37-39). Asthmatic infant girls who are immunocompromised are more at risk of severe viral infections of viruses such as coronaviruses (40, 41). Regarding influenza viruses, the studies have shown that influenza is safe for asthmatic patients (42). The low rate of flu-B and negative case of flu-A in the current study are consistent with the results that showed flu viruses caused respiratory diseases in young and middle-aged individuals more than children (43). Some studies have shown that the spread of flu with *S.pneu* causes pneumonia, bronchitis, sinus infections and ear infections (44, 45). However, in the present study, although *S.pneu* was frequent in asthmatic children and was associated with the exacerbation of the asthma attacks, there were no flu infections in our study population.

Similar to previous studies, a low rate of HPIV in asthmatic children was observed. However, the incidence of H1N1 infection was significantly higher in asthmatic children than non-asthmatic children (46, 47). This result is inconsistent with our results which also cover autumn. The present study also showed that children seem to have lower rate of respiratory viral infection than adults, but the asthma attacks could occur in the presence of infection colonization. In addition, viral infections associated with the opportunistic bacterial flora are more likely to be symptomatic, and in children with asthma, they are more likely to be associated with asthma exacerbations. A recent pooled meta-analysis of 60 studies across all ages and continents found the prevalence of respiratory viruses associated with asthma exacerbations was <15% (16). However, the present study showed that around 39% of microbial asthma is viral. Notably, the low rates of positive viruses in asthmatic older children in our study could be explained by the fact

that exposure to some bacterial or viral products has increased the maturity of innate and acquired immunity.

Our study indicated a predominant circulation of H1B bacteria in asthmatic individuals. This opportunistic bacterium was detected in 41.5% of the studied children with asthmatic exacerbations, which is consistent with some other studies on adulthood(48). Although H1B, S.aur, S.pneu and C.pneu are more detected in infant and school-aged children, other infection agents are less common in adolescence (45).

Mixed etiology and different pathogenic roles of viruses and bacteria lead to existence in respiratory secretions in combination or alone. The findings of our study are shown in Fig. 1 and 2, and, surprisingly, mixed bacterial infections of S.aur, S.pneu and C.pneu have been more common in 15% of the infections, and in a single infection the S.aur was predominant. It seems that S.aur alone or in the mixed bacterial infection has been the main cause of triggering asthma attacks in this region.

S.aur develops the chronic rhinosinusitis via a TH2-biased immune response to staphylococcal enterotoxins (SE) through both IgE-independent and IgE-dependent inflammatory reactions (49). Nasal colonization with S.aur is known to worsen eczema (49, 50). Aside from eczema, S.aur has been implicated in the development and severity of allergic rhinitis, asthma, and food allergy. Building on these findings, S.aur nasal colonization could be nominated as a risk factor for a range of asthma-associated outcomes, including diagnosis, symptoms, and exacerbations, among the population.

Furthermore, although HIB has been more frequent bacterial infection in asthmatic children, it could only exacerbate the asthma attacks around 5% and 41% as mixed with other bacterial or viral infections, respectively. The close association of bacteria and, to lesser extent, opportunistic respiratory viruses is little known in asthmatic children. Although evaluating the overall contribution of each virus/bacteria to disease severity is complicated by the presence of many confounding factors in clinical studies, understanding the role of each virus/bacteria in defining the asthma outcome will potentially reveal novel treatment and prevention strategies and also improving patient outcomes (51, 52).

Conclusions

In conclusion, while there is no doubt that age, race, genetic, gender, and environmental factors are involved in the onset of asthma respiratory symptoms, the results of the present study provide further evidence that these risk factors per se are insufficient to cause an exacerbation and highlight the importance of viruses as pathogens in asthmatic children.

Abbreviations

Respiratory syncytial virus (RSV), Influenza type A (FluA), Influenza type B (FluB), Human para-influenza viruses (HPIV), Human coronaviruses (HCoV), Human metapneumoviruses (HMPV), Human

parechoviruses (PV), Mycoplasma pneumoniae (M.pneu), Chlamydia pneumoniae (C.pneu), Haemophilus influenza Type B (HIB), Staphylococcus aureus (S.aur), Streptococcus pneumoniae (S.pneu)

Declarations

- Ethics approval and consent to participate

The study was approved by the Ethics Committee for Biomedical Research with approval code of MUMS: 931307.

- Consent to publish

Not applicable

- Availability of data and materials

The datasets generated and/or analyzed during the current study are included in this paper and available from the corresponding author (SAR. Rezaee)

- Competing interests

The authors declare that they have no competing interests.

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- Authors' Contributions

HA is a specialist in Allergy and Clinical Immunology examined, confirmed and referred the children to the molecular diagnostics lab, and co-supervised the study. FS, and SB handling the proper sampling of asthmatic children, performing the experiments and data collection and entering data in the software. FSM compiling the clinical data. AM and MM did the compiling and analyzed the data, wrote the draft of manuscript. SAR designed and supervised the study and did academic proof reading. All authors concur with the submission and their informed consent prior to their inclusion in the study. All authors have read and approved the final manuscript.

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Tables

Table1. *Frequencies of microbial agents* in asthmatic children with respiratory infection and productive cough.

Respiratory Pathogens	Positive outcomes, n (%)
S.aurs	18 (43.9)
H1B	17 (41.5)
S.pneu	16 (39)
C.pneu	12 (29.3)
HRSV-A,B	3 (7.3)
PV	2 (4.9)
EV	2 (4.9)
HCoV-43	2 (4.9)
HCoV-HKU	2 (4.9)
FluB	2 (4.9)
H1N1	1 (2.4)
HPIV-1	1 (2.4)
HBoV	1 (2.4)
M.pneu	1 (2.4)
Rhino	0
HMPVA.B	0
HPIV 2,3,4	0
HCoV 63,229	0

Table2. The frequency of infections agents according to the gender of studied children

	Male (boy)		Female (girl)	
	Positive outcomes		Positive outcomes	
	Frequency	percent	Frequency	Percent
<i>HIB</i>	11	37.9	6	50
<i>S.aur</i>	12	41.4	6	50
<i>S.pneu</i>	10	34.5	6	50
<i>Cpneu</i>	7	24.1	5	41.7
<i>AV</i>	3	10.3	1	8.3
<i>HRSV-A,B</i>	3	10.3	0	0
<i>HCoV-HKU</i>	2	6.9	1	8.3
<i>EV</i>	1	3.4	1	8.3
<i>HBoV</i>	1	3.4	0	0
<i>HCoV-43</i>	1	3.4	1	8.3
<i>FluB</i>	1	3.4	1	8.3
<i>H1N1</i>	1	3.4	0	0
<i>PV</i>	1	3.4	1	8.3
<i>M.pneu</i>	1	3.4	0	0

Table3. *Frequencies of infectious agents in different age groups of children with asthma*

Respiratory Pathogens	1 to 5 years		6 to 10 years		11 to 16 years	
	Positive outcomes		Positive outcomes		Positive outcomes	
	Frequency	percent	Frequency	percent	Frequency	Percent
<i>FluB</i>	0	0	1	4.3	1	33.3
<i>H1N1</i>	0	0	1	4.3	0	0
<i>HIB</i>	4	26.7	12	52.2	1	33.3
<i>HRSV-A,B</i>	0	0	3	13	0	0
<i>PV</i>	1	6.7	1	4.3	0	0
<i>EV</i>	0	0	2	8.7	0	0
<i>AV</i>	1	6.7	3	13	0	0
<i>HBoV</i>	1	6.7	0	0	0	0
<i>HPIV-1</i>	1	6.7	0	0	0	0
<i>HCoV-43</i>	1	6.7	1	4.3	0	0
<i>HCoV-HKU</i>	1	6.7	2	8.7	0	0
<i>S.aur</i>	7	46.7	11	47.8	0	0
<i>S.pneu</i>	6	40	9	39.1	1	33.3
<i>C.pneu</i>	4	26.7	8	34.8	0	0
<i>M.pneu</i>	1	6.7	0	0	0	0

Figures

Figure 1

The frequency of mixed or single respiratory pathogens in symptomatic children

Figure 2

The frequency of signs and symptoms in studied subjects