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Nasopharyngeal aspirate & blood cytokine profile in infants hospitalized for respiratory syncytial virus bronchiolitis: A pilot study from south India

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***Background & objectives*: Following a respiratory syncytial virus (RSV) bronchiolitis, only some infants develop serious illness, and a proportion of them develop recurrent wheeze subsequently. Studies have revealed that cytokine expression following RSV infection may influence the severity and also the risk for subsequent reactive airway disease. This present study was conducted to determine the blood, and nasopharyngeal aspirate (NPA) cytokine profile among infants admitted for RSV bronchiolitis.**

***Methods*: In this prospective pilot study, a sample size of 15 cases and 15 controls was included. Detailed history, physical examination, blood sample and NPA collection were done. Cytokines (IFNγ and IL-4) estimation was done in the blood and NPA samples of cases and blood samples of controls.**

***Results*: The mean levels of interferon gamma in controls (blood) and cases (NPA and blood) were 5.95, 9.54 and 32.02 pg/ml, respectively. The mean levels of interleukin-4 in controls (blood), and cases (NPA and blood) were 1280.77, 956.08 and 692.37 pg/ml, respectively (P<0.05).**

***Interpretation & conclusions*: Our study showed that infants with RSV bronchiolitis evoked a Th1 response in both blood and NPA. Large multicentre studies are needed to validate our findings.**

Key words Bronchiolitis - cytokines - immunology - respiratory syncytial virus

Respiratory syncytial virus (RSV) infection may be associated with short and long-term morbidity that includes recurrent wheeze, reactive airway disease and pulmonary function abnormalities. The main problem in the case management of RSV bronchiolitis is the availability of only supportive treatment (oxygen, intravenous fluids, saline nebulization, *etc.*). No safe and effective drugs/vaccine recommended for treatment and prevention of the cases of RSV bronchiolitis are available[1]. Among those infants who experience RSV bronchiolitis, only some infants tend to develop a serious illness, and a proportion of them also develop recurrent wheeze subsequently for reasons unknown. The mechanisms underlying the highly variable course of the disease and risk of recurrent wheeze in infants with RSV bronchiolitis is poorly understood.

Accumulating evidence suggests that the spectrum of (Th1/Th2) cytokine expression associated with RSV infection affects the balance between virus elimination and disease pathogenesis, and may influence the severity of clinical manifestations, and also has a role in the subsequent reactive airway disease[2]. Studies from various parts of the world regarding the Th1/Th2 cytokines expression in infants with RSV infection have yielded conflicting results. While some studies observed a Th2 response, other studies documented a Th1 response[1]. In India, Hemalatha *et al*[3] observed a high concentration of interleukin-8 (IL-8) in the nasopharyngeal aspirate (NPA) of children with RSV infection. However, interferon gamma (IFNγ) and IL-4 which represent the archetypal cytokines of the Th1/Th2 paradigm, need to be studied in the Indian patients. Hence, this study was conducted with the aim of studying the peripheral blood and NPA cytokine profile in infants hospitalized for RSV bronchiolitis in a tertiary care hospital in south India.

Material & Methods

This prospective study was conducted in the Pediatric Pulmonology Department of Kanchi Kamakoti CHILDS Trust Hospital (KKCTH), a paediatric tertiary care hospital situated in Chennai, in collaboration with the ICMR-National Institute for Research in Tuberculosis (NIRT), National Institutes of Health-International Centre for Excellence in Research (NIH-ICER), Chennai, India. The study population was from KKCTH whereas the cytokine analysis was done in NIRT, NIH-ICER.

Sample selection: Considering the cost involved in the cytokine kit procurement and non availability of previous studies on Th1/Th2 cytokines response in RSV bronchiolitis in the Indian scenario, this pilot study was planned with a minimum sample size. Román *et al*[4] studied the Th1 (IFNγ)/Th2 (IL-4) levels in 15 infants with RSV bronchiolitis in Chile. This pilot study was also planned with a minimum sample of 15 cases and 15 controls. Controls included the healthy infants attending the surgery/immunization/well baby clinic of the hospital during the study and were matched for age group and sex over the cases.

Inclusion & exclusion criteria: Consecutive infants aged one month to one year admitted at KKCTH, Chennai, during January 2011 - December 2011 with the diagnosis of bronchiolitis and whose NPA was positive for RSV by dipstick immunoassay (QuickVue RSV test, Quidel, San Diego, USA) were included as cases. Bronchiolitis was defined as the first episode of wheezing along with prodrome of upper respiratory tract infection including rhinorrhoea, cough and sometimes low-grade fever. The exclusion criteria included infants aged less than one month or greater than one year, those with the previous episodes of wheeze, those not requiring hospital admission, where parents were unwilling to undertake RSV Rapid Antigen test or unwilling to give blood and NPA for the study, those who received steroids and those with pneumonia and congenital heart disease.

The Institutional Ethics Committee of KKCTH, Chennai, approved the study protocol. Written informed consent was obtained from parents/guardians. A detailed history was obtained and a thorough physical examination was done. The baseline data (demography, socio-economic status, atopy/asthma & smoking in parents) were collected. Blood sample (3 ml for complete blood count & cytokines estimation) and NPA (1 ml for cytokine estimation) were taken within 24 h of hospitalization. Serum and NPA samples for the cytokine analysis were stored in a deep freezer at -70^0 C.

Cytokine assay: Cytokines (IFNγ and IL-4) estimation was done in the NPA of cases and peripheral blood sample of cases and controls using ELISA kit (R&D Systems, Minneapolis, MN, USA). The reference values for the cytokines were as follows: IFNγ=15.6-1000 pg/ml, IL-4=31.2-2000 pg/ml.

Statistical analysis: Statistical analysis was performed using software SPSS 11.5 version (SPSS Inc., Chicago, IL, USA). Independent *t*-test was used to look out the statistical significance in the cytokine levels among the cases and controls.

Results & Discussion

Among the 15 cases, eight (53%) had moderate bronchiolitis and seven (47%) had severe bronchiolitis. The age and sex distribution among cases [median (range) 116 (48-225) in days] and controls [97 (45-210) in days] was comparable with a male : female ratio of 2.8:1 for both cases and controls. The mean levels of Th1 cytokine (IFNγ) in controls (blood sample), cases (NPA sample) and cases (blood sample) were 5.95, 9.54 and 32.02 pg/ml, respectively (Table). The difference in the mean blood IFNγ levels between the cases and controls was significant (P<0.01). The mean levels of Th2 cytokine (IL-4) in controls (blood sample), cases (NPA sample) and cases (blood sample)

Table. Mean cytokines levels in the study groups

Study group (Sample type)	IFNγ levels (pg/ml)	IL-4 levels (pg/ml)
Control (blood)	5.95±3.43	1280.77±552.32
Cases (nasopharyngeal aspirate)	9.54±7.63	956.08±354.58*
Cases (blood)	32.02±36.77**	692.37±382.38***

Values are mean±SD (n=15). SD, standard deviation; IFNγ, interferon gamma; IL-4, interleukin 4.
*P**<0.05 ***<0.01 ****<0.001 compared to control

were 1280.77, 956.08 and 692.37 pg/ml, respectively. The difference in the mean blood IL-4 levels between the control and cases was significant (*P*<0.001).

Th1 cells produce IFNγ and IL-2 which play an essential role in the antiviral immune response. Th2 cells produce IL-4, IL-5, IL-9 and IL-10 which have a prominent role in asthma[5]. The RSV infection induces a predominant Th2 mediated response (elevated IL-4, IL-5, IL-9 levels over IFNγ, IL-2 levels) in various body fluids among the infected infants and this may be responsible for the recurrent wheeze observed in 50 per cent of cases during their childhood[6-8].

Studies in mice have demonstrated that RSV infection induces a Th2 response with increased IL-5 production[9]. Román *et al*[4] from Chile reported an increased IL-4/IFNγ ratio, a Th2 response in the peripheral blood of infants with bronchiolitis. Kim *et al*[7] also observed a Th2 type of response in the bronchoalveolar lavage fluid of RSV infected Korean infants who were of slightly older age (4 months - 1.8 yr). In our study RSV bronchiolitis evoked a Th1 response (predominantly IFNγ elevation over IL-4) in both peripheral blood and NPA which was contrary to the above studies. Similar to our study, Flores *et al*[10] from Portugal reported Th1 cytokine response in nasopharyngeal secretions among infants with RSV bronchiolitis while van Schaik *et al*[11] have reported an increased IFNγ levels over IL-4 levels in nasopharyngeal secretions from infants with RSV infection. They also suggested the possibility of double hit hypothesis[8] stressing the role of host (genetic) and environmental factors in addition to the RSV infection in deciding the cytokine response. Welliver[12] suggested that the infants with atopic predisposition predominantly developed asthma after RSV infection. Considering the small sample size in this preliminary study and the paucity of data in the Indian infants, further studies with large sample size with a follow up are required.

***Conflicts of Interest*:** None.

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