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Original Article

Clinical Value of Toll Like Receptor 4 and CD14 in Children with Acute Lower Respiratory Tract Infection

Houriah Ahmed Allam, Manal Abd EL-Salam*, Amany Mohammad, Alshaymaa Gamal Aboulkhair

Pediatric Department, Faculty of medicine for girls Al-Azher University, Cairo, Egypt.

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Toll like receptors (TLRs) with a myeloid differentiation antigen (CD14) recognize and bind various structures from invading microbes and then trigger cell activation. They initiate a variety of effectors' functions, including cytokine secretion, proliferation, co-stimulation or phagocyte maturation. The aim of this study was to evaluate Toll-like receptor 4 (TLR4) and CD14 expression in children with acute lower respiratory tract infections and their relation to severity. The study was carried on 50 children under 3 years of age; 25 of them with lower respiratory tract infection (LRTI) with mean age 11.9± 6.7 months, the other 25 children were healthy controls, age and sex matched. TLR4 and CD14 expression were assessed in both case and control groups. There was no statistical significant difference between cases and controls regarding the mean TLR4 level. mCD14 level among cases was significantly higher than that of the control group with more increase in bacterial LRTI. There was a positive correlation between CD14 with TLC and ESR in bacterial group. There was a positive correlation between respiratory distress severity with CD14, TLC, and ESR levels in patients group. TLR4 was not involved in the development of lower respiratory tract infection in the studied cases. mCD14 might be involved in the development of LRTI, and changes in mCD14 expression are parallel with the levels of TLC and acute phase reactants including CRP, ESR and with respiratory distress severity.

Keywords: Respiratory tract infection, CD14, toll like receptor

Lower respiratory tract infections (LRTI) are generally more serious than upper respiratory infections and are the leading cause of deaths among all infectious diseases. They include all infections of the lungs and the large airways below the larynx, mainly bronchiolitis and pneumonia (1, 2). The main burden of childhood respiratory diseases and associated deaths occur in developing countries especially in Africa where almost half of pneumonia-associated deaths occur (3).

Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs) derived from bacteria, viruses, fungi and protozoa. The binding of a PAMP to a TLR results in cellular activation and initiates a variety of effectors functions, including cytokine secretion, proliferation, co-stimulation or phagocyte maturation. To facilitate microbial recognition and to amplify cellular responses, certain TLRs require additional proteins, such as lipopolysaccharide (LPS) binding protein (LBP) and

*Correspondence: Pediatric Department, Faculty of medicine for girls Al-Azher University, Cairo, Egypt. E-mail: manal24969@gmail.com

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CD14 (4, 5).

CD14 is a myeloid differentation antigen that is mainly produced by monocytes and macrophages. CD14 recognizes and binds various structures from invading microbes, such as lipopolysaccharide from gram-negative bacteria, lipoteichoic acid from gram-positive bacteria, lipoarabinomannan from mycobacteria, viral double stranded RNA and F glycoprotein from respiratory syncytial virus (RSV). CD14 subsequently transfers these bound components to TLRs which then trigger cell activation (6, 7).

CD14 exists in two forms. The membrane associated form (mCD14) is present on the surface of monocytes, macrophages, dendritic cells and neutrophils; and the soluble form (sCD14) is present in the circulation and other body fluids. sCD14 may result from secretion of the protein before coupling to the glycosyl phosphatidyl inositol anchor or from shedding or cleavage from the surface of monocytes. Levels of sCD14 in plasma increase during inflammation and infection (2).

We aimed to study and evaluate TLR4 and mCD14 expression in children with acute lower respiratory tract infections and their relationship to disease severity.

Materials & methods

This cross sectional comparative study was conducted on 50 children under 3 years of age; 25 cases (19 males and 6 females) with lower respiratory tract infection with mean age 11.9± 6.7 months, who were hospitalized for LRTI in Pediatric Department, Al-Zahraa hospital, Al-Azhar University, and 25 healthy controls, age and sex matched with cases group. Children under 3 years of age with a diagnosis of LRTI, fever, cough, tachypnea, chest retractions, and rhonchi or crackles up on chest auscultation, according to WHO criteria (1), were included in the study after obtaining oral consent from parents in adherence with the guidelines of the

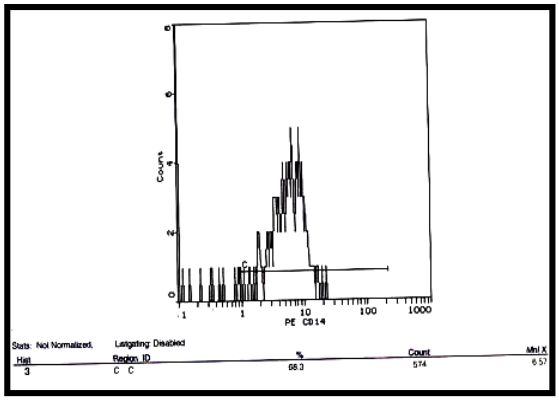

Figure 1. CD14 staining of one sample from the control group.

ethical committee of AL-Zahraa hospital, AL-Azher University, Cairo, Egypt.

The studied cases were divided into: bacterial LRTI and non bacterial LRTI. The bacterial LRTI was diagnosed when the following criteria were given: infiltrates in chest radiographs, increased C-reactive protein (CRP), elevated white blood cell count (WBC) and/or accelerated erythrocyte sedimentation rate (ESR) (6). Newborn. children with chronic systemic illness (chest, heart, renal,…), primary immune disorders, receiving immuno suppressive therapy and with acute illness other than respiratory tract infections were excluded from the study.

All infants and children in the study were subjected to the followings: history of fever, cough, dyspnea, grunting, feeding refusal, cyanosis. Similar attacks, history of vaccination and similar attacks and family history of chest disease. Full general and local chest examination including vital signs such as temperature, heart rate, respiratory rate and thresholds for fast breathing depending on child's age (50 or more breaths/minute for children between 2-12 months; 40 or more breaths/minute for children between 12-60 months) (8). Complete blood count (CBC), ESR, CRP, CD14 and TLR4 expression on peripheral blood monocytes were investigated using flow cytometry, and chest X ray (for cases only).

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Sampling

Blood samples were obtained by vein-puncture of about 5 ml, and divided into 3 parts; two parts were collected on ethylene-diamine-tetra-acetic acid (EDTA) tubes, one for CBC, ESR, and one for CD14, TLR4 analyzes**.** The third tube was plain vaccutainer for CRP testing. CD14 and TLR4 were measured in the blood by flow cytometry.

Flow cytometry analysis

Mononuclear cells were isolated from blood samples by centrifugation on a separate medium and were washed twice with PBS, pH 7.2-7.4. Then, 100 µl of the cell suspension was mixed with 10 µl fluor-escein isothiocyanate (FITC)-conjugated TLR4 (Abcam, Cambridge) and R-Phycoerythrin (RPE)-conjugated CD14 (Dako, Denmark). A non-reactive monoclonal antibody of the same isotype conjugated with the same fluorochrome (FITC and RPE) was used as a negative control. The mixture was incubated in the dark at 4 °C for 30 min, then washed twice with PBS containing 2% BSA and the cells were resuspended in 0.3 ml 1% paraformaldhyde (fixative) in 0.01 M PBS, pH 7.4 for flow cytometry. Flow cytometry was performed on a coulter (Coulter Epics xl-Beckman) with an argon laser and fluorescence channels. Leukocyte populations were selected for fluorescence analysis based on a combination of forward angle and side angle light scatter characteristics. Fluorescence gates for the determination of positive cells were set based on the determination of positive cells were set based on the isotype control samples. Pulses of scattered and emitted light received by appropriate detectors were converted into a form suitable for computer analysis and interpretation. Calculations were performed with Cell Quest analysis software (9). Figures 1, 2A and 2B show the staining of CD14 in control, non bacterial LRTI and bacterial LRTI children, respectively.

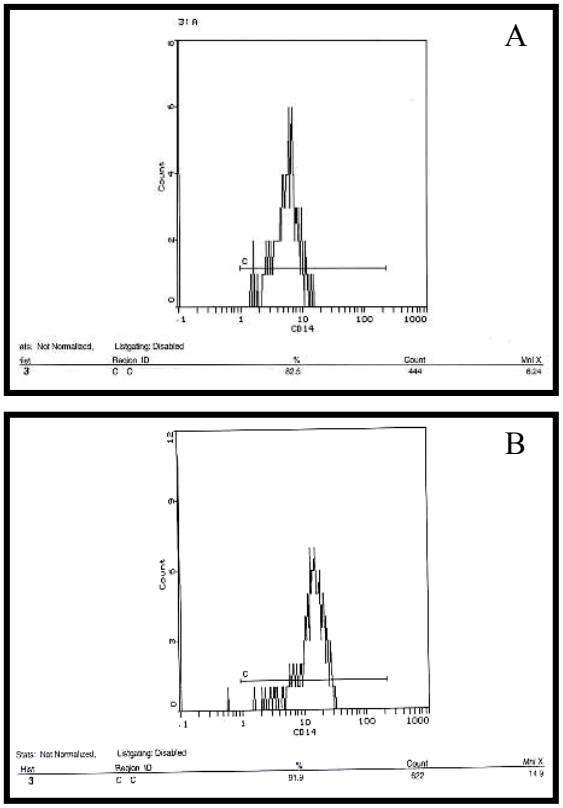

Figure 2. A: CD14 staining in children with non bacterial LRTI. B: CD14 staining in children with bacterial LRTI.

Statistical analyzes

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 12). Chi-square test was used to compare qualitative variables between groups.T-test was used for comparisons between 2 groups and ANOVA (F-test) test was used to compare between more than 2 groups to test for significance of quantitative variables. Correlation coefficient test was used to rank positively or inversely different variables against each other. $P< 0.05$ was considered as statistically significant.

Results

Table 1 shows the demographic data, CBC, ESR, CD14 and TLR4 in the study groups. It revealed a significant increase in the total leukocytes, platlet counts and ESR level in patients with acute LRTI in comparison with the control group. Also it shows a significant increase in the

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Table 1. Comparison between case and control groups regarding demographic data, CBC, ESR, CD14 and TLR4

Variable	Case (no=25)		Control (no=25)		P-value	Sig.
Mean age (months)	11.9± 6.7		11.5± 5.7		0.8	NS•
Gender	n	%	n	%		
Male	19	76	18	72	0.9	NS•
Female	6	24	7	28		
TLC (x10^3/µl)	17.1± 6.3		9.1± 6.3		0.001	HS**
PLT (x10^3/µl)	446.1± 124.9		319.6± 56.6		0.001	HS**
ESR (mm/h)	25.1± 16.9		8.1± 2.0		0.001	HS**
CD14	87.2± 3.6		69.8± 3.6		0.000	HS**
TLR4	9.1± 10.1		8.7± 9.4		0.9	NS•

NS•: not significant (P> 0.05); HS**: highly significant.

mean values of CD14 in patient group when compared to the controls, while no significant difference was detected regarding TLR4. Revealed a significant increase in the total leukocytes, platlet counts and ESR level in patients with acute LRTI in comparison with the control group. Also it shows a significant increase in the mean values of CD14 in patient group when compared to the controls, while no significant difference was detected regarding TLR4.

The distribution of non bacterial and bacterial LRTIs was 14(56%) and 11(44%), respectively.

Figure 3 shows the classification of LRTIs in the studied cases. It revealed that bronchopneumonia is the most common LRTI in the studied cases with 13 out of 25 cases.

Table 2 shows a statistical significant increase of the mean levels of total leukocyte count (TLC) and ESR level in lobar pneumonia and bronchopneumonia cases when compared to bronchiolitis cases. Also, a significant increase in CD14 level was observed in cases with lobar pneumonia in comparison with bronchopneumonia and bronchiolitis cases, while the mean level of TLR4 shows no statistical significant difference between cases group.

Regarding CRP, it is mainly positive in cases with lobar pneumonia followed by bronchopneumonia cases while 100% of bronchiolitis cases have negative CRP.

The correlation between respiratory distress severity in children with LRTI and related variables, revealed a positive correlation between TLC, PLT, ESR and CD14 with respiratory distress severity Table 3. No statistical significant correlation was detected regarding TLR4.

The sensitivity and specificity of mCD 14 in prediction of bacterial LRTI were 100% with the best cut off point≥ 88.7 Figure 4.

A positive correlation between the mean of TLC

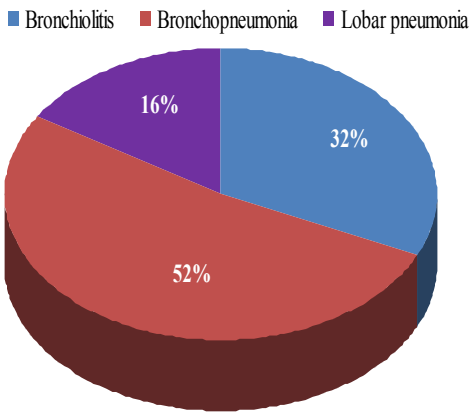

Figure 3. CD14 staining in children with non bacterial LRTI.

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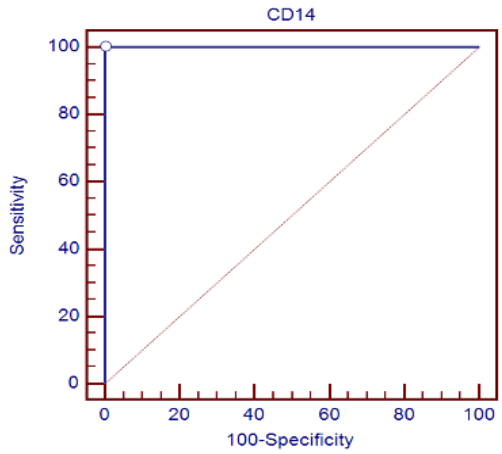

Figure 4. Positive correlation between CD14 level with TLC in children with bacterial LRTI.

and ESR with CD14 was observed in children with bacterial LRTI Fig. 5 & 6.

Discussion

Pneumonia and bronchiolitis are considered to be leading contributors to the global burden of acute respiratory infection in young children and are responsible for the greater part of these deaths, of which the most majority occurs in the developing world (10). In the present study, cases with lower respiratory tract infections were classified as bacterial LRTI when all CRP, WBC and ESR levels were elevated and combined with alveolar infiltrates in chest radiograph. The present study demonstrated increased TLC and ESR levels in cases with lobar and bronchopneumonia than those with acute bronchiolitis. Our results are in consistence with Moulin et al. who found that WBC and CRP levels in bacterial pneumonia were significantly higher than viral LRTI (11). Moreover, Lee et al. revealed that patients with community acquired pneumonia had significantly higher serum CRP, ESR and WBC levels than those in healthy controls, with more significant increase of CRP and ESR levels in lobar pneumonia when compared to bronchopneumonia (12). Contrary to our results, WBC level in the same study did not show significant increase in lobar pneumonia when compared to bronchopneumonia. On the other hand, Furer et al. found that 16.7% of children with pneumococcal pneumonia presented a normal WBC count on admission and 70% of them developed leukocytosis within a few days after admission (13). Also Toikka et al. reported that 17% of children with pneumococcal pneumonia presented normal WBC and elevated CRP on admission (14); suggesting that it is practical to use both indices in screening for pneumococcal pneu-

Table 2. TLCs, ESR, CD14 and TLR4 in cases

Variable	Bronchiolitis no = 8 Mean ± SD	Bronchopneumonia no = 13 Mean ± SD	Lobar pneumonia no = 4 Mean ± SD	F-test	P-value	Sig.
TLC (x10^3/µl)	11.8± 0.42	17.6± 5.0	27.7± 2.4	18.2	0.000	HS**
ESR (mm/h)	10.5± 3.6	27.5± 14.4	51.0± 3.6	14.3	0.000	HS**
CD14	82.8± 1.9	88.8± 1.4	91.3± 0.5	42.5	0.000	HS**
TLR4	12.6± 15.4	7.7± 7	6± 1.7	0.6	0.5	NS•
negative CRP	8 (100%)	6 (46%)	0 (0)		P -0.007**	
positive CRP	0 (0)	7 (54%)	4 (100%)			

NS•: not significant (P> 0.05); HS**: highly significant.

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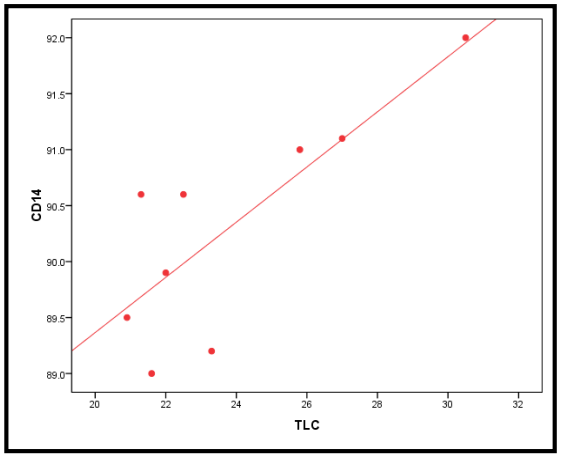

Figure 5. Positive correlation between CD14 level with TLC in children with bacterial LRTI.

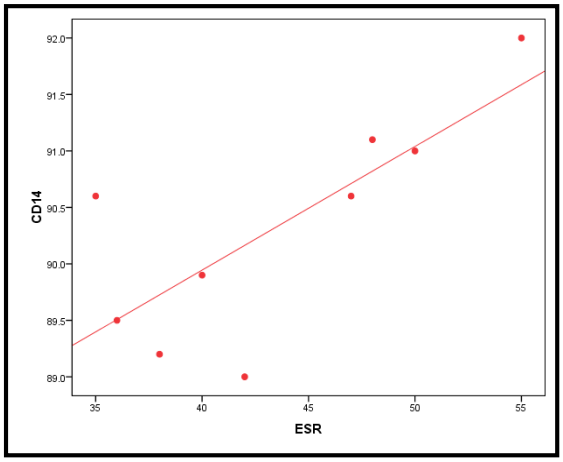

Figure 6. Positive correlation between CD14 level with TLC in children with bacterial LRTI.

monia.

Our results showed no statistical significant difference between cases and control group regarding mean TLR4 level .This was in accordance to Chen et al. (15). Also Ehl et al. demonstrated that TLR4 had no impact on RSV elimination or NK cell activity or on recruitment of other pulmonary inflammatory cells (16). These findings clearly argue against a significant role for TLR4 in RSV infection. Moreover, Faisca et al. demonstrated that pneumovirus infection is independent of the presence of TLR4 (17). The pneumovirus of mice, shares many similarities with RSV, the leading cause of lower respiratory tract infections in infants and young children. The serological and structural relationships that exist between pneumovirus and RSV suggest that the immune response to these viruses may be similar in their respective natural hosts.

Table 3. Correlation between respiratory distress severity and related variables

Variable	Respiratory distress severity		Sig.
	r	P-value	
TLC	0.718	0.000	HS**
ESR	0.737	0.000	HS**
PLT	0.610	0.003	HS**
CD14	0.826	0.000	HS**
TLR4	-0.139	0.547	NS•

HS**: highly significant; NS•: not significant.

The present study demonstrated that the mean mCD14 level among cases with LRTIs was significantly higher than that of the control group. Moreover, there was more increase of mCD14 level in cases with lobar pneumonia. This suggests that $CD14^+$ monocytes might be involved in the development of LRTI. This was in accordance to Marcos et al. who demonstrated that mCD14 and sCD14 levels significantly increase in serum and bronchoalveolar lavage fluid of children with pneumonia compared to the controls, cystic fibrosis and asthma subjects (6). Moreover, the highest levels of sCD14 and mCD14 were found in children with bacterial pneumonia when compared with nonbacterial.

The present study demonstrated positive correlation between respiratory distress severity and CD14, TLC, PLT and ESR levels in cases, indicating that the high levels of these variables might be related to the severity of illness. This was in contradiction to the findings of Gluck et al. who emphasized that mCD14 expression level was depressed in patients with sepsis and low levels correlated with the severity of infection, whereas persistently low levels correlated with fatal outcome (18). This indicates that sepsis is associated with a

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profound dysregulation of monocyte function and decreased immunity.

The present study demonstrated positive correlation between CD14 with TLC and ESR in bacterial group, while no correlation was detected in nonbacterial group. Marcos et al. detected also a positive correlation between sCD14 levels and TLC in pneumonia subjects but not in asthmatics or control subjects (6).

The ROC curve revealed that, the cut off value of mCD14 to predict bacterial LRTI was ≥ 88.7 with 100% sensitivity and specificity. Chalupa et al. demonstrated that increased serum levels of sCD14, as well as increased mCD14 expression on blood monocytes had a high sensitivity and specificity for the diagnosis of bacterial infection and its discrimination from viral infection (19), while the monocyte expression of TLR4 revealed the lack of a difference between the patients with bacterial infections versus the patients with viral infections. In conclusion, mCD14 would serve as the predictive parameter in early diagnosis and severity evaluation of children with LRTI and has a high sensitivity and specificity for the diagnosis of bacterial infection and its discrimination from viral infection.

Conflict of interest

The authors declared no conflict of interests.

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