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ORIGINAL ARTICLE

SERUM HUMAN NEUTROPHIL LIPOCALIN IN PEDIATRIC CHRONIC LUNG DISEASES: A NOVEL MARKER OF ONGOING NEUTROPHILIC INFLAMMATION

Magda Y El-Seify,[1] Terez B Kamel,[1] Mahera I El-Mogy,[2] Seham H Ossman[1]

[1]Departments of Pediatrics, [2]Clinical Pathology, Faculty of Medicine, Ain Shams University, Egypt

Correspondence to: Terez B Kamel, Email: terez2003@hotmail.com

Background: ***Pediatric chronic lung diseases include bronchiectasis, interstitial pneumonitis, cystic fibrosis and sequestrated lung disease. They are characterized by endobronchial chronic bacterial infections and predominantly neutrophilic inflammatory response. Human neutrophil lipocalin (HNL) is a newly discovered protein from human neutrophil secondary granules.***

Objective: ***The present study was performed to evaluate serum human neutrophil lipocalin (HNL) as a specific marker of ongoing neutrophilic inflammation in chronic lung diseases.***

Methods: ***This was a case-control study that included 40 children with chronic lung diseases (thirty were in exacerbation and ten were in stable state) recruited from the Pediatric Chest clinic, Children's hospital, Ain Shams University hospitals and ten healthy children as negative control.***

Results: ***Patients' mean age was 6.7±2.88 years; Twenty two (55%) were females and eighteen (45%) were males. The mean lipocalin (HNL) level among patients during exacerbation was 189.8±68.64 ng/ml; while in patients not in exacerbation was 76.2±29.13 ng/ml and in healthy control group was 47.4±23.17 ng/ml. Serum HNL cut-off value was 84ng/ml. HNL sensitivity was 87% and specificity was 90%.***

Conclusion: ***Human neutrophil lipocalin can be used as a novel biomarker in detection of increasing neutrophilic inflammation during exacerbation of pediatric chronic respiratory diseases.***

Keywords: ***Chronic lung diseases, exacerbation, children, Human neutrophil lipocalin.***

INTRODUCTION

Pediatric chronic lung diseases may include any of the following; bronchiectasis, interstitial pulmonary fibrosis, bronchopulmonary dysplasia, bronchiolitis obliterans, sequestrated lung disease and cystic fibrosis. They can be primary or secondary. They can be congenital or acquired.[1,2] Exacerbation of chronic pulmonary diseases represents an increase in inflammation that is present in stable state, with increased numbers of inflammatory cells particularly neutrophils, cytokines, protein and proteases in the airways and blood.[3] Clinically, it can be suspected in patients with fever, increased cough, increased sputum production over baseline, increased viscidity of sputum, a foul odor of the sputum and less specific symptoms include dyspnea, pleuritic chest pain, and wheezing.[4]

Human neutrophil lipocalin (HNL) is a newly discovered protein from human neutrophil secondary granules. It is located in bone marrow cells as well as lung, bronchial and colonic epithelial cell.[5] Its concentrations increase in the sera of patients with acute bacterial infections, and it appears to be more specific and sensitive in the distinction between viral and bacterial infections.[6] This newly discovered protein HNL has been shown to distinguish between chronic bacterial colonization or infection and acute bacterial pulmonary exacerbation in cystic fibrosis, with higher specificity and sensitivity than other neutrophil markers such as myeloperoxidase or lactoferrin.[7]

Aim of the work: The present study aimed at evaluating serum human neutrophil lipocalin (HNL) as a novel marker of ongoing neutrophilic inflammation in chronic lung diseases.

PATIENTS AND METHOD

Study: This was an observational case-control study, that was carried out during the period from October, 2007 till May, 2009 at the Pediatric Chest clinic, Children's hospital; Ain Sham University hospitals.

Subjects: Study included forty children with chronic pulmonary diseases; their mean age was 6.7±2.88 years, twenty two (55%) were females and eighteen (45%) were males. Thirty were in exacerbation (group I). Acute pulmonary exacerbation was defined as a significant increase of C-reactive protein (CRP), increase of cough and purulent sputum production, fever, new lung infiltrates on chest radiography and deterioration of oxygen saturation and of lung function.[7] Ten patients were not fulfilling these criteria and were considered to be in stable condition (group II). Another ten -age and sex matched- healthy children without evidence of infection were included as a healthy negative control group (group III), (six males and four females with a mean age of 5.9±3.49 years).

Parental agreement was obtained in all cases and drawing of blood was only performed in routine sampling for clinical examination. The Pediatric Department Board ethically approved the study.

Exclusion criteria: Patients who received antibiotic treatment during the last 2 weeks, asthmatic patients and tuberculous patients were excluded from the study.

Assessment of clinical conditions and routine laboratory investigations

Patients were reviewed for demographic features and clinical presentation (e.g. fever, difficulty of breathing, cough and sputum amount, odor and color). Laboratory investigations for active infection: complete blood picture using coulter counter (T660), with total and differential leukocytes count, erythrocyte sedimentation rate by Westergren method and C-reactive protein (CRP) assayed in batches, using a nephelometer analyzer (Behring Diagnostics, Montreal, Quebec).

Assessment of serum human neutrophil lipocalin

Blood sampling, handling and storing were performed according to the manufacturer's instructions. HNL, in serum was analyzed by an enzyme-immunoassay technique (Pharmacia CAP System HNL FEIA; Pharmacia, Uppsala, Sweden). In brief, anti-HNL is coupled to Immuno-CAP. Standards and samples are then added and incubated at room temperature for 30 min. After washing, b-galactosidase-conjugated anti-HNL is added and incubated at room temperature for 2.5 h. After washing, 4-methylumbelliferryl b-D-galactoside is added to form a fluorescent product which can be detected after 10 min. The measuring range of this test is 20±600 mg.L-1. The inter-assay coefficient of variation ranged 4.6±6 % for HNL concentrations 53±462 mg.L-1.

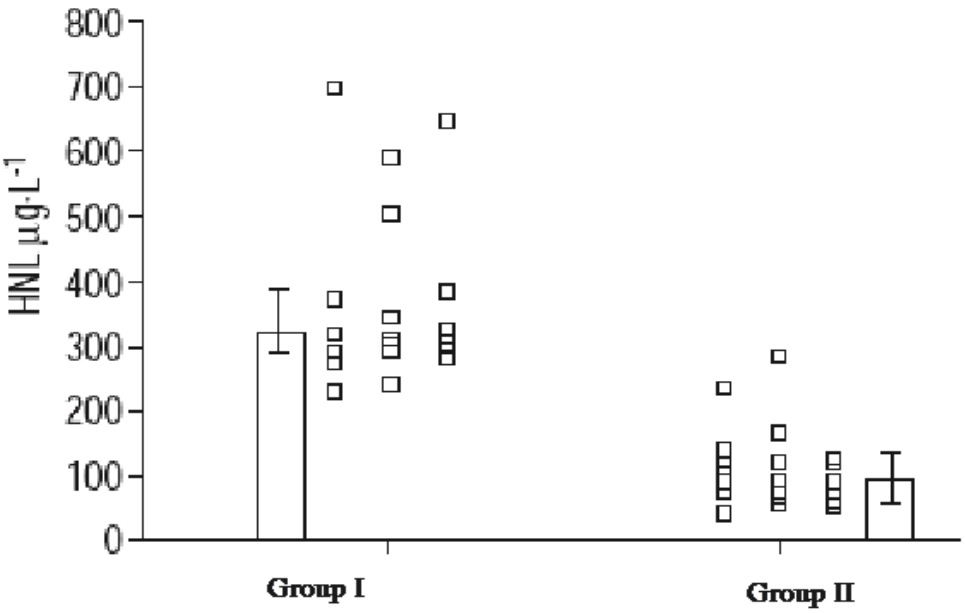
Statistical analysis

Collected data entered to an IBM computer using SPSS (Statistical Program for Social Science) version 15 to be analyzed as follows: quantitative variables as mean (SD) and range, qualitative variables as number and percentage. Chi-Square test X^2, ANOVA, Fisher exact test and correlation coefficient test were performed. P values less than or equal to 0.05 were considered statistically significant. ROC curve was used to assess cut-off value, specificity and sensitivity.

RESULTS

Study included forty children with chronic pulmonary diseases, seven had cystic fibrosis (CF) (17.5%), fifteen were non-CF bronchiectasis (37.5%), while eighteen were diagnosed as interstitial lung disease (45%). Thirty were in exacerbation (group I) and ten were in stable condition (group II). Twenty two (55%) were females and eighteen (45%) were males. Their mean age was 6.7±2.88 years. Ten healthy children included as negative control (six males and four females with a mean age of 5.9±3.49 years) (group III).

Symptoms of exacerbation among group I (n=30) were; increased cough in 100%, increased sputum production in 86.7%, whilst both fever and dyspnea represented 80% Table 1. Laboratory investigations done for them showed that total leucocytes count mean ±SD was 16985.77 ±5344 cells/106, of them neutrophils count mean ±SD represented 8888.6 ±2237.35 cells/106. Erythrocyte sedimentation rate first hour (ESR) ranged from 14 to 23 mm/hour. C-reactive protein (CRP) was more than 5 IU/liter in only 18 patients out of 30 (60%). The mean level of serum lipocalin (HNL) among these patients in exacerbation was 189.8±68.64 ng/ml.



Data are presented as the median value and the quartile 1, quartile 3; range and individual values.
Fig 1. Serum human neutrophil lipocalin in acute pulmonary exacerbation (Group I) in comparison with patients in stable clinical condition (Group II).

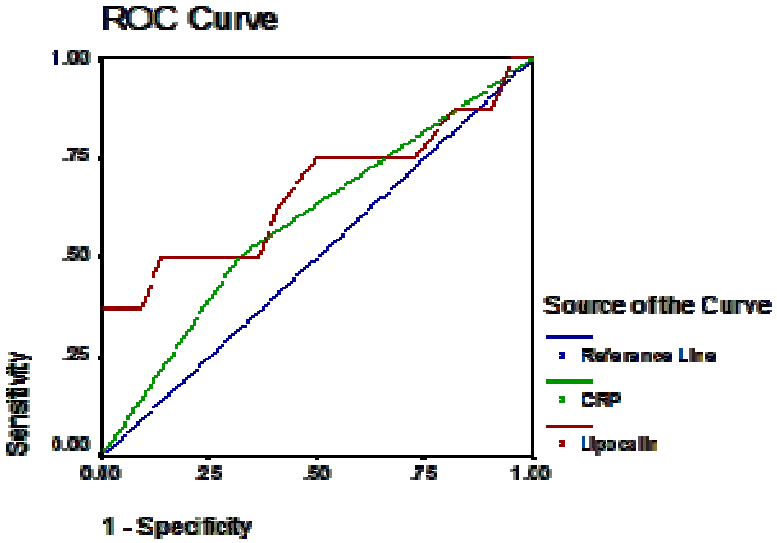


Fig 2. ROC curve demonstrates sensitivity and specificity of lipocalin and C-reactive protein (CRP) among studied patients (Groups I and II, n=40).

Table 1. Distribution of Exacerbation's Symptoms and Signs among Patients in Group I (n=30).

Symptoms and Signs	No. of patients	%
Cough	30	100%
Increased sputum	26	86.7 %
Fever	24	80.0 %
Dyspnea	24	80.0 %
Wheezes	22	73.3 %
Chest retraction	20	66.7 %
Diminished air entry	18	60.0 %
Crepitations	18	60.0 %
Chest deformity	10	33.3 %
Clubbing	8	26.7 %

Table 2. Comparison of Mean Lipocalin level among group I (n=30), group II (n=10) and group III (n=10).

Lipocalin level in ng/ml	Group I	Group II	Group III	P
Mean ±S.D.	189.87±68.64	76.2±29.13	47.4 ±23.17	0.000*

P: Highly significant.

Table 3. Correlation Between Lipocalin And CRP Among All Patients (n=40).

	Mean ± SD	R	P
CRP (IU/L)	3.77±0.490	0.473	0.008*
Lipocalin (ng/ml)	138.64 ±84.526		

* Significant positive correlation.

In contrast to stable patients group (group II, n=10) whose negative CRP (< 5 IU/Liter) considered as an inclusion criterion, erythrocyte sedimentation rate first hour (ESR) ranged from 11 to 18 mm/hour and mean level of lipocalin (HNL) was 76.2±29.13 ng/ml . As regards the healthy control (group III, n=10), mean level of lipocalin (HNL) was 47.4±23.17 ng/ml. This showed a very highly statistical significant difference among the three studied groups (P<0.05) Table 2. (Fig. 1).

ROC curve (Fig. 2) showed that serum human neutrophil lipocalin (HNL) cut-off value was 84 ng/ml. HNL sensitivity was 87% and specificity was 90%, while C-reactive protein (CRP) showed sensitivity 50% and specificity 32% with a cut-off value of 5 IU/liter among studied patients' group (groups I and II, n=40).

Correlation coefficient test showed a positive correlation between CRP and lipocalin in the studied patients' group (n=40) $p < 0.05$ Table 3.

DISCUSSION

Chronic lung disease is a condition in which damaged tissue in the lung causes breathing and health problems.[8] Warning signs of chronic lung diseases are shortness of breath after little or no exertion, difficulty in drawing air or breathing out, cough lasting more than a month, regardless of the age, chronic sputum production lasting a month or more, wheezing or noisy breathing and coughing up blood.[9] Chronic inflammation causes lung damage which, in turn, facilitates persistent infection. Acute inflammation is an important host defense against bronchial infection, but if it fails to clear the infection and becomes chronic it can cause lung damage and lead to disease progression.[10-13]

Since pulmonary inflammation, neutrophil activity and tissue destruction were found to directly correlate, a sensitive and specific marker for the degree of pulmonary inflammation, which can also be used as a therapeutic monitoring tool, seems warranted. In addition, the ongoing chronic inflammatory process within the lungs makes it difficult to accurately diagnose an acute pulmonary exacerbation.[7] Recently, a new protein of the secondary granules, human neutrophil lipocalin (HNL), was discovered.[14] Further studies[15,16] have shown that HNL was secreted more readily from the neutrophils than any other specific marker protein. Therefore, the present study aimed at evaluating the diagnostic value of human neutrophil lipocalin (HNL) as a novel inflammatory marker for ongoing neutrophilic inflammation during acute exacerbation of chronic lung diseases in children. It included 30 children in exacerbation (group I) and 10 patients in stable condition (group II). Ten healthy children age and sex matched were included as negative control group (group III).

Patients' age (n=40) ranged between 1 year and 13 years with mean age 6.7±2.88 years. Twenty two (55%) were females and eighteen (45%) were males. Although the mean age at presentation was of school age, children had

been symptomatic since early childhood. This agreed with both studies done by Karadag et al[17] and Ibrahim et al.[2] In contrast, Eichler and coworkers[7] reported a median age of 14.5 yrs (range 8±17 yrs) among children with CF–bronchiectasis.

In previous studies,[18,19] pediatric bronchiectasis without an identified cause accounted for between 26 and 63% of all cases of non-CF pediatric bronchiectasis .In addition, Ibrahim and coworkers[2] reported 43% of studied children with chronic lung diseases had non-CF bronchiectasis, 20% confirmed to be cystic fibrosis and 37% had interstitial lung diseases . While current study included fifteen children with non-CF bronchiectasis without identified cause (37.5%) and seven with cystic fibrosis (CF) (17.5%), while eighteen were diagnosed as interstitial lung disease (45%).

Current results also disagreed with Ehrenkranz et al[20] who found difference in clinical diagnoses of randomly selected patients with chronic lung diseases (n=35); 45% of cases were diagnosed as cystic fibrosis (CF), 30% with non-CF bronchiectasis, and 25% with interstitial lung disease and unresolved pneumonia. This may be attributed to racial, environmental and genetic differences. Moreover, we depended only on sweat chloride test to survey for CF while other studies used both sweat chloride test and genetic survey.

The present study showed that 100% of cases in exacerbation were presented by cough followed by increased sputum production (86.7%) and fever (80%).Similar results were found by Barker[21] who studied 50 patient during exacerbation of chronic lung diseases and demonstrated that a worsening of cough and an increase in the volume and purulence of sputum are often used as criteria for identifying exacerbations (90% of cases) followed by fever, then other manifestations.

In agreement with several studies,[19,22,23] present results showed that erythrocyte sedimentation rate first hour (ESR) ranged from 14 to 23 mm/hour in children with exacerbation (n=30); of them only 60% of cases had positive CRP (> 5 IU/Liter) while 40% had negative CRP. On contrary, Silvestre et al (24) who studied prognostic value of CRP in 40 septic adult patients with chronic pulmonary diseases and found that 95% of them were positive CRP while 5% had negative CRP which can be attributed by different age category.

In agreement with both studies done by Wilson et al[25] and Konstan et al,[26] mild elevation of ESR first hour in the stable group II (n=10) was reported. This could be due to continuous inflammatory process resulting in airway injury and remodeling.[26]

In the present study, there were highly statistical significant differences between mean values of lipocalin levels in the patients with chronic lung diseases in exacerbation over patients not in exacerbation and control healthy group (P=0.000). The mean lipocalin level in group I was 189.8±68.64ng/ml, in group II was 76.2±29.13ng/ml and in group III was 47.4±23.17 ng/ml. These data came in concordance with the studies done by Eichler et al[7] and Metso et al.[27]

The fact that CRP and blood neutrophil counts were taken into account in the classification of children into exacerbation or stable condition. Thus, the study was biased towards CRP and/or blood neutrophils. It should be emphasized that the purpose of the study was not that of a diagnostic study with the intention of a head-to-head comparison of HNL and CRP. A study on the diagnostic sensitivity and specificity of any test requires the identification of a gold standard that enables the accurate diagnosis of both positive and negative cases.[7]

However, study found a significant positive correlation between CRP and serum lipocalin. That comes in agreement with study of Fjaertoft et al;[6] but they reported that differences in the kinetics of CRP and HNL make HNL a better marker for ongoing infection and the additional diagnostic power of HNL over neutrophil counts is likely to be a result of the active secretion of HNL from the activated neutrophils.

The study showed that the cut-off value of serum lipocalin level was 84ng/ml which was similar to the study done by Eichler et al[7] who found that the cut-off value of lipocalin level was 86.4ng/ml. In contrary, the study done by Fjaertoft et al[6] who reported that the cut-off limits for HNL was 155 ng/ml but they selected studied patients all with age above 9 years. Moreover, the present study revealed that sensitivity of lipocalin (HNL) was 87% and its specificity was 90%; while C-reactive protein (CRP) showed a sensitivity of 50% and a specificity of 32%.

In concordance, Seveus et al[28] who found that sensitivity of lipocalin was 100%, its specificity was 90% while sensitivity of CRP was 66% and specificity 78%. Moreover, Fjaertoft et al[6] concluded in their study that HNL is a promising diagnostic tool in the distinction of acute infections caused by bacteria or virus. The differences in the kinetics of CRP and HNL make HNL a better marker for monitoring antibacterial treatment, since HNL is probably elevated only when an active bacterial infection is at hand.[6]

Current study was limited in having no definite gold standard for comparison. However, in clinical studies, this gold standard is usually not available, and diagnosis of positive and negative cases is therefore often based on the "next best thing", i.e. the judgment of skilled and unbiased clinicians based on clinical and laboratory data. The accurate diagnosis of exacerbation in chronic lung illness is no exception to these difficulties.[7]

CONCLUSION

Pulmonary inflammation, neutrophil activity and tissue destruction were found to directly correlate, a sensitive and specific marker for the degree of pulmonary inflammation seems warranted. In addition, the ongoing chronic inflammatory process within the lungs makes it difficult to accurately diagnose an acute pulmonary exacerbation so clinical assessment is not enough. Thus human neutrophil lipocalin (HNL) can be a new sensitive marker of exacerbation and increased neutrophilic inflammation in children with chronic respiratory diseases. However, more studies are needed to evaluate its prognostic value and its role in follow up therapeutic tools among this category of diseased children. Further studies to assess serum HNL in relation to sex and age are also needed.

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