Research Article

Prognostic value of serum proadrenomedullin level in children with community-acquired pneumonia

Osama G. Mohammed, Asmaa N. Reyad, Mahmoud M. Moussa, and Dalia M. Shams El-din
Department of Pediatrics, El-Minia Faculty of Medicine

Abstract
Introduction & Aim of the work: Community-acquired pneumonia (CAP) is one of the most common infectious diseases addressed by clinicians. CAP is an important cause of mortality and morbidity worldwide A number of pathogens can give rise to CAP. Typical bacterial pathogens that cause the condition include Streptococcus pneumoniae (penicillin- sensitive and -resistant strains), Haemophilus influenzae (ampicillin-sensitive and -resistant strains), and Moraxella catarrhalis (all strains penicillin-resistant). These 3 pathogens account for approximately 85% of CAP cases (1).
Subjects and Methods: The study included 80 children who were collected from the Pediatric department at Minia Health Insurance Hospital during the period from August 2015 to February 2016. They **were divided into two groups:- Results:** This study was done in pediatric department at Minia Health Insurance Hospital during the period from August 2015 to February 2016 and included 80 children. They were divided into 2 groups: **Group I:** (Study group) included 50 children with the age ranged from 18 months to 48 months with Mean age (32.6±8.2), 32 were males (64%) and 18 were females (36%) as shown in table (1) **Group II:** (control group) included 30 apparently healthy children who were selected from outpatient clinic with age ranged from 24 months to 48 months with Mean age (32.2±9.1), 24 were males (80%) and 6 females (20%) as shown in table (1).
Keywords: CGRP: Calcitonin gene related peptide, CDC: Center of disease control and prevention, CAP: Community acquired pneumonia

Introduction
Community-acquired pneumonia (CAP) is one of the most common infectious diseases addressed by clinicians. CAP is an important cause of mortality and morbidity worldwide A number of pathogens can give rise to CAP. Typical bacterial pathogens that cause the condition include Streptococcus pneumoniae (penicillin- sensitive and -resistant strains), Haemophilus influenza (ampicillin-sensitive and -resistant strains), and Moraxella catarrhalis (all strains penicillin-resistant). These 3 pathogens account for approximately 85% of CAP cases (Howard et al., 2010).

The most common symptoms of pneumonia are cough, fever which may be mild or high, shacking chills, shortness of breath and loss of appetite (Limper et al., 2011).
Peptide hormone Adrenomedullin (ADM or AM) is a gene that in humans is encoded by the ADM Adrenomedullin consists of 52 amino acids. lt may function as a hormone in circulation control because it is found in blood in a considerable concentration. The precursor, called preproadrenomedullin, is 185 amino acids long. By RNA-blot analysis, human adrenomedullin mRNA was found to be highly expressed in several tissues (Kitamura et al., 2000).
AM was initially identified as a vasodilator, some have cited this as the most potent endogenous vasodilatory peptide found in the body. Differences in opinion regarding the ability of AM to relax vascular tone arises from the differences in the model system used (Hamid and Baxter, 2005)

ProADM is a prognostic marker and predicts the severity of community acquired pneumonia. Two main mechanisms might be responsible for the increase of circulating proADM in infections, including CAP. Firstly, as a member of the calcitonin gene family, ADM is widely expressed and extensively synthesized during severe infections similar to other calcitonin peptides, namely procalcitonin and calcitonin-gene related peptides (Becker et al., 2004)
Bacterial endotoxins and proinflammatory cytokines up-regulate ADM gene expression in many tissues (Shoji et al., 1995).

In addition, a decreased clearance by the kidneys may be responsible in part for the increased proADM levels in infections (Hirata et al., 1996)

Subjects and methods

The study included 80 children who were collected from the Pediatric depar tment at Minia Health Insurance Hospital during the period from August 2015 to February 2016. They were divided into two groups:

Group I: (Study group) included 50 children with proven diagnosis of community acquired pneumonia which was defined as an acute pulmonary infection in a previously healthy individual acquired in the community (Esposito and Principi, 2012).

It can be defined as the presence of at least one of the following symptoms: cough, expectoration, dyspnea, chest pain, fever, abnormal lung auscultation or leukocytes > 10,000 or < 4,000 cells/p, L in combination with a new infiltrate on a chest X-ray (Christ-Crain et al., 2005).

Group II: (Control group) included 30 apparently healthy children who were selected from outpatient clinic.

Inclusion criteria:

1- Children aged 1-4 years

2- Children already diagnosed as community acquired pneumonia (clinically, CRP, WBCs count).

Exclusion criteria

1- Children under 1 year

2- Children who need ventilator

3- Features suggestive of:

* Immuno-compromised patients
* Chronic medical conditions predisposing to severe pneumonia as chronic cardiopulmonary disease
* Pneumonia complicated at time of admission
* Patients receiving antibiotics for previous diagnosis of pneumonia

4- Hospital-acquired pneumonia patients

All studied groups were subjected to the following:

1- Careful history taking including demographic and epidemiological data, symptoms of the current disease and previous antibiotic treatment

2- Through clinical examination including chest examination which revealed tachypnea, increased work of breathing, crackles (rales), retractions, rhonchi, and nasal flaring.

3- Chest X-ray (for group I only)

4- Laboratory investigations

Indications for hospitalizations and intensive care admission (Esposito and Principi, 2012).

• Hypoxia (oxygen saturations < 90%-92%)

• Infants < 3-6 months with suspected bacterial infection (unless a viral cause or Chlamydia trachomatis is suspected and they are normoxemic and relatively asymptomatic)

• Tachypnea:

* Infants <12 months of age: respiratory rate >70 breaths/min C
* hildren: respiratory rate >50 breaths/min

• Respiratory distress: apnea, grunting, difficulty breathing, and poor feeding

• Signs of dehydration

• Inability to maintain hydration or oral intake

• Capillary refill time >2 seconds

• Infants and children with toxic appearance or suspected or confirmed to have infection with a virulent organism (CA-MRSA or group A Streptococcus)

• Underlying conditions comorbidities that:

* May predispose patients to a more serious course (eg, cardiopulmonary disease, genetic syndromes, neurocognitive disorders)
* May be worsened by pneumonia (eg, metabolic disorder)
* May adversely affect response to treatment (eg, immunocompromised host, sickle cell disease)

• Complications (eg, effusion/empyema)

• Failure of outpatient therapy (48-72 hours with no response)

• Caretaker unable to provide appropriate observation or to comply with prescribed home therapy

Indications for intensive care admission include:

• Severe respiratory distress or impending respiratory failure requiring

B Intubation and mechanical ventilation

B Positive pressure ventilation

• Recurrent apnea or slow irregular respirations

• Cardiopulmonary monitoring due to cardiovascular compromise secondary to:

* Sustained tachycardia
* Inadequate blood pressure
* Requires pharmacologic support of blood pressure or perfusion
* Altered mental status due to hypercarbia or hypoxemia

Septic shock is defined as serious medical condition that occurs when sepsis, which is organ injury or damage in response to infection, leads to dangerously low blood pressure and abnormalities in cellular metabolism. The primary infection is most commonly by bacteria, but can also be by fungi, viruses, or parasites, and can be located in

any part of the body, but most commonly in the lungs, brain, urinary tract, skin, or abdominal organs (Vincent et al., 2013).

Sampling

5m1 of venous blood were withdrawn by sterile venipuncture then the whole blood was divided into:

1- One ml of venous blood was evacuated into EDTA-containing tube then mixed well for estimation of complete blood count

2-Four ml of venous blood were evacuated into plain tube, allowed to clot for 30 minutes then centrifuged at 3000 rpm for 15 minutes to separate serum. Half ml was used to estimate CRP. The remaining serum was kept frozen at -40°C until analysis of pro-adrenomedullin.

-Complete blood count (CBC)

-C-reactive protein (CRP)

-Measurement of serum pro-adrenomedullin during the first 24 hours of admission to the pediatric department.

A) Routine investigations

C-reactive protein (CRP):

CRP Test is based on the latex-agglutination method. The principle of this test is based on the immunological reaction between CRP as an antigen the corresponding antibody coated on the surface of biologically inert latex particles.

Complete blood count (CBC):

Complete blood count was determined by automated cell counter (coulter), SYSMEX-KX-2IM (TAO Medical Incorporation, Japan).

B) Special investigations

Serum pro-adrenomedullin

a) Principle of the test

This ELISA kit uses Sandwich-ELISA as a method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Pro-ADM. Samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Pro-ADM and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively incubated. Only those one that contain ProADM, biotynilated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by addition of sulphuric acid solution and color turns yellow. The optical density (OD) is measured spectophotometrically as a wave length of 450nm±2nm.The OD value is proportional to the concentration of Pro-ADM

b) Reagent preparation

All reagents were brought at room temperature (18-25)

-Wash Buffer: We diluted 30 ml of Concentrated Wash Buffer into 750 ml of Wash Buffer with distilled water and the unused solution was put back at 4°C

- Standard: we prepared standard within 15 minutes before use. First, we centrifuged it at 4000 rpm then we reconstituted the Standard with 1 ml of Reference standard &Sample Diluent. We tightened the lid and let it stand for l0 minutes and then we turned it upside down for several time.

After it dissolved fully we mixed it with a pipette. This reconstitution produced a stock solution of 50 pmol/ml. Then we made serial dilutions. The concentrations were as follows:

0.78 ⟶ 1.65 ⟶ 3.13 ⟶
12.5 ⟶ 25 ⟶ 500 Pmol/ml

The undiluted standard serves as the highest standard (50pmol/m1). The Reference Standard & Sample Diluent serves as zero (0 pmol/ml)

C) Assay procedure

1- We added 100 [1.L of Standard, or Sample per well. The blank well was added with Reference Standard & Sample Diluent. Solutions were added to the bottom of micro ELISA plate well then we mixed gently. We covered the plate with sealer we provided and left in the incubator for 90 minutes at 37°C

2- We removed the liquid of each well then immediately we added 100pi, of Biotinylated Detection Ab working solution to each well. We covered them with the plate sealer then we tapped the plate to ensure through mixing then left in incubator for 1 hour at 37°C

3- Wash: All contents of wells were removed by decanting. We washed by filling each well with Wash Buffer (Approximately 350gL). After the last wash, we removed remained Wash Buffer by decanting then we inverted the plate and pat it against thick clean absorbent paper.

4- We added 100pL of HRP Conjugate working solution to each well and then we covered them with the plate sealer and left in the incubator for 30 minutes at 37°C.

5- Wash: we repeated the wash process for 5 times as conducted in step3

6- We added 901AL of Substrate Solution to each well and covered them with a new plate sealer then we left them in the incubator for 15 minutes at 37°C.

7- We added 501AL of Stop Solution to each well. Then the color turned to yellow immediately

D) Calculation of results

We created a standard curve by plotting the mean OD value for each standard on the Y-axis against the concentration on the X axis then we drew a best fit curve through the points on the graph. A best fitting equation of standard curve was calculated using OD values and concentrations of standard sample The software will calculate the concentration of samples after entering the OD values of samples Statistical analysis: Statistical analyses were performed using the SPSS statistics version 19. Differences in the mean of continuous variables were analyzed using parametric test (independent sample T. test). And differences between categorical variables were analyzed using Chi Square test. The associations between continuous variables were determined using Pearson Product-Moment Correlation. For all tests, the values P<0.05 were regarded statistically significant.

Pediatric respiratory severity score (Reed et al., 2012)

Score Component	Operational definition	Scoring
Respiratory rate	Respiratory rate at rest, on room air*	0 or 1
Wheezing	High-pitch expiratory sound heard by auscultation	0 or 1
Accessory muscle use	Any visible use of accessory muscles	0 or 1
Sp02	Oxygen saturation <95% on room air	0 or 1
Feeding difficulties	Refusing feedings	0 or 1
	Sum of fie components	
PRESS score	0-1: mild 2-3: moderate 4-5: severe	0-5
Criteria of Tachypnea	Month Respiratory rate	

Results

Comparison between studied group and control group as regard Age and Sex

Variable	Studied group NO. =50	Control group NO. =30	P-VALUE
Age (months) Min-Max Mean ± SD	 18-48 32.6±8.2	 24-48 32.4±9.1	0.9
Sex No. (%) Males Females	 32 (64%) 18 (36%)	 24 (80%) 6 (20%)	0.1

***: Significant difference in between groups (p value 5_ 0.05)**

Comparison between cases and control as regard HB, WBCs and PLTs

Variable	Studied group NO. =50	Control group NO. =30	P-VALUE
Hemoglobin (g/dl)	9.5±1.1	12.9±0.4	0.001*
WBCs (billion/L)	12.7±1.9	8.20.8	0.001*
PLI's (billion/L)	299±68.6	313.8±51.6	0.3

***: Significant difference in between groups (p value 5 0.05)**

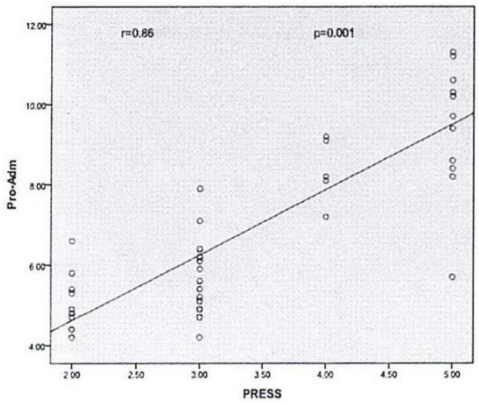
Comparison between studied group and control group as regard pulse, RR, Systolic BP and Diastolic BP

Variable	Studied group NO. =50	Control group NO. =30	P-VALUE
Pulse (beat /minute)	134±8.7	77±4.1	0.001*
RR (cycle/minute)	65.6±4.2	22.4±1.7	0.001*
Systolic BP (mmHg)	98.1±10.8	115±4.5	0.001*
Diastolic BP (mmHg)	65.6±10.5	76.7±4.8	0.001*

Shows correlation between Pro ADM level and Pediatric respiratory Severity Score (PRESS)

	PRESS	
Pro- ADM(nmol1L)	R	P-VALUE
	0.86	0.001*

shows correlation between Pro ADM and Pediatric respiratory Severity Score (PRESS)



Discussion

Globally each year, 1.5 million children 5 years of age and younger suffer a pneumonia-related death, particularly in developing countries **(**Bradley et al., 2011). CAP can be defined as the presence of signs and symptoms of pneumonia in a previously healthy child caused by an infection that has been acquired outside the hospital (Bradley et al., 2011).

The clinical presentation of childhood pneumonia varies depending upon the responsible pathogen, the particular host, and the severity. The presenting signs and symptoms are nonspecific; no single symptom or sign is pathognomonic for pneumonia in children (Murphy et al., 2007).
Proadrenomedullin (ProADM) is a peptide with vasodilatory, antimicrobial and anti-inflammatory properties. Specifically, its midregional fragment (MRproADM) has been associated with mortality in patients with CAP (Seligman et al., 2012).

Predicting the risk of death is crucial in the emergency department, but at the same time, the level of care required by patients cannot be assessed only in terms of mortality (Renaud et al., 2012).
Our study was carried on 80 children, who were collected from the Pediatric department at Minia Health Insurance Hospital and divided into 2 groups, group I included 50 children with proven diagnosis of community acquired pneumonia and group II included 30 apparently healthy children who were selected from outpatient clinic. Our study aimed to evaluate the prognostic value of Pro-adrenomedullin level in children with community acquired pneumonia.

Our results show statistical significant higher WBCs count and statistical significant lower HB level in studied group than control group (P-value=<0.001) which is parallel to the study done by Theodore and Charles 2010 and this is explained as it is a well-known response for sepsis as CAP is a main cause of sepsis community acquired pneumonia.

In accordance to our study we found that our patients had significantly higher Pro-adrenomedullin level than controls and this is in agreement with study done by Huang et al., 2009 who studied Midregional Proadrenomedullin as a Prognostic Tool in Community-Acquired Pneumonia and found that MR-proADM levels correlate with increasing severity of illness and death. High MR-proADM levels offer additional risk stratification in high-risk CAP patients and they explained that as MR-proADM is one of the most potent vasodilating agents and has additional immune-modulating metabolic properties, also it has a bactericidal action which is further enhanced by modulation of complement activity and regulation.
Also another study done by Linscheid et al., 2005 who studied autocrine/paracrine role of inflammation-mediated calcitonin gene-related

peptide and adrenomedullin expression in human adipose tissue and founded that human adipose tissue is a contributor to inflammation- and sepsis-induced elevation of serum procalcitonin (ProCT). Several calcitonin (CT) peptides, including ProCT, CT gene-related peptide (CGRP), and adrenomedullin (ADM) are suspected mediators in human inflammatory diseases and they explained that by two main mechanisms might be responsible for the increase of circulating proADM in infections, including CAP. Firstly, as a member of the calcitonin gene family, ADM is widely expressed and extensively synthesized during severe infections, that is, sepsis, similar to other calcitonin peptides, namely procalcitonin and calcitonin-gene related peptides and the second one is a decreased clearance by the kidneys may be responsible in part for the increased proADM levels in infections.

our study we found respiratory rate is significantly higher in patients than in control which is similar to a study done by Culvert et al., 2006 who studied community acquired pneumonia in children and found that physical examination may reveal respiratory rate may be faster than normal, and this may occur a day or two before other signs and they explained it by that the invading organism causing pneumonia provokes an immune response in the lungs that causes inflammation of the lung tissue (pneumonitis), a condition that actually makes the lung environment more ideal for infection. Small blood vessels in the lungs (capillaries) begin to empty protein-rich fluid into the alveoli, a condition that results in a less functional area for oxygen-carbon dioxide exchange. The individual becomes relatively oxygen deprived, while retaining potentially damaging carbon dioxide.
This results in rapid respiration (tachypnea or faster and faster breathing) in an effort to bring in more oxygen and blow off more carbon dioxide.

The PRESS scoring system is useful for initial assessment in respiratory tract infection to identify the need for hospitalization and further examination in emergency settings, also used for assessing the severity of infection caused by several pathogens (Reed et al., 2012). In our study we found a strong positive correlation between Pro-ADM and PRESS (r=0.86, p=0.001) as PRESS can predict the severity of CAP so, Pro-ADM can be used as a predictor of pneumonia severity because the elevated level of it is significantly associated with both short-term mortality and complications in patients with CAP as reported in the study done by Christ-Crain et al., 2006 who studied Pro-adrenomedullin to predict severity and outcome in community-acquired pneumonia. In our study we found that Pro-ADM level was significantly higher in studied cases that need ICU admission than studied cases that did not need ICU admission (P-value 0.001).

Similar to us, a multicenter study done by Huang DT et al., 2009 who found that higher levels of pro-ADM at admission were associated with an increased need for intensive care. In contrast to us, one pediatric study done by Sarda Sanchez et al., 2012 who studied Pro-adrenomedullin usefulness in the management of children with community-acquired pneumonia and found that there was no statistical significant relation between pro-ADM levels and admission to ICU (1.4 vs. 0.9, pl1=0 0.5). As regarding Pro-ADM and mortality, we found the level of Pro-ADM was significantly higher in studied cases who died than improved cases so, Pro-ADM can also predict mortality. Similar to us a study done by Huang DT et al., 2009 who found that higher levels of pro-ADM at admission were associated with increased mortality rate.
As regard our study we found that there was weak positive correlation between WBCs count and Pro-ADM level ($r=0.24$, $p < 0.001$), which is the same finding in the study done by Christ-Crain et al., 2006 who found that ProADM levels correlated with total leukocyte count in a weak or no correlation ($r = 0.23$, $p < 0.001$).

As regard length of hospitalization, there was a moderate positive correlation between Pro-ADM level and length of hospitalization($r=0.71$, $p= 0.001$) which increase with increasing severity of CAP so, Pro-ADM correlate with severity of CAP.
In contrast to us a study done by Sarda Sanchez et al., 2012 who found that there was no statistical significant relation between pro-ADM levels and length of hospital stay ($r =0.2$; p 0.2)
According to our study ROC curve was done as regard Pro-ADM in predicting complications of CAP as pleural effusion and empyema we found AUC (area under curve) 0.813, sensitivity of Pro-adrenomedullin in predicting complications was 81.3%, specificity of Pro-adrenomedullin in predicting complications was 90.2%, positive predictive value was 88.2%, negative predictive value was 81.9%, cutoff value was <1.1

In the study done by Bello et al., 2012 who studied Prognostic power of proadrenomedullin in community-acquired pneumonia is independent of aetiology and ROC curve was done as regard Pro-ADM in predicting complications of CAP and found The optimal cut-off for predicting complications for MR-proADM was 0.933 nmo1L-1 (sensitivity 77.35%; specificity 86.23%) the AUC for Pro-ADM was 0.706

Another study done by Dan et al., 2016 who studied Prognostic value of mid-regional pro-adrenomedullin (MR-proADM) in patients with community-acquired pneumonia: a systematic review and meta-analysis and ROC curve was done as regard MR-proADM in predicting complications of CAP which displayed moderate diagnostic accuracy for predicting complications in CAP (AUC) 0.74 and the optimal cut-off for predicting complications for MR-proADM was 0.833 nmol•L-1

Also a study done by Susana et al., 2015 who studied Usefulness of Midregional Proadrenomedullin to Predict Poor Outcome in Patients with Community Acquired Pneumonia and ROC curve was done as regard MR-proADM in predicting complications of CAP.
The optimal cut-off point for MR-proADM for predicting adverse event was 0.85 nmol/L, 97% sensitivity and 66% specificity.

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