Original Research Article

The Egyptian Journal of Immunology
Volume 30 (4), 2023: 111–120.
www.Ejimmunology.org

Calcitonin gene-related peptide and its correlation with prognosis in severe pneumonia among children below 6 years at PICU of Al Zahraa university hospital

Sara Saad[1], Soheir Ibrahim[2], Shimaa Moustafa[2], and Fatma Elzhraa AE Diab[3]

[1]Department of Pediatrics, Ministry of Health, Cairo, Egypt.
[2]Department of Pediatrics, Faculty of Medicine (for Girls) Al-Azhar University, Cairo, Egypt
[3]Department of Clinical Pathology, Faculty of Medicine (for Girls) Al-Azhar University, Cairo, Egypt

Corresponding author: Sara Saad, Department of Pediatrics, Ministry of Health, Cairo, Egypt.
Email: s_motawea@yahoo.com

Abstract

Pneumonia is known to be the biggest cause of death in children younger than five years old. In pulmonary diseases such as pulmonary arterial hypertension, asthma, acute lung injury, and pulmonary fibrosis, calcitonin gene related peptide (CGRP) has been linked to the regulation of inflammation, proliferation, and fibrosis. However, its ability to foretell the emergence of severe pneumonia is questionable. We aimed to determine whether blood levels of CGRP correlate with the outcome of critically ill children. This case-control study included 45 children with severe pneumonia admitted to the pediatric intensive care unit and 45 children with matched age and sex as controls. We investigated the serum level of CGRP as well as routine laboratory investigations of both groups. The CGRP level was lower in the patient group with median of 77 ng/L ranged from 55 to 183 as compared to control group with median of 230 ng/L ranged from 133 to 664 ($p \leq 0.001$). Also, CGRP level was significantly higher in the survived group with median of 96.1 ng/L ranged from 55 to 183 than the non-survived group with median of 63.4 ng/L ranged from 55.5 to 120.9 (p=0.022). In conclusion, we found that serum level of CGRP was extremely low in critical and extremely critically ill patients, and thus can be used as a predictor of mortality in children with severe pneumonia.

Keywords: Severe pneumonia, Pediatric intensive care unit, Calcitonin Gene-Related Peptide.

Date received: 10 June 2023; **accepted:** 07 September 2023

Introduction

The most common cause of morbidity and mortality in pediatric patients worldwide is acute respiratory infection, placing a significant strain on healthcare systems. Infections with severe acute lower respiratory infections accounted for approximately 11.9 million hospital admissions of young children worldwide.[1] More than 95% of pediatric pneumonia deaths worldwide, which affect children under the age of 5, take place in underdeveloped nations.[2]

Untreated acute respiratory infections in Egypt frequently result in pneumonia, a more dangerous condition that kills 15% of children under the age of five.[3] When the innate immune system fails to eliminate a pathogen from the distal bronchi and alveoli, pneumonia develops. Pneumonia is an infection of the alveoli. [4] The usage of broad-spectrum antibiotics and hospitalization among low-risk persons may be decreased thanks to prognostic models and studies. However, there are no reliable models that can forecast how children with pneumonia would fare clinically.[5]

Calcitonin Gene-Related Peptide (CGRP) is a 37 amino acid vasoactive neuropeptide derived from Calcitonin gene localized in chromosome 11 and widely distributed in the central and peripheral nervous system and the cardiovascular system. It has two forms, α and β CGRP. α- CGRP is found in neuronal tissues and a potent vasodilator and β- CGRP predominant in the enteric nervous system and pituitary gland.[6] In this study, we will only refer to α-CGRP when describing CGRP.

CGRP has several functions where it has a role in the central nervous system and modulates acetylcholine receptor function. It also modulates antigen presentation in Langerhans cells. It blocks tolerance to morphine. It is a potent inhibitor of gastric acid secretion. It has a role in cardiovascular system where it has a potent vasodilator, positive ionotropic and chronotropic effect. It is well recognized that CGRP plays a key role in the series of circumstances that result in a migraine episode.[7]

Alveolar epithelial type II cells are protected by CGRP against DNA damage, oxidative stress injury, and apoptosis brought on by hyperoxia. [8] In China, Tao et al., 2020[9] investigated CGRP to determine how children diseased with severe pneumonia would fare. To our knowledge, changes in CGRP expression and their significance for diagnosis or prognosis of severe pediatric pneumonia have never been studied in Egypt. Therefore, the purpose of this research was to determine whether elevated blood levels of CGRP are associated with a better prognosis for children with severe pneumonia.

Subjects and Methods

Study setting and design

This longitudinal case control study included 45 children with severe pneumonia admitted at the pediatric critical care unit, Al Zahraa University hospital during the period from October 2020 to December 2021. Patients included both sexes, their ages ranged from one month to 6 years. In addition, 45 age and sex matched apparently healthy children, attended to the outpatient clinic with non-respiratory condition, were included in the study as a control group.

The study protocol was ethically reviewed and approved by the Research Ethics Committee of the Faculty of Medicine for Girls Al Azhar University, Egypt (approval date: August 2020). The importance of the study was explained to the parents of the participating children. An informed written consent was taken from the parents before enrolling the children in this study.

Inclusion criteria

Age range between one month to 6 years, patients hospitalized at the intensive care unit with pneumonia, in light of the WHO diagnostic criteria for childhood pneumonia 2020 [10] the subsequent:

Quick breathing or lower chest wall indrawing, which occurs when the chest slides in or out with inhalation and can occur with or without fever, are signs of pneumonia (in a healthy person, the chest expands during inhalation). Viral infections are more likely to cause wheezing. Infants that are really seriously unwell may not be able to eat or drink, and they may also become unconscious, develop hypothermia, and go into convulsions.

Exclusion criteria

These included Infants with malignant tumors, infants with severe malnutrition, Infants with autoimmune diseases, Infants with heart failure, liver failure, acute kidney injury or chronic kidney disease and infants with chronic central nervous system disease.

Methods

All patients and controls were subjected to full clinical taking of children history, demographic data including sex, age, and residence, socio-economic standard, and special habits.

Complete clinical examination, which included, thorough general examination including face, built, decubitus, colors, vital signs (pulse, blood pressure, temperature, and respiratory rate), head, neck, upper limb, lower limb, heart, abdomen, and anthropometric measurement (weight, height, head circumference, and body mass index, calculated by dividing weight by height in meter square.[11] Local chest examination including inspection (movement and shape), palpation (tracheal shift and tactile vocal fremitus), percussion and auscultation (breath sounds, adventurous sounds including rhonchi "sonorous or sibilant" and crepitation "fine, medium-sized and coarse" and vocal resonance).

Pediatric respiratory severity score (PRESS) for each patient was calculated[12]. The PRESS assessed tachypnea, wheezing, retraction (accessory muscle use), peripheral capillary oxygen saturation (SpO2), and feeding difficulties, with each component given a score of 0 or 1, and total scores were classified as mild (0–1), moderate (2–3), or severe (4–5).

Pediatric critical illness score (PCIS)[13] was calculated within 24 hours after admission, and the values were averaged as demonstrated in Table 1.

The total possible score is 100 points, and the severity of pneumonia is ranked as follows: >80 points, noncritical; 71–80 points, critical; and <70 points, extremely critical.[9]

Laboratory investigations

Within 24 hours after admission general laboratory tests were performed. These included complete blood count (CBC) which was done using fully automated cell counter (Sysmex KX21N, Kobe, Japan), according to the manufacturer's instructions. C-reactive protein (CRP) was done using latex agglutination kits (lot # A3256/1, CRP visilatex–slide assay), blood gases which was done by blood gas analyzer (Gem premier 3000, Werfen, Belgium), according to the manufacturer's instructions. Blood urea nitrogen (BUN), serum creatinine, Sodium and Potassium were done using a fully automated chemistry analyzer (Cobas c 311, Germany), using commercial kits supplied by (Roche Diagnostics, Germany), according to the manufacturer's instructions.

In addition, serum CGRP was determined by enzyme linked immunosorbent assay (ELISA) commercial kits (Catalog #: 90404, Glory Science Co., Ltd, USA), according to the manufacturer's instructions. An ELISA washer (106ff41412 Bio Tek, USA) was used during the process. The optical densities of the final ELISA products were measured using a microtiter reader (1851 Das, Italy), according to the manufacturer's instructions. Finally, a standard curve was obtained by plotting the concentration of the standards versus their absorbance values.

Statistical Analysis

The Statistical Package of Social Science (SPSS) application for Windows (version 21) was used to analyze data of this study. The Kolmogorov-Smirnov test was initially used to determine whether the data were normal. The subsequent tests were utilized, Chi square test to compare qualitative variables; Monte Carlo test and Fisher exact test to compare qualitative variables when expected count less than 5; Student t test to compare two quantitative variables (parametric); Mann Whitney test to compare two quantitative variables (non-parametric); Spearman correlation for comparison of numerical data (non-parametric). The receiver operating characteristic (ROC) curve analysis was used to determine the sensitivity and specificity at different cutoff points. The confidence interval was set at 95%, while the allowed margin of error was set at five %. Therefore, the *p*-value was considered significant at $p<0.05$.

Table 1. Pediatric critical illness score (PCIS).[13]

Variables	Measurement		Score
	<1-year-old	≥1-year-old	
Heart Rate (beats per minute)	<80 or >160	<60 or >160	4
	80-100 or 160-180	60-80 or 140-160	6
Other values			10
Systolic blood pressure kPa (mmHg)	<7.3 (55) or >17.3 (130)	<8.7 (65) or >20.0 (150)	4
	7.3-8.7 (55-65) or 13.3-17.3 (100-130)	8.7-10 (65-75) or 17.3-20.0 (100-130)	6
Other values			10
Respiratory Rate (breaths per minute)	<20 or >70 or irregular respiratory rate	<15 or >60 or irregular respiratory rate	4
	20-25 or 40-70	15-20 or 35-60	6
Other values			10
PaO2 kPa (mmHg)	<6.7 (50)	<6.7 (50)	4
	6.7-9.3 (50-70)	6.7-9.3 (50-70)	6
Other values			10
PH	<7.25 or >7.55	<7.25 or >7.55	4
	7.25-7.3 or 7.5-7.55	7.25-7.3 or 7.5-7.55	6
Other values			10
Sodium(mmol/L)	<120 or >160	<120 or >160	4
	120-130 or 150-160	120-130 or 150-160	6
Other values			10
Potassium(mmol/L)	<3.0 or >6.5	<3.0 or >6.5	4
	3.0-.5 or 5.5-6.5	3.0-.5 or 5.5-6.5	6
Other values			10
Creatinine (µmol/L)	>159	>159	4
	106-159	106-159	6
Other values			10
Blood Urea Nitrogen (mmol/L)	>14.3	>14.3	4
	7.1-14.3	7.1-14.3	6
Other values			10
Hemoglobin (g/L)	<60	<60	4
	<60-90	<60-90	6
Other values			10

(Collaborative PCIS Trail Group, 1998).

Results

There were 90 children in this case-control study, their age ranged from one month to six years. There was no difference in age and sex between the pneumonia patients and the control group (Table 2).

Table 3 shows a significant decrease in hemoglobin level (p<0.001) and absolute lymphocytic count (p=0.003) in the pneumonia patient group as compared to the control group. However, there was a significant increase in counts of platelet (p<0.001), monocytes (p=0.001), eosinophils (p<0.001), and basophils (p<0.001) in the pneumonia patient group when compared to the control group.

Table 2. Comparison of age and gender between the patient and control groups.

Variable	Patient group (n=45)	Control group (n=45)	*p* value
Age (Month) Median (Min-Max)	4 (1-36)	8 (1-48)	^{Z}NS
Sex			
Male	25 (55.6%)	20 (44.4%)	χ^2NS
Female	20 (44.4%)	25 (55.6%)	

Z: Mann Whitney test, $\chi^{2:}$ Chi square test, $P > 0.05$ is not significant (NS).

Table 3. Comparison of complete blood count (CBC) parameters between the patient and control groups.

CBC	Patient group (n=45)	Control group (n=45)	^{Z}p value
Hemoglobin (g/dl) Median (Min-max)	10.1 (6.9-14.4)	12.2 (8.8-13.6)	<0.001
WBCS (10^3/ µl) Median (Min-max)	8.7 (2.9-27.7)	6.8 (4.54-13.5)	NS
Neutrophils (10^3/ µl) Median (Min-max)	3.6 (0.1-24.2)	2.78 (1.25-7.50)	NS
Lymphocytes (10^3/ µl) Median (Min-max)	2.3 (0.9-7.0)	3.49 (1.5-5.6)	0.003
Monocytes (10^3/ µl) Median (Min-max)	0.8 (0.1-1.8)	0.57 (0.34-0.94)	0.001
Eosinophils (10^3/ µl) Median (Min-max)	0.2 (0.0-0.5)	0.0 (0.0-0.0)	<0.001
Basophils (10^3/ µl) Median (Min-max)	0.1 (0.0-0.5)	0.0 (0.0-0.0)	<0.001
Platelets (10^3/ µl) Median (Min-max)	400 (61-869)	254 (160-434)	<0.001

Z: Mann Whitney test, $P > 0.05$ is not significant (NS).

A significantly higher CRP positives (62.2%) were seen in the pneumonia patient group compared to the control group (15.6%, $p<0.001$) (Table 4). Data in Table 5 demonstrates that there was no difference in serum concentrations of Na, K, creatinine, and BUN between the pneumonia patient group and the control group. CGRP level was lower in the pneumonia patient group as compared to control group ($p<0.001$) Table 6. The pneumonia cases were classified according to the PCIS into non-critical, critical, and extremely critical sub-groups (Table 6).

Table 4. Comparison of CRP level between the pneumonia patient and the control group.

	Patient group (n=45)	Control group (n=45)	$\chi^2 p$ value
CRP			
Positive	28 (62.2%)	7 (15.6%)	<0.001
Negative	17 (37.8%)	38 (84.4%)	

$\chi^{2:}$ Chi square test, $^*P \leq 0.05$ is significant.

Table 5. Comparison of laboratory data between pneumonia patient group and the control group.

Variable	Patient group (n=45)	Control group (n=45)	^{t}p value
Na (mmol/L)	136.96±8.13	139.40±5.24	NS
K (mmol/L)	4.90±0.65	4.88±0.50	NS
Creatinine (µmol/L)	100.07±22.68	93.60±8.95	NS
Blood Urea Nitrogen (BUN) (mmol/L)	7.15±2.19	6.49±0.90	NS

t: student t- test $P > 0.05$ is not significant (NS).

Table 6. Calcitonin gene related peptide (CGRP) levels in the studied groups.

CGRP level	Patient group (n=45)	Control group (n=45)	^{Z}p value
Median (ng/l) (Min-Max)	77 (55-183)	230 (133-664)	≤0.001

Z: Mann Whitney test, $P \leq 0.05$ is significant.

A positive association was observed between CGRP levels and PCIS as shown in Figure 1. In addition, according to their outcome, the pneumonia patients were further classified into surviving and non-surviving groups Table 7. Serum CGRP levels were significantly higher in the survived group than the non-survived group (p=0.022) (Table 8).

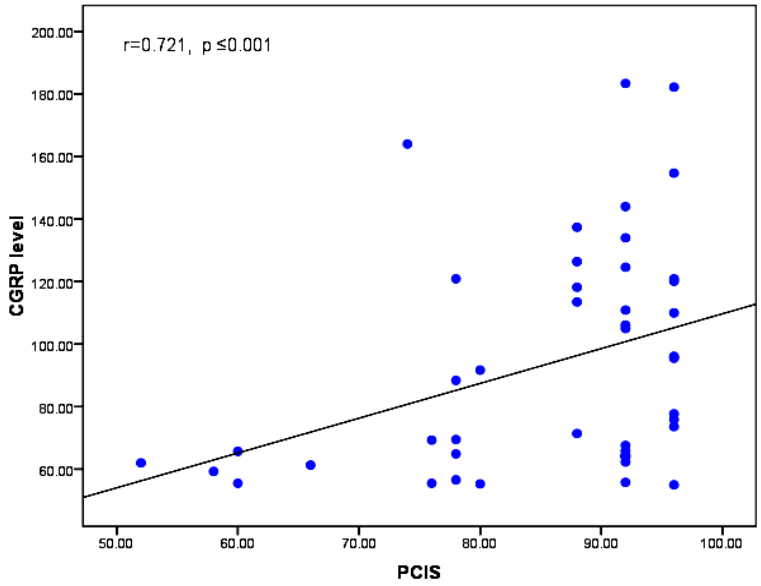

Figure 1. A scatter diagram showing the positive correlation between calcitonin gene related peptide (CGRP) level and pediatric critical illness score (PCIS).

Table 7. Pediatric critical illness score (PCIS) risk factors and pneumonia disease outcome in the 45 study patients.

PCIS risk factors and outcome	Patient group
PCIS Risk factors	
Non-critical	30 (66.7%)
Critical	10 (22.2%)
Extremely critical	5 (11.1%)
Outcome	
Survived	35 (77.8%)
Non-survived	10 (22.2%)

Table 8. Relation between calcitonin gene related peptide (CGRP) level and the pneumonia disease outcome.

	Survived (n=35)	Non-survived (n=10)	^{Z}p value
CGRP level (ng/l) Median (Min-Max)	96.1 (55-183)	63.4 (55.5-120.9)	0.022

Z: Mann Whitney test, $P > 0.05$ is not significant (NS).

At the area under the curve (AUC) of 0.978 with 95 % confidence interval (CI) between 0.95 and 1.0, a cutoff point of 145.25 ng/L can be used as a predictor for the pneumonia patient group. The sensitivity was 97.8%, specificity 91.1 %, positive predictive value (PPV) 97.6%, negative predictive value (NPV) 91.7% and accuracy 94.4% as shown in Figure 2. At AUC of 0.834, with a 95% CI between 0.711 and 0.958 and a cutoff value of 93.60 ng/L can be used as a predictor for mortality. The sensitivity, specificity, PPV, NPV, and accuracy were 93.3%, 63.3%, 56%, and 95%, respectively, (Figure 3).

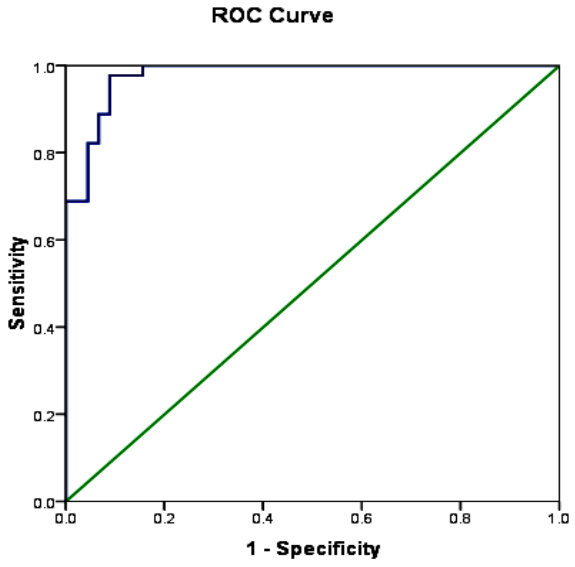

Figure 2. Receiver operating characteristic (ROC) curve for prediction of patients group by calcitonin gene related peptide (CGRP) level.

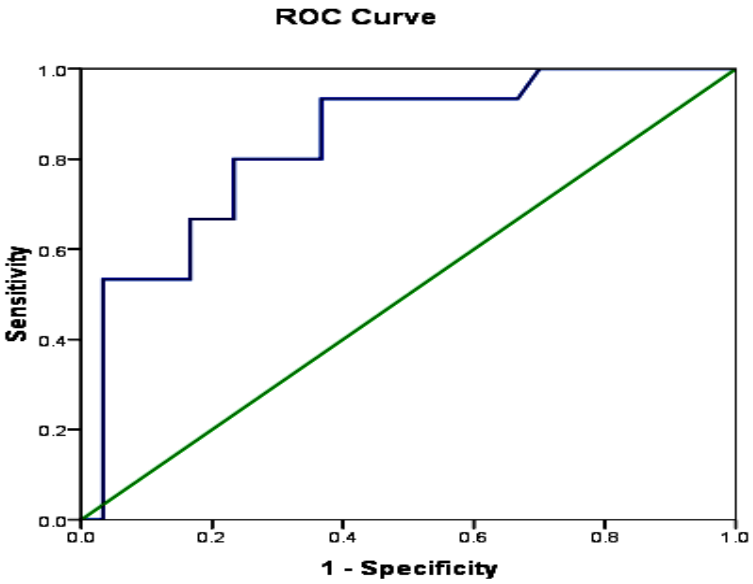

Figure 3. Receiver operating characteristic (ROC) curve for prediction of critical and extremely critical cases according to calcitonin gene related peptide (CGRP) level.

Discussion

Globally, pneumonia is a leading cause of morbidity and mortality in children younger than the age of 5 years.[14] The purpose of this investigation was to determine whether serum levels of the calcitonin gene-related peptide were related to the prognosis of pediatric patients with severe pneumonia.

In the present study, the median age the pneumonia group was 4 months, ranged between 1 month to 36 months, which agreed with a study by Moustafa et al., 2019,[15] that the majority of included patients (62.7%) were below the age of three years old. Also, Bolursaz et al., 2017,[16] noted that 1% of pneumonia cases began before the age of three months, 6% between the ages of three and 12 months, 65% between the ages of one and five years, and 28% after the age of five years. This finding can be explained by the significantly higher prevalence of co-morbid conditions in the age group of 0 to three years, such as congenital anomalies, congenital heart disease, and undernutrition A study by Tarhani et al., 2020,[17] determined the frequency of different laboratory tests for the diagnosis of childhood pneumonia in Khorramabad (Iran), provided different results. They stated that 40% of the patients were under the age of two years old, making up 63.8% of the studied patient population.

In our study, there was a male predominance (55.6%). Our results agreed with those of Moustafa et al., 2019,[15] who reported that 65.5% of recurrent pneumonia cases in Assiut university children hospital were males. Bolursaz et al., 2017,[16] also found that 55.0% of cases were males in Iran, they explained the tradition of favoring male to female in the community makes parents seek medical advice for male children earlier and more frequent. Ullah et al., 2019,[18] reported that male patients were more vulnerable to infections and the possibility could be that the testosterone suppressing the immune response.

In our study there was a significant decrease in the hemoglobin level and absolute lymphocytic count in the patient group as compared to the control group, while there was a significant increase in the platelet count, monocytes, eosinophils, and basophils in patient group as compared to control group. In agreement with our results Huang et al., 2018,[19] reported that platelet to lymphocyte ratio, white blood cell (WBC), neutrophil and monocyte levels in the community-acquired pneumonia group were higher than that of

control group, while hemoglobin and lymphocyte levels were lower ($p< 0.05$). In addition, they suggested that viral infection is expected to lead to an increased number of lymphocytes, although most other studies have contrarily shown a decrease in lymphocytes in these patients. This finding suggests that one of the causes may be lymphocyte consumption. A study by Stepan et al., 2018,[20] reported a significant association of acute lower respiratory tract infections in toddlers with iron deficiency anemia.

Our study showed that positive CRP was higher in the patient group (62.2%) as compared to the control group (15.6 %). In agreement with our results Wu et al., 2015,[21] demonstrated that serum levels of CRP were elevated in patients with severe and mild pneumonia. Their results demonstrated that the proportion of cases with severe pneumonia was higher compared with the mild pneumonia group. This also agrees with Higdon et al., 2017,[22] who reported that elevated CRP was associated with confirmed bacterial pneumonia and negatively associated with the respiratory syncytial virus pneumonia in a multicenter case-control study of pneumonia etiology research for child health.

The present study revealed that the mean serum Na was (136.96±8.13), K (4.90±0.65), creatinine (100.07±22.68), and BUN (7.15±2.19). These agreed with findings of a study by Tarhani et al., 2020,[17] who reported that, the levels of BUN, creatinine, Na, and K were normal in all patients.

In our study, the PCIS score was used to classify the patients into three groups: non-critical (66.7% of the cases), critical (22.2% of the cases), and highly critical (11.1% of the cases). These agreed with those of Tao et al., 2020,[9] who aimed to evaluate the relationship between serum levels of CGRP and the prognosis of pediatric patients with severe pneumonia. They reported that, the majority of their patients were in the non-risk group (PCIS>80), whereas approximately 22.4% and 10.5% of the patients classified into the risk and high-risk groups, respectively.

As regard the survival outcome, 77.8% of our cases have survived and (22.2%) died. Kallander et al., 2016,[23] reported that the case–mortality rate in untreated children with pneumonia was high, sometimes reaching as high as 20%, and deaths can occur as early as 3 days after illness onset.

The current study showed that there was a statistically significant positive correlation between CGRP level and PCIS score. In agreement with our results Tao et al., 2020,[9] reported that the risk and high-risk groups showed decreased serum CGRP levels relative to the non-risk group.

In conclusion, we found that among severely and extremely critically ill pneumonia patients, serum CGRP levels can be suggested as useful markers to predict mortality in children with severe pneumonia.

Author Contributions

SS collected the data, examined the patients, and collected the samples. SI and SM made a substantial contribution to the concept and design of the work and draft of the manuscript. FAD performed the laboratory work. All authors participated in writing and reviewing the paper.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was ethically reviewed and approved by the Research Ethics Committee of the Faculty of Medicine for Girls Al Azhar University, Egypt (approval date: August 2020).

Informed consent

The importance of the study was explained to the parents of the participating children. An informed written consent was taken from the parents before enrolling the children in this study.

References

1. Chen, J., Hu, P., Zhou, T., et al. (2018). Epidemiology and clinical characteristics of acute respiratory tract infections among hospitalized infants and young children in Chengdu, West China, 2009-2014. *BMC Pediatr*, 18(1): 216.
2. Ferdous, F., Ahmed, S., Das, SK., et al. (2018). Pneumonia mortality and healthcare utilization in young children in rural Bangladesh: a prospective verbal autopsy study. *Trop Med Health*, 46: 17.
3. Ekram, M., Abdel, k., Doaa, M., (2016). Acute respiratory tract infections in children under 5 years of age in Upper Egypt. *Int J Community Med Public Health*, 3(5): 1161-1166
4. Rider, AC., Frazee, BW., (2018). Community-Acquired Pneumonia. *Emerg Med Clin North Am*, 36(4): 665-683.
5. Williams, DJ., Zhu, Y., Grijalva, CG., et al. (2016). Predicting Severe Pneumonia Outcomes in Children. *Pediatrics*, 138(4): e20161019.
6. Russell, F. A., King, R., Smillie, S. J., et al. (2014). Calcitonin gene-related peptide: physiology and pathophysiology. Physiological reviews, 94(4), 1099–1142. https://doi.org/10.1152/physrev.00034.2013
7. Barrett, JE., Flockerzi, V., Michael, A., et al. (2019). Calcitonin Gene-Related Peptide (CGRP) Mechanisms Focus on Migraine. Handbook of Experimental Pharmacology, Susan D. Brain and Pierangelo Geppetti Editors. *Springer Nature Switzerland AG*, 255.
8. Dang, HX., Li, J., Liu, C., et al. (2017). CGRP attenuates hyperoxia-induced oxidative stress-related injury to alveolar epithelial type II cells via the activation of the Sonic hedgehog pathway. *Int J Mol Med*, 40(1): 209-216.
9. Tao, B., Jiang, L., Chen, L., (2020). Aberrant expression of calcitonin gene-related peptide and its correlation with prognosis in severe childhood pneumonia. *Clinics (Sao Paulo)*, 75: e1448.
10. World Health Organization. Pneumonia [Internet]. Who.int. 2020. Available from: https://www.who.int/news-room/fact-sheets/detail/pneumonia [Accessed at 2020 Oct 1]
11. Nuttall F. Q. (2015). Body Mass Index: Obesity, BMI, and Health: A Critical Review. *Nutrition today*, *50*(3), 117–128.
12. Miyaji, Y., Sugai, K., Nozawa, A., et al. (2015). Pediatric respiratory severity score (PRESS) for respiratory tract infections in children. Austin Virol Retrovirol, 2(1), 1009.
13. Collaborative PCIS Trail Group (1998). Evaluation of pediatric critical illness scoring system in clinical application. Chin J Pediatr; 36:579e82.
14. Ebeledike, C., Ahmad, T., (2022). Pediatric Pneumonia. In: Stat Pearls [Internet]. *Treasure Island (FL): StatPearls Publishing*.
15. Moustafa, M., Zienab, M., Gamal, A., (2019). Recurrent Pneumonia in Children Admitted to Assiut University Children Hospital. Magnitude of the Problem and Possible Risk Factors. *Medical Research Journal*, 4(1): 13-24.
16. Bolursaz, MR., Lotfian, F., Ghaffaripour, HA., et al. (2017). Underlying Causes of Persistent and Recurrent Pneumonia in Children at a Pulmonary Referral Hospital in Tehran, Iran. *Arch Iran Med*, 20(5): 266-269.
17. Tarhani, F., Nezami, A., & Heidari, G. (2020). Evaluating the Usefulness of Lab-Based Test for the Diagnosis of Pneumonia in Children. *International journal of general medicine*, *13*, 243–247.
18. Ullah, M. B., Mridha, M. K., Arnold, C. D., et al. (2019). Factors associated with diarrhea and acute respiratory infection in children under two years of age in rural Bangladesh. *BMC pediatrics*, *19*(1), 386.
19. Huang, Y., Liu, A., Liang, L., et al. (2018). Diagnostic value of blood parameters for community-acquired pneumonia. *International immunopharmacology*, *64*, 10–15.
20. Stepan, D., Dop, D., Moroşanu, A., et al. (2018). Implications of the Iron Deficiency in Lower Tract Respiratory Acute Infections in Toddlers. *Current health sciences journal*, 44(4), 362–367.
21. Wu, J., Jin, Y. U., Li, H., Xie, et al. (2015). Evaluation and significance of C-reactive protein in the clinical diagnosis of severe pneumonia. *Exp. & Therap. Med.*, *10*(1), 175–180.
22. Higdon, M. M., Le, T., O'Brien, K. L., et al. (2017). Association of C-Reactive Protein with Bacterial and Respiratory Syncytial Virus-Associated Pneumonia Among Children Aged <5 Years in the PERCH Study. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, 64(suppl_3), S378–S386.
23. Kallander, K., Burgess, D. H., & Qazi, S. A. (2016). Early identification and treatment of pneumonia: a call to action. *The Lancet. Global health*, *4*(1), e12–e13.