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## **Study of Procalcitonin and neutrophil/lymphocyte count ratio in children infected with Community-acquired pneumonia**

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**Abstract**---Objective: Investigate the expression of procalcitonin (PCT) in children infected with Community-acquired pneumonia (CAP), and its correlation to the number of white blood cells (WBC), and neutrophil/lymphocyte count ratio (NLR). The current study included sixty (60) patient suffering from acquired community pneumonia aged range between one year to larger than 6 years, gander (30) female and (30) male Childs in Al Zahara Teaching Hospitals in Najaf Provenance, during November 2020 – M arch 2021. The (30) control Child without suffering any of symptoms or criteria of acquired community pneumonia our results show a significant increment ( $p < 0.05$ ) in total count of leukocytes, Neutrophils, N/L ratio, lymphocytes ( $10^9/L$ ) and PCT level in Pneumonia patients group compared with control group respectively, and non-significant ( $p > 0.05$ ) different in biomarker procalcitonin level between female and male pneumonia child patients. Serum procalcitonin level demonstrate a statistically significant elevation ( $p < 0.05$ ) in the pneumonia child patient among the age (1-3 y) when compare with other ages. Also results indicate there is a significant increase ( $p < 0.05$ ) in serum procalcitonin at leukocyte count (5000-10000) and (10000-20000) of pneumonia child patient as compared with control group, and there is no significant ( $p > 0.05$ ) different in procalcitonin at leukocyte count  $\leq 5000$  of pneumonia child patient as compare with control group. Significant ( $p < 0.05$ ) increase in procalcitonin of pneumonia child patient as

comparison with control group among different groups of NLR (4-5) and (6-7).

**Keywords**---Procalcitonin, neutrophil/lymphocyte, children infected, pneumonia.

## Introduction

Pneumonia is a lung illness caused by an infection of the Acute respiratory tract (ARTI). Alveoli in the lungs become clogged with pus and fluid, making it difficult for the patient to breathe and limiting the amount of oxygen they can take in. Pneumonia is most often caused by bacteria or viruses. *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib), and respiratory syncytial virus (RSV) are the most common pathogens. *S. pneumoniae* In developing countries, is the most common cause of bacterial pneumonia in children under the age of five (Hema Latha *et al.*, 2017). According to the World Health Organization, pneumonia accounted for 15% of all fatalities in children under the age of five in 2015, 922,000 children are estimated to have died. In the first two years of life, the majority of deaths occur, with poverty presenting the greatest burden, particularly in South Asia and Sub-Saharan Africa (World Health Organization., 2015). While bacteria, viruses, and fungi can all cause pneumonia, the vast majority of scientists agreed in 2010 that bacterial pneumonia was the leading cause of death (Al-Fatlawi., 2020 ; Zar ., 2013).

Procalcitonin (PCT) is a 116 amino acid protein with an approximate molecular weight of 13 kDa (Prucha., 2015). PCT is a biomarker for sepsis and infection in postoperative patients, as it is one of the inflammatory mediators implicated in the inflammatory response (Wacker *et al.*, 2013; Hammod *et al.*, 2016). Although PCT has been found to be an effective indicator of infection in most studies (Jebali *et al.*, 2007). In the diagnosis of sepsis, PCT has been shown to be more clinically beneficial than routinely used clinical characteristics and laboratory tests. The University of Geneva Hospitals in Geneva, Switzerland, carried a clinical trial to investigate the diagnostic efficacy of procalcitonin (PCT), IL-6, and IL-8 as standard measurements in detecting critically extremely sick patients with sepsis (Torres *et al.*.,2019 ). Furthermore, it has been demonstrated that PCT values are related to the extent and severity of microbial invasion (Al-Kraity *et al.*.,2017). The neutrophil-to-lymphocyte ratio (NLR) is well-known as a biomarker of infection and systemic inflammation. As a bacterial infection predictor, it has been found to be more accurate than other standard inflammatory indicators (Harbarth *et al.*, 2001). NLR has also shown good prediction value for pneumonia and dose-response findings on the incidence of CAP (Curbelo *et al.*, 2017). NLR is a promising candidate predictor of death in CAP (AL-Nafakh *et al.*, 2020).

## Material and Methods

The current study included sixty (60) patient suffering from acquired community pneumonia aged range between one year to larger than 6 years, gander (30) female and (30) male Childs with Cough, sputum production, Dyspnea, leukocytes count > 100000 / mm<sup>3</sup>, positive sputum culture with more than 25

polymorphonuclear leukocyte for bacterial infection only with 10 squamous cells in field also chest radiography and ultrasonography . Male and female patients Childs diagnose by specialized physician in Al Zahara Teaching Hospitals in Najaf Provenance, during November 2020 – M arch 2021. The (30) control Child were selected from same hospital without suffering any of symptoms or criteria of acquired community pneumonia.

### **Blood samples**

Three milliliters of venous blood were taken from each patient and control subject using a disposable needle and plastic syringes. The blood was train into EDTA tube for estimated of total leukocyte and procalcitonin, after centrifugation at 3000 pm for 15 minutes, the serum was suctioned, divided and stored at -20 C ° in ependroff tubes separated into aliquots.

### **Total white blood cell count**

According to this question, a blood cell counter and Turks fluid were used to determine the total leucocytes count by microscopy:

Total Leucocytes count /mm<sup>3</sup> = The count cells ×50

### **Differential white blood cell count**

Preparation of blood smear and staining dye with Leishman stain then diagnosis under oil immersion lens were used to measure differential leucocytes count.

### **Calculate of neutrophil-to-lymphocyte ratio NLR**

The NLR were calculated by dividing neutrophil count by lymphocyte count.

### **Determination procalcitonin (PCT) concentration**

PCT concentrations in the serum of patients and control was examined by using ELISA according to prepare processed from Elabscience, China-Cat-No. E-EL-H1492.

### **Statistical analysis**

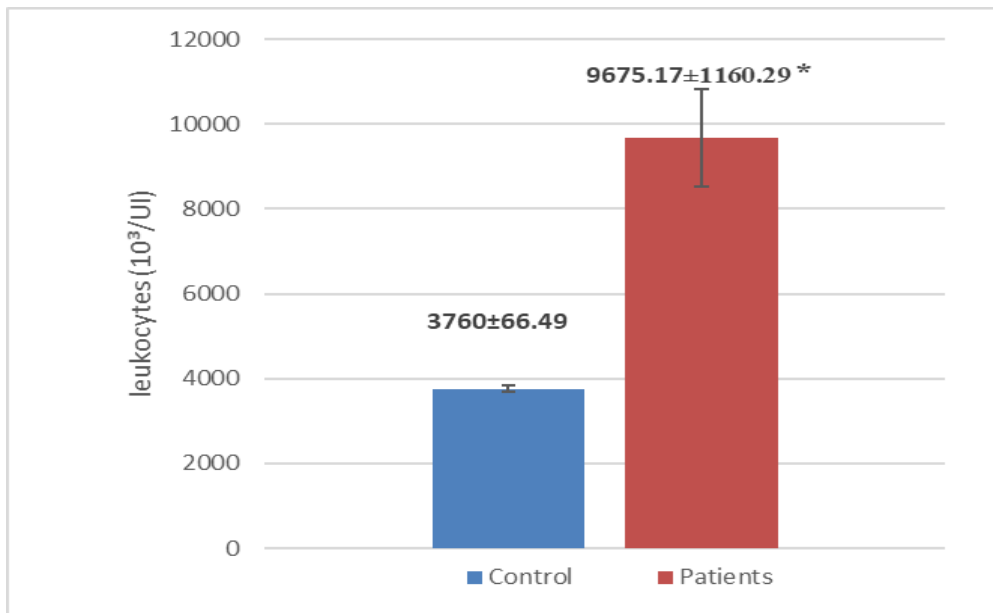
Every value was express as means ± standard Error (SE). The computerized SPSS application was used to examine the data.The Student's t-test was used to investigate differences between and within groups. Firstly a comparisons between patients and control was done by using un paired t-test. The unpaired t-test and ANOVA test were use to make comparisons between and within patient groups. A statistically significant p value is < 0.001.

## **Result**

### **Comparison of hematological parameters between patient and control**

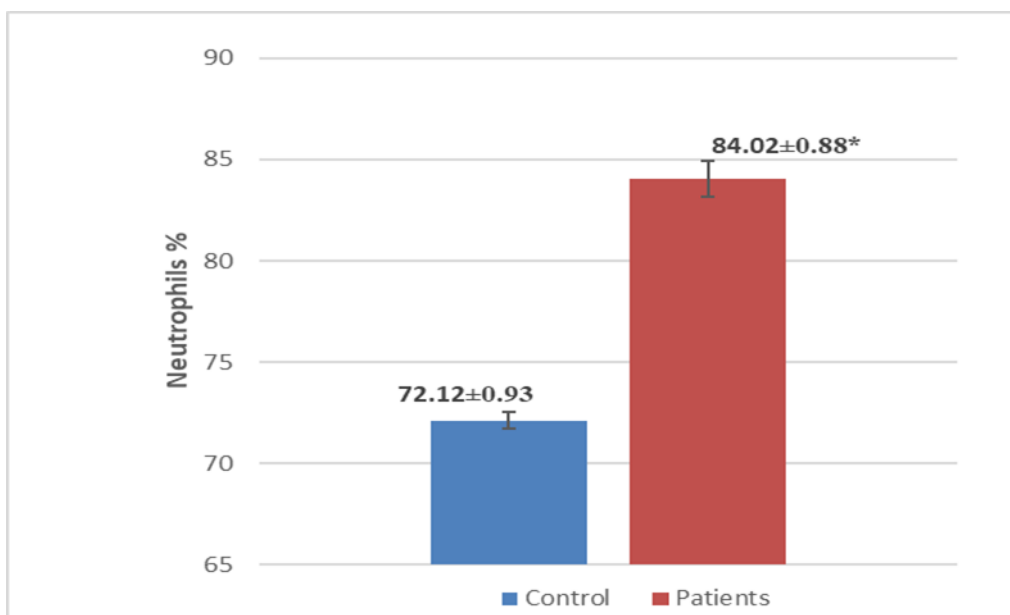
The result in figures (1, 2, 3, 4) exhibit a statistically significant (p< 0.05) increase in the count of total Leukocytes, Neutrophils , N/L ratio, and

lymphocytes( $10^9/L$ ) in Pneumonia patients group by comparison with control group respectively.



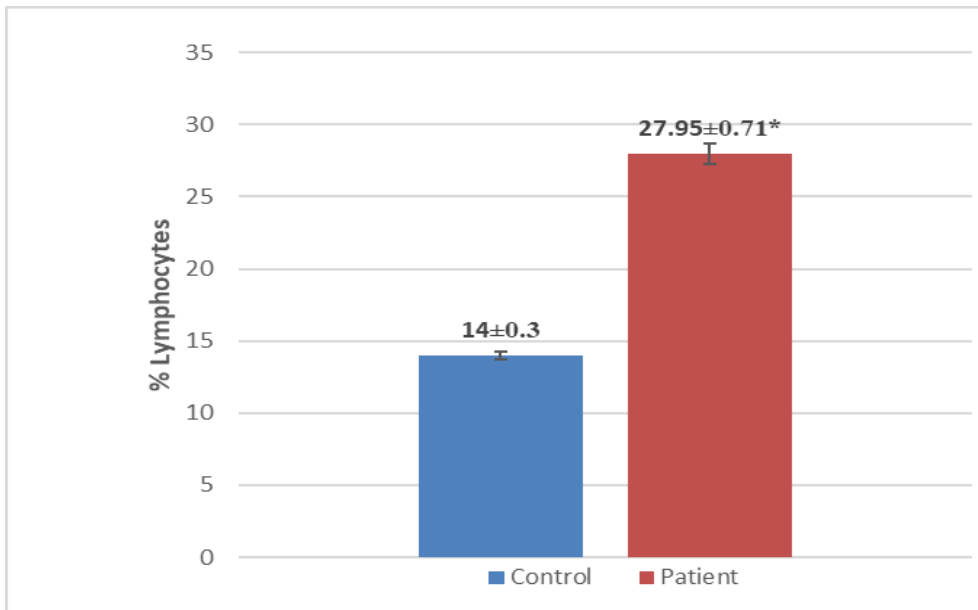
(\*): Statistically significant differences ( $p < 0.05$ ) mean  $\pm$  standard Error

Fig. 1. Total Leukocytes count in healthy controls and Pneumonia patients group

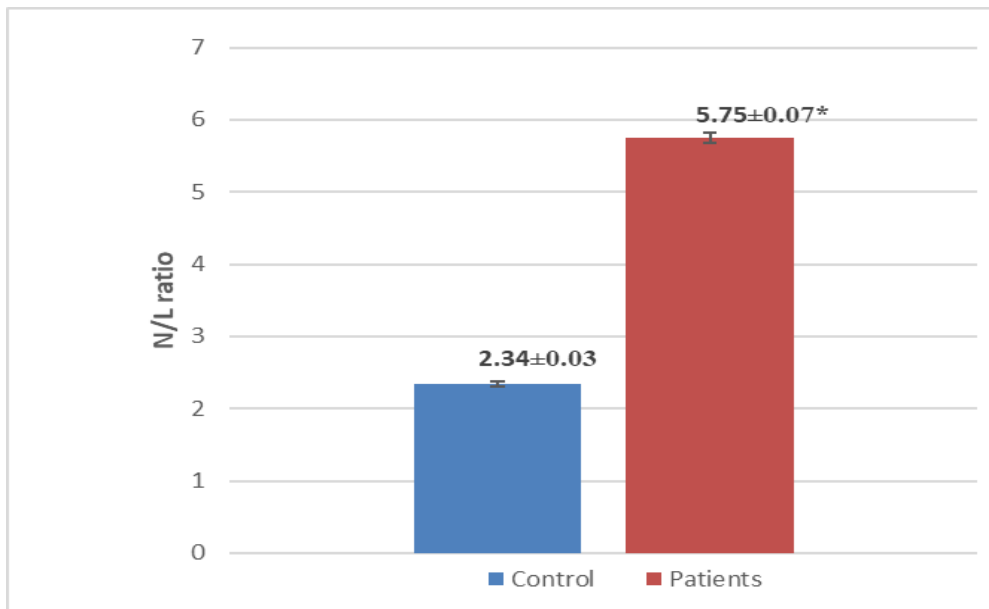


(\*): Statistically significant differences ( $p < 0.05$ ) mean  $\pm$  standard Error

Fig. 2. Comparison of Neutrophils between healthy controls and Pneumonia patients group



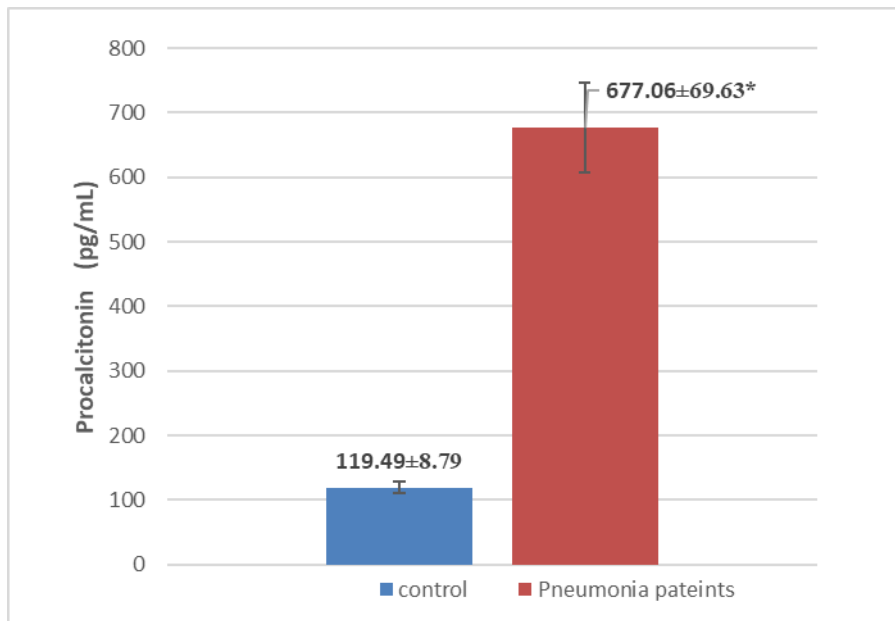
(\*): Statistically significant differences ( $p < 0.05$ ) mean  $\pm$  standard Error  
Fig. 3. Distribution of Lymphocytes % in healthy controls and Pneumonia patients group



(\*): Statistically significant differences ( $p < 0.05$ ) mean  $\pm$  standard Error  
Fig.4. Distribution of N/L ratio in healthy controls and Pneumonia patients group

### Compare chemical procalcitonin biomarker between pneumonia patient and control group

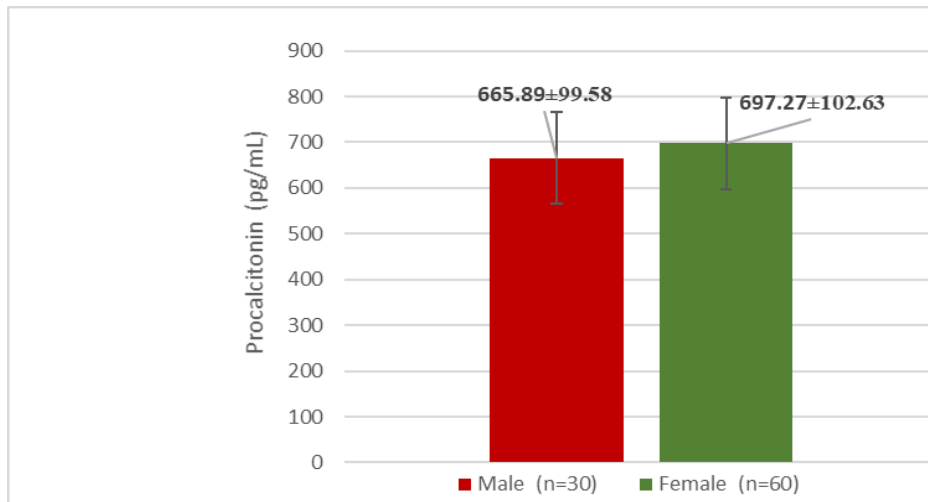
In the pneumonia patient group, there was a significant rise ( $p < 0.05$ ) in the chemical biomarker PCT in comparison to the control group as show in the figures (5).



(\*): Statistically significant differences ( $p < 0.05$ ) mean  $\pm$  stander Error  
 Fig. 5. Procalcitonin levels in pneumonia patients and healthy controls

### Estimation of procalcitonin biomarker in pneumonia child patients according to gander

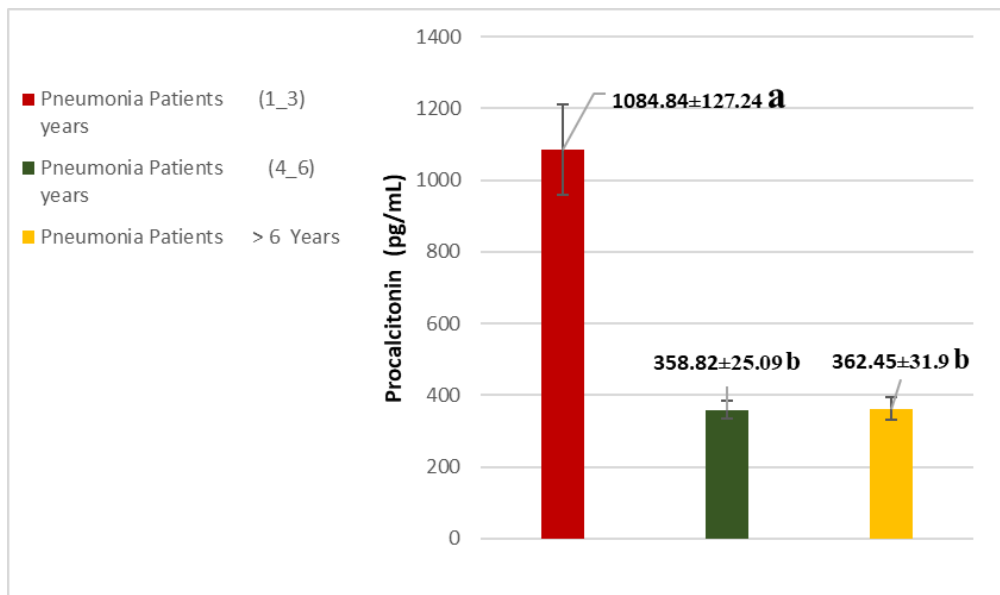
The result in figures ( 6) show no significance ( $p > 0.05$ ) different in biomarker procalcitonin level between female and male pneumonia child patients respectively.



(\*): Statistically significant differences ( $p < 0.05$ ) mean  $\pm$  standard Error  
 Fig. 6.) Male and female pneumonia patients' serum procalcitonin levels

### Estimation of procalcitonin l biochemical markers in pneumonia child patient according to age

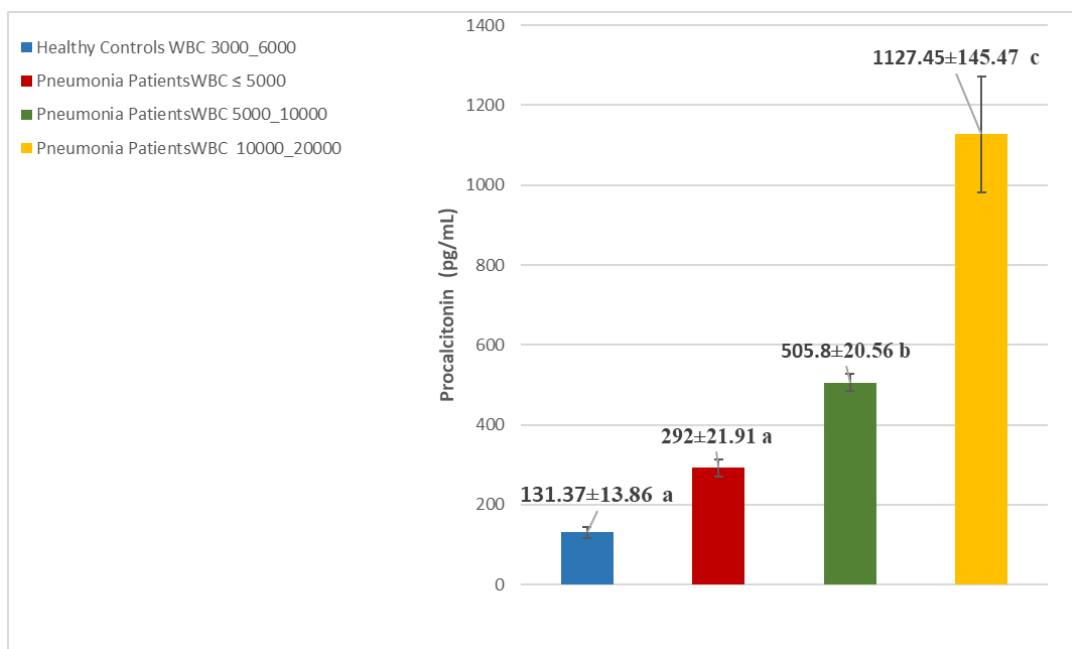
The results show significant rise ( $p < 0.05$ ) in the serum procalcitonin levels of pneumonia children (1-3 y) as shown in figure (7) when compare with among the ages (4-6 y) and (>6y) of pneumonia child patient, And there is no significant ( $p > 0.05$ ) different in procalcitonin between the ages (4-6y) when compare with ages (>6y).



The different letters mean significant differences ( $p < 0.05$ ) mean  $\pm$  standard Error  
 Fig. 7. Serum procalcitonin levels in pneumonia children of different ages

### Estimation of procalcitonin biochemical markers in pneumonia child patient and control group according to total count of leukocytes

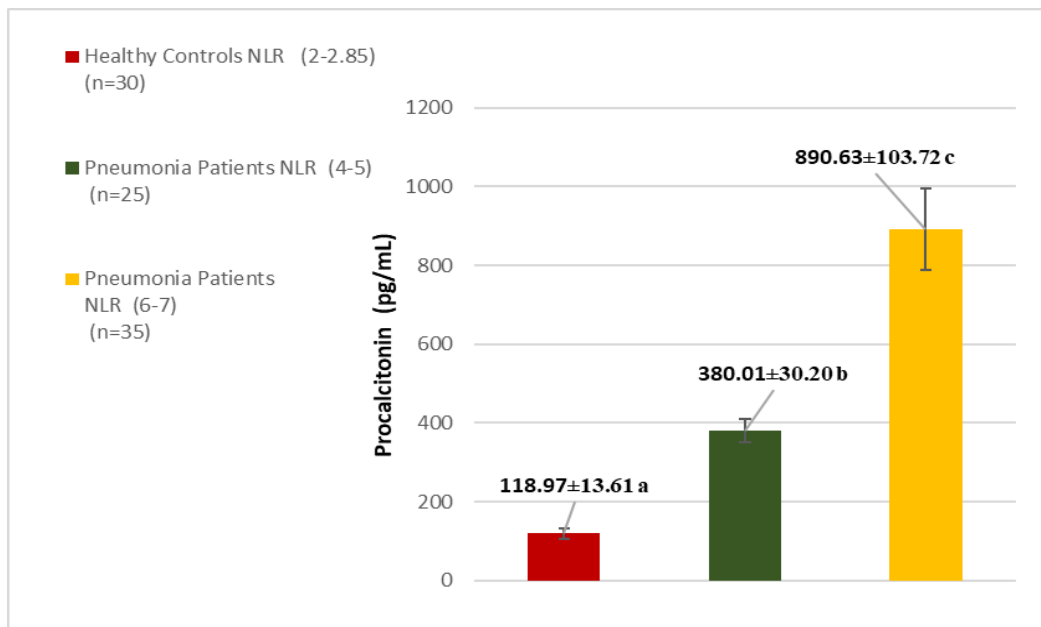
Serum procalcitonin levels have risen significantly ( $p < 0.05$ ) as shown in figure (8) at leukocyte count (5000-10000) and (10000-20000) of pneumonia child patient as compare with control group. And there is no significant ( $p > 0.05$ ) different in procalcitonin at leukocyte count  $\leq 5000$  of pneumonia child patient as compare with control group.



The different letters mean significant differences ( $p < 0.05$ ) mean  $\pm$  standard Error  
 Fig. 9. Comparison of serum procalcitonin level in pneumonia child patient and control group according to total of leukocytes

### Comparison of biochemical markers in pneumonia child patient and control group according to neutrophils lymphocyte ratio NLR

The results in figure (10) indicate there is significant ( $p < 0.05$ ) increase in procalcitonin of pneumonia child patient as comparison with control group among different groups of NLR (4-5) and (6-7).



The different letters mean significant differences ( $p < 0.05$ ) mean  $\pm$  standard Error  
 Fig. 10. Procalcitonin level in pneumonia child patient and control according NRL

## Discussion

CAP is a commonly known infection in children caused by viruses, 'typical' respiratory bacteria (such as *Streptococcus pneumoniae*), or 'atypical' respiratory bacteria (such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*) (Nam *et al.*, 2018). Pneumonia is the most common cause of death in children around the world, accounting for approximately 30% of all childhood mortality. In most cases, no specific pathogen is identified, but both viruses (mostly respiratory syncytial and influenza viruses) and bacteria (most particularly *Streptococcus pneumoniae* and *Haemophilus influenzae*) are significant pathogens (Al-Fatlawi *et al.*, 2022). This infection can be caused by a variety of bacteria, viruses, and their combinations, but most agents lack rapid and commercially available diagnostic assays, and this could explain why the specific cause is rarely diagnosed in clinically and antibiotic therapy is essentially experimental in the majority of cases. Because up to 60% of cases involve viral infections, antibiotics may be administered that are unnecessary and ineffective (Kartal *et al.*, 2017). In children, rapid detection and systemic bacterial infection treatment are extremely crucial. Delay in treating serious bacterial infections can have a negative impact on the patient's recovery. When it relates to treatment options, it is critical to distinguish between a severe bacterial disease and a localized bacterial or viral infection (Don *et al.*, 2007; AL-Nafakh *et al.*, 2022).

The result indicated significant increment in the count of leukocyte and NLR in CAP patients, previous research had been showed that the levels of neutrophils, lymphocytes, monocytes, and platelets play a key role in systemic inflammation and infection. A considerable rise in NLR levels was found in CAP patients,

suggesting that NLR levels could be utilized to predict the occurrence of CAP (Adler-Shohet *et al.* , 1998).

Infections (particularly sepsis), trauma, poor nutrition, physiologic stress, and chronic psychological stress are all factors to consider all can cause a significant increase in total leukocyte count. As previously demonstrated, an increased leukocyte count could be a good indicator of chronic systemic inflammation and subclinical disorder ( Virkki *et al.* ,2002).

As a well-known indicator of systemic inflammation and infection, the neutrophil-to-lymphocyte ratio (NLR) is measured, and it has been studied as a bacterial infection diagnostic, and its predictive value significantly exceeds that of traditional inflammatory markers ( van Rossum *et al.* , 2004). Furthermore, the NLR has shown excellent pneumonia prediction potential as well as CAP burden dose-response data (Pantzaris *et al.* , 2018) .

The current study indicates significant increase in procalcitonin(PCT) in CAP. According to findings, PCT, a precursor peptide of the hormone calcitonin (CT), is cleaved following translation from CT messenger RNA (mRNA) and plays a crucial role in pneumonia (Chmielowski *et al.* , 2018). In contrast, it is likely that cytokines will play a role in the host's defense. CT precursors, such as PCT, can rise up to several thousand-fold when an infection or inflammation occurs, but mature CT isn't affected, and the rate of increase, in particularis associated with mortality and disease severity (Curbelo *et al.* , 2017). Extrathyroidal CALC-I gene transcription is suppressed and restricted to neuroendocrine cells in the thyroid and lungs in the absence of infection (Nam *et al.* , 2018 ) .

Serum procalcitonin (PCT) levels deal with interest by recent study as biological indicators in respiratory tract infections patients for several reasons. One was correlated with septic shock and, bacterial pneumonia and, this relation was positively with pneumonia infection severity (Jain., 2017 ) . Septic shock patients with bacteremia had higher serum PCT levels, according to a series of clinical observations. Additional researches revealed PCT as a component of the innate immune system's complex pro-inflammatory response (MULGUND *et al.* , 2020).

The current study show there is no different in PCT level between female and male of current study may be explained by presence of similar immune system in both male and females that activate macrophage also all biomarkers to resist of bacterial infection also hormonal system were not developed so the concentration of testosterone and estrogen at low level to contribute a differences between two gender in addition to same exposure to risk and environmental factor also bacterial infection.

The current study show increase of PCT at early age and this agree with another study that showed PCT levels have been raised shortly after birth14–21, concentration of serum PCT must be carefully evaluated for assessing infection, and a new point of reference must be established for children at early age (Giannetta *et al.* , 2020) . A previous study found an age-specific reference for serum PCT in term newborns from birth to 48 hours, with a peak around 20 ng/mL (the 95-percentile value) around 24 hours after birth and decreasing

subsequently. As a result of these findings, researchers have established cut-off values for neonatal sepsis or bacterial infection in neonates at an early age, and they have revealed that serum PCT is a highly accurate marker ( Yan *et al.* , 2017).

Taken together, serum PCT, WBC and NLR levels in severe pneumonia patients were found to be increased significantly, as one of the most important cells in human blood, WBC are an important line of defense in immunity. Thus, the rise of WBC is clinically regarded as one of the diagnostic indicators of infection level because it indicates acute/chronic infection in the body or tissues, inflammation or tissue injury. However, the detection of WBC lacks specificity, as the rise of WBC levels can even be induced by physiological fluctuations. Previous study explored the expression of PCT, WBC and CRP in different pneumonia groups and the research results showed that the levels of PCT, WBC and CRP in patients with pneumonia and severe pneumonia were significantly higher than that in healthy people, and the levels were strongly related to the disease's severity, and infection also suggest that PCT, WBC and CRP are all involved in the inflammatory response of severe pneumonia patients (Chen *et al.*, 2021).

The authors of a study conducted in Italy discovered a significant positive correlation between PCT levels and CRP, neutrophil percentage, and WBC counts (Huetz *et al.*, 2020). In agreement with these findings Serum PCT levels strongly associated with CRP, ESR, and WBC count values in 76 hospitalized patients with CAP and 18 healthy controls in a study conducted in South Korea (Fukuzumi *et al.* , 2016) .

A variety of inflammatory mediators, including cytokines, PCT, chemokines, and growth factors regulate infection and immunity were highly related with pulmonary immunity and play a curtail role in lung against microbe (Wang *et al.*, 2020).

Numerous studies have been conducted to assess the neutrophil/lymphocyte count ratio's (NLCR) as diagnostic and evaluative performance in a variety of clinical situations, including sepsis (Agnello *et al.*, 2016). Septic shock, bacteremia (Lee *et al.*, 2010). Lung, renal, colorectal carcinomas and intracranial tumors. Furthermore, NLCR has a higher predictive accuracy in predicting the severity and outcome of CAP than standard infection markers in the emergency unit (Chen *et al.*, 2020).

An increase in neutrophils and a decrease in lymphocytes number is one of the immune system's physiological responses to systemic inflammation. This is because apoptosis kinetics and regulation differ in an inflammatory state as against a non-inflammatory state (Westerdijk *et al.*, 2019). Toxic metabolic elaboration will be prolonged if neutrophil apoptosis is delayed, which in turn will prolong neutrophil function in inflammation (Jiang *et al.*, 2019). Increased apoptosis of lymphocytes, on the other hand, will result in a reduction in the number of effector inflammation (Wilson *et al.*, 2018). Activated neutrophils release toxic metabolic and inflammatory cytokines that can induce tissue injury and organ dysfunction. As a result, adaptive immune responses may be suppressed, As a result, the patient is more susceptible to nosocomial infection

and opportunistic microorganism invasion, which could result in a further systemic inflammatory response (Torres *et al.* , 2021). Recent findings have shown that neutrophil-lymphocyte ratio (NLR) and lymphocytopenia are stronger predictors of bacteremia in patients than leukocyte count, neutrophil count, and CRP (Hafez *et al.* , 2021).

According to a previous study PCT persists better compared to the NLR in identifying patients with sepsis and those with severe sepsis. This differs from prior research that concluded that NLR was superior to PCT (Arbogast *et al.*, 2018). The measuring time of neutrophils, lymphocytes, and PCT is one potential for explaining the variation in results. Neutrophils remain in circulation for seven to ten hours before traveling to the network, where they can remain for days. After a microorganism releases endotoxin, the number of neutrophils in the blood and inflammatory tissue will rise within a few hours. Within two hours of stimulation, PCT levels rise quickly, peak at 12 to 48 hours, and then gradually decline over the duration of 48 to 72 hours (Patricio *et al.* , 2019). Another study founded that the NLR parameter predicts disease severity, as well as the CAP result 10 times more effective than leukocytes, absolute neutrophil count (ANC), and c-reactive protein (CRP) ( Yan *et al.* , 2020) .

## Conclusion

The general sensitivity and specificity of PCT and NLR as early indicators of bacterial infections in children are appropriate. It can be used as an addition toll to other methods for detecting bacterial infections in CAP patients.

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