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Prospective Study

Salivary C reactive protein and mean platelet volume as possible diagnostic markers for late onset neonatal pneumonia: A prospective control study

Possible Diagnostic Markers in Neonatal Pneumonia

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Abstract

BACKGROUND

Neonatal sepsis, a formidable threat to newborns, is a leading cause of neonatal mortality, with late-onset sepsis manifesting after 72 h post-birth being particularly concerning. Pneumonia, a prevalent sepsis presentation, poses a significant risk, especially during the neonatal phase when lung defenses are compromised. Accurate diagnosis of pneumonia is imperative for timely and effective interventions. Saliva, a minimally invasive diagnostic medium, holds great promise for evaluating infections, especially in infants.

AIM

This prospective case-control study delves into the potential of serum C-reactive protein (CRP), salivary C-reactive protein (sCRP), and mean platelet volume (MPV) as diagnostic markers for late-onset neonatal pneumonia (LONP).

METHODS

Eighty full-term neonates were systematically examined, considering anthropometric measurements, clinical manifestations, radiology findings, and essential biomarkers, including Serum CRP, sCRP, and MPV.

RESULTS

The study reveals noteworthy distinctions in serum CRP levels, MPV, and the serum CRP/MPV ratio between neonates with LONP and healthy controls. MPV exhibited a robust discriminatory ability (AUC = 0.87) with high sensitivity and specificity at a cutoff value of >8.8. Correlations between serum CRP, salivary CRP, and MPV were also identified. Notably, sCRP demonstrated excellent predictive value for serum CRP levels (AUC = 0.89), underscoring its potential as a diagnostic tool.

CONCLUSION

This study underscores the diagnostic promise of salivary and serum biomarkers, specifically MPV and CRP, in identifying and predicting LONP among neonates. These findings advocate for further research to validate their clinical utility in larger neonatal cohorts.

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Core Tip: This prospective study explores the potential of salivary C-reactive protein (sCRP) and mean platelet volume (MPV) as diagnostic markers for late-onset neonatal pneumonia (LONP). Analyzing 80 neonates, significant differences in serum CRP

levels, MPV, and the serum CRP/MPV ratio were observed between LONP cases and healthy controls. MPV demonstrated strong discriminatory ability with high sensitivity and specificity at a cutoff value of >8.8. Salivary CRP displayed notable predictive value for serum CRP levels. These findings highlight the diagnostic potential of salivary and serum biomarkers in identifying and predicting LONP among neonates.

INTRODUCTION

Neonatal sepsis is a serious infection in newborns, with a very high risk of neonatal death, and occupies the third rank among the causes of neonatal death [1]. It can manifest in various forms, like septicemia, pneumonia, meningitis, osteomyelitis, arthritis, and urinary tract infections. Late-onset sepsis occurs after 72 h of birth and is a significant cause of infant mortality [2]. Despite medical advances, diagnosing and managing neonatal infections remains challenging. Childhood mortality due to pneumonia carries the highest risk during the neonatal phase since the fetus and neonate have compromised lung defenses, making them more prone to infections [3]. Neonatal pneumonia can be classified according to its onset into early-onset (within the first week of life) and late-onset (onset of symptoms after the first week of life within the first 28 days). Lat-onset neonatal pneumonia is further classified into hospital- or community-acquired. The community-acquired neonatal pneumonia occurs in term and near-term neonates who were discharged home after the initial birth hospitalization. Hospital-acquired late-onset pneumonia occurs in newborns who remain hospitalized since birth (e.g., preterm infants) [4,5].

It is crucial to accurately diagnose pneumonia to assess the disease's impact, implement suitable preventive or treatment measures, and develop more efficient interventions [6]. Saliva has been found to have excellent potential as a diagnostic fluid over the years. Its easy and non-invasive collection method makes it the most attractive diagnostic medium to examine vulnerable populations such as infants, toddlers, and children [7]. C-reactive protein (CRP), which is a major acute phase protein, is a member of the pentraxin family and plays a central role in innate and adaptive immunity. It takes 10-

12 h for CRP to rise after the onset of an infection significantly [8]. Since CRP shows an increase in several conditions, it is better to use it in combination with other biomarkers. Platelets, small anuclear cells derived from precursor megakaryocytes, have multiple functions and play a vital role in hemostasis by forming blood clots. They are a natural source of growth factors, including platelet-derived growth factor (PDGF) and transforming growth factor-β, which are essential for connective tissue repair and regeneration [9]. Platelet-rich plasma (PRP) has been used to increase the concentration of these growth factors and aid wound healing. Thrombopoiesis, the production of platelets, is driven by thrombopoietin and several transcription factors. In inflammatory states, IL-6 enhances the process of proplatelet formation by increasing thrombopoietin levels [10]. Mean platelet volume (MPV) is one of the hemogram parameters that is affected by many inflammatory conditions. In neonates, MPV can predict the development of sepsis and its severity [11]. Therefore, the combined measurement of CRP and MPV can be used to diagnose bacterial *vs* viral pneumonia and predict its complications [12]. This study aims to assess the effectiveness of salivary C-reactive protein (sCRP) and mean platelet volume (MPV) in identifying late-onset pneumonia in newborns.

MATERIALS AND METHODS

The present research was a prospective case-control study conducted on eighty full-term neonates recruited serially from the Neonatal Intensive Care Unit (NICU) and Clinic, Pediatric Department, the tertiary care hospital of Tanta University between June 2021 and May 2022, to evaluate the usefulness of salivary C-reactive protein (sCRP) and mean platelet volume (MPV) in identifying Late Onset Neonatal Pneumonia (LONP). The recruited neonates were divided into two comparable groups: Group I included neonates who developed late- onset neonatal pneumonia (who developed pneumonia after 3rd day and before 28th days of life). Group II included healthy neonates with no clinical manifestation of infection or other systemic diseases.

We included full-term neonates (gestational age ≥ 37 wk and birth weight ≥ 2.5 kg) with post-natal age between 7 and 28 days with clinical suspension of late-onset pneumonia. All eligible neonates underwent comprehensive assessments of their prenatal, perinatal, and postnatal history, thorough clinical examinations, a complete blood cell count (CBC) including differential, evaluation of CRP levels, urine analysis and culture, blood culture, cerebrospinal fluid (CSF) analysis and culture, and relevant infection markers. Blood gases, chest imaging (Plain X-ray and/or ultrasonography), echocardiography, and abdominal X-ray were conducted based on specific clinical indications. We followed Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI) for reporting neonatal infection [13] and Gerdes' sepsis screen (>2) to screen for neonatal sepsis, including pneumonia [14]. Pneumonia was suspected in the presence of fever or temperature instability, irritability, lethargy, feeding difficulty, apnea, or respiratory distress. Other systemic manifestations, such as hepatomegaly, abdominal distention, convulsion, hypotonia, hemodynamic instability, and bleeding diathesis, were considered general manifestations of neonatal sepsis. We classified the patients as mild (RD 1: tachypnoea >60/min and flaring nostrils), moderate (RD 2: RD2 + intercostal and subcostal retractions), severe (RD2 + expiratory grunting), and advanced (RD3 + central cyanosis); according to the degree of respiratory distress. Chest X-ray findings varied from normal chest X-ray to localized or diffuse alveolar densities, reticular opacities, homogenous ground glass opacities, and dense bilateral air space-filling process with air bronchograms. Any pneumonia complication findings were also recorded, such as interstitial emphysema, pleural effusion, pneumomediastinum, or pneumothorax.

We excluded premature infants and neonates with inflammation other than pneumonia, congenital heart conditions, hypoxic-ischemic encephalopathy, liver or kidney issues, hereditary coagulopathies, or any other systemic disorders unrelated to pneumonia that could impact CRP or platelet size levels. We also excluded neonates exposed to antibiotics before admission, neonates younger than seven days, or infants older than 28 days of life. According to Neonatal Intensive Care Unit protocol, all children with

suspected pneumonia receive the appropriate management. All parents, guardians, or next of kin signed informed consent for the minors to participate in this study. The Institutional Ethical and Research Review Board of the Faculty of Medicine, Tanta University, approved the study.

Laboratory investigations, including salivary and serum CRP measurement and MPV measurement. We collected salivary samples just before feeding to avoid milk contamination. With gentle handling of the baby, we stimulated the saliva secretion by allowing the baby to suck on a clean, sterilized pacifier for a few minutes. The head of the baby was elevated to allow the saliva to collect on the floor of the mouth, under the neonates' tongues, for accessible collection. The saliva samples were collected using a one-ml syringe without a needle with the suction pressure applied manually for about 10–15 s, collecting about 0.5 mL of saliva. Then, the samples were transferred to sterile polypropylene tubes to avoid contamination and stored at −20 °C until analysis and measuring CRP using ELISA. Serum CRP levels were determined using a fully automated auto-analyzer Cobas c501 (Roche Diagnostics, Manheim, Germany).

A peripheral blood sample was collected just before feeding (to avoid the effect of feeding on the platelet volume) into a clean, sterile EDTA Vacutainer tube to measure MPV value. The sample was handled gently without unnecessary agitation to minimize platelet activation and analyzed using an automated blood cell counter (Cell-Dyn 3700, Abbott Laboratories, IL, USA) within 60 minutes of collection to avoid platelet swelling and pseudo increase in MPV value. The analyzer calculates the MPV by dividing the total platelet volume by the number of platelets in the blood sample.

Statistical Analysis:

We used the Power and Precision V3 program (http://www.Power-Analysis.com, Englewood, New Jersey) to determine the study's power level. The collected data were organized, tabulated, and subjected to statistical analysis using the Statistical Package for the Social Sciences (SPSS) version 20 (SPSS, Chicago, IL, USA) to determine the sensitivity, specificity, and predictive value of MPV, sCRP, serum CRP, and the serum CRP/MPV ratio for diagnosing LONP cases. We used the Shapiro-Wilk test to test the

normality of data distribution. Mann-Whitney U-test assessed the differences between groups regarding nonparametric quantitative data. Receiver operating characteristic (ROC) curves were used to identify optimal cutoff values for differentiating patients with LONP from healthy controls. We used the mean and standard deviation to characterize the quantitative data. We considered the findings to be statistically significant when the P value is < 0.05.

RESULTS

In this study, we compared two groups of neonates: one with LOP comprising 40 neonates and a control group of 40 healthy neonates. Our analysis in Table 1 revealed no significant differences in sex distribution, age, mode of delivery, APGAR score, and the presence or absence of maternal illness during pregnancy between the neonates with LOP and the control group. However, notable variations in anthropometric measurements were observed, with the neonates with LOP neonates exhibiting lower weight, length, and head circumference than the control group. Additionally, the LOP group demonstrated substantially elevated levels of serum C-reactive protein (CRP), platelet volume (MPV), and the CRP/MPV ratio, indicating the potential diagnostic value of these markers for LOP among neonates.

Table 2 shows LOP's clinical and radiological features in a cohort of 40 neonates. Clinical manifestations were prominent, with 65% and 70% of neonates exhibiting fever and cough, respectively. Various degrees of respiratory distress were observed, ranging from RD1 to RD4 in 10% to 40% of neonates. Abnormal auscultatory findings were prevalent, including decreased air entry (82.5%) and fine crepitations (87.5%). Radiologically, pneumonic patches with air bronchogram were the most common pattern (75%), followed by homogenous ground glass shadow (10.0%) and interstitial pneumonia (10.0%). A smaller percentage showed a complete white lung (2.5%), and complications were noted in 2.5% of cases. Oxygen support was crucial, with 77.5% requiring a nasal cannula, 12.5% supported by nCPAP, and 10% necessitating mechanical ventilation. These comprehensive findings offer valuable insights into LOP's

clinical and radiological spectrum, enabling more precise diagnoses and tailored treatment approaches for affected neonates.

In addition to the clinical and radiological findings, the study evaluated the diagnostic validity of key markers in discriminating patients from controls, as shown in Table 3. The area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for MPV, Serum CRP, and sCRP. MPV exhibited a high discriminatory ability (AUC = 0.87) with a sensitivity of 86.67% and specificity of 80.0% at a cutoff value of >8.8. Serum CRP also showed good discriminative power (AUC = 0.81), with a sensitivity of 76.67% and specificity of 60.0% at a cutoff value of >6. Similarly, sCRP demonstrated notable discriminatory ability (AUC = 0.80) with a sensitivity of 76.67% and specificity of 83.33% at a cutoff value of >3.5. Furthermore, correlations were explored between serum CRP, salivary CRP, and MPV in Table 4, revealing significant positive correlations. The study also assessed the diagnostic validity of salivary CRP in predicting serum CRP levels, demonstrating a high AUC of 0.89 with a sensitivity of 91.3% and specificity of 71.4% at a cutoff value of >3.2, as shown in Table 5. These comprehensive assessments highlight the potential diagnostic utility of MPV and CRP markers, both serum and salivary, in discriminating and predicting disease severity in neonates with LOP.

DISCUSSION

Late-onset pneumonia (LOP) in neonates is a critical condition, presenting unique challenges in diagnosis and management that demands a thorough understanding of its clinical, radiological, and biochemical features to enhance diagnostic precision, facilitate tailored therapeutic interventions, and improve clinical outcomes [15]. This study aimed to comprehensively explore these aspects and evaluate the diagnostic potential of specific biomarkers in discriminating between neonates with LOP and healthy controls. The clinical manifestations observed in neonates with LOP encompassed fever, cough, varying degrees of respiratory distress, decreased air entry, and fine crepitations. These clinical findings align with the well-documented respiratory symptoms associated with

pneumonia. These findings agreed with Omran *et al*, who studied 35 full-term neonates diagnosed with LOP and 35 controls. They found fine crepitation in 32 (91.4%), decreased air entrance in 24 (74.3%), and intercostal retractions in 25 (71.4%) [12]. Furthermore, the varying degrees of respiratory distress, observed as thoracic retractions in Omran *et al*'s study and as RD1 to RD4 in our study, indicate the diverse respiratory involvement in LOP. Additionally, radiological examinations provided valuable insights into the lung pathologies present in LOP, notably pneumonic patches with air bronchograms, ground glass shadows, and interstitial pneumonia. These findings agree with that of Haney *et al*, who found that bilateral alveolar densities were the most commonly identified X-ray abnormality, noted in 77% of cases. One-third of their patients had typically dense and extensive alveolar changes with frequent air bronchograms [16]. Our findings resonate with existing literature, further validating the robustness of our study.

Intriguingly, our study delved deeper into the potential diagnostic value of biomarkers. Mean Platelet Volume (MPV), Serum C-reactive protein (CRP), and Salivary CRP (sCRP) were identified as promising, readily available candidates for discriminating patients from controls. MPV, a marker often associated with inflammatory conditions, demonstrated high discriminative ability (AUC = 0.87) with significant sensitivity and specificity, suggesting its potential as a diagnostic tool for LOP.

Platelets play a significant role in neonatal sepsis-induced coagulopathy. During systemic inflammation, P-selectin is expressed on the surface of platelets, enhancing platelet adherence to leukocytes, platelet aggregation, and expression of tissue factor on monocytes [17]. MPV is considered a marker of platelet function and activation, associated with larger and more reactive platelets. During inflammatory states, platelets become activated, leading to an increase in MPV. This inflammation observed in LOP triggers platelet activation, resulting in larger platelets (higher MPV). Therefore, MPV could be used as a marker that indicates systemic inflammation and infection, including LOP [18]. It is being studied in various conditions in adults and children, including acute

coronary syndrome and acute appendicitis [19,20]. In the current study, MPV was shown to have a high level of discriminative ability (AUC = 0.87), with a sensitivity of 86.67% and specificity of 80.0% at a cutoff value of >8.8 for neonates with LOP. The high AUC score indicates that MPV is an excellent diagnostic tool for distinguishing neonates with LOP from healthy controls. An AUC of 0.87 signifies a strong ability to correctly classify patients and controls based on MPV levels. In addition, MPV has a sensitivity of 86.67%, which means it accurately identified the majority (86.67%) of neonates with LOP, reducing false negatives. On the other hand, the specificity of 80.0% suggests that MPV effectively excluded a significant portion (80.0%) of healthy neonates, reducing false positives. With a cutoff value of >8.8 for MPV, neonates with MPV levels exceeding this threshold are likelier to have LOP.

Our results agree with many previous works. Omran and colleagues found a significant difference in MPV levels between neonates suffering from pneumonia and those who didn't and established a noteworthy association between MPV and CRP in both serum and saliva [21]. We have confirmed these findings. MPV, with a cut-off value of 9.0 fl exhibited an excellent diagnostic accuracy of 80% in identifying infants with pneumonia. Similarly, Pamudji *et al* reported that an MPV of 7.44 fl had 80% sensitivity and 84.2% specificity in diagnosing neonatal sepsis [22]. In addition, Wang *et al* conducted a meta-analysis which found that MPV was significantly higher in patients with neonatal sepsis than in the control group, suggesting that MPV could be used as an early indicator for diagnosing neonatal sepsis in clinical practice [23]. This can help clinicians make diagnostic decisions based on MPV levels. With a high AUC and its balanced sensitivity and specificity, MPV holds promise as a diagnostic marker for neonatal LOP. It may offer a relatively simple and cost-effective method to diagnose neonatal pneumonia [24]. However, further research and validation in larger and more diverse cohorts are needed to definitively establish MPV's diagnostic accuracy and clinical utility.

CRP is one of the most utilized biomarkers to monitor infection and inflammation in the pediatric and neonatal populations [25]. Serum CRP, a well-established inflammation

marker, exhibited a substantial discriminative ability (AUC = 0.81) and meaningful sensitivity and specificity, as observed in the current study. These findings are consistent with previous research indicating the diagnostic significance of serum CRP in respiratory infections. Kumar *et al* found higher overall serum CRP accuracy in diagnosing late-onset neonatal sepsis, ranging from 96.5% in proven sepsis to 99.1% in probable sepsis with a specificity of 85.3 %, using a CRP cut-off value of 5 mg/L. This suggests that CRP has a high diagnostic accuracy in identifying neonates at risk of sepsis [26]. In addition, Omran *et al* found a significant increase in serum CRP with a mean of 29.4 ± 13 mg/L neonates with late-onset sepsis [27].

Serum CRP also exhibited considerable discriminative ability, with notable sensitivity and specificity for neonatal infection, including pneumonia. Its diagnostic significance in respiratory infections aligns with the observed potential in discriminating LOP. A meta-analysis by Xiao *et al* revealed that infants with pneumonia exhibited higher serum CRP levels than healthy infants. Therefore, serum CRP levels might be an independent diagnostic tool for pneumonia in children [28]. In addition, Li *et al* found a close correlation between higher serum CRP levels and the progression of neonatal pneumonia [29]. Several studies have reported different cut-off values for serum CRP levels in diagnosing neonatal sepsis. These values range widely, from 1.5 to 20 mg/L, and are associated with varying sensitivities and specificities. For instance, sensitivity values range from 74% to 98%, while specificities range from 71% to 94%, whether using a single measurement at least 12 h after the onset of symptoms or serial CRP determinations [30-33].

The detection of CRP in saliva is a new and promising diagnostic method that has recently gained attention as an emerging biomarker. It shows potential in diagnosing various medical conditions, such as pneumonia. One of the significant advantages of using sCRP in diagnosing neonatal pneumonia is its non-invasive nature. Saliva collection is less intrusive and more feasible than obtaining blood samples, especially in neonates, where it can be challenging to draw blood [34,35]. Iyengar *et al* conducted a study on y sCRP detection and its usefulness in neonates, which is considered the first

study to detect, quantify, and demonstrate that sCRP is a good measure of discrimination for clinically relevant serum CRP thresholds. The study included most salivary samples obtained from neonates suffering from necrotizing enterocolitis (NEC) or spontaneous intestinal perforation (SIP), infectious diseases, and post-operative monitoring. The median sCRP concentration was found to be 3.1 ng/mL, whereas the median serum CRP concentration was 106.1 mg/L [36]. Interestingly, we used sCRP in our study as a non-invasive alternative that displayed noteworthy discriminatory ability (AUC = 0.80) and strong correlations with serum CRP and MPV. Our results emphasize the potential of sCRP as a viable diagnostic marker in neonates with LOP and agree with many previous studies. A study conducted by Omran, and colleagues found a result similar to ours. They observed a significant difference in the mean level of salivary C-reactive protein between septic neonates (12.0±4.6ng/L) and the control group (2.8±1.2ng/L). The sensitivity of salivary C-reactive protein was 94.3%, and specificity was 80% at a cut-off point of 3.48ng/L [27]. Barekatain and colleagues also reported a significant increase in sCRP levels in neonates with sepsis compared to healthy controls. The AUC value was 0.63, with a sensitivity of 44.9%, specificity of 80%, PPV of 73.3%, NPV of 54.2%, and diagnostic accuracy of 61% at a cutoff of 4.55 ng/L [25].

The current study has shown a significant correlation (r = 0.59, p < 0.001) between serum CRP and sCRP levels in neonates with LOP. This suggests that sCRP levels reflect those in serum and can serve as a non-invasive diagnostic biomarker for neonatal LOP. sCRP can potentially be used as a proxy for serum CRP, indicating the systemic inflammatory response associated with pneumonia [37]. The positive correlation strengthens the case for considering sCRP as a reliable diagnostic tool for neonatal LOP. In a study by Iyengar *et al*, an sCRP concentration of 4.84 ng/L was found to have 64% sensitivity and 94% specificity for predicting a serum CRP of 5 mg/L. It was also found to have 54% sensitivity and 95% specificity for predicting a serum CRP of 10 mg/L [36]. On the other hand, Tosson *et al* found no significant correlation between sCRP and serum CRP levels in neonates with late-onset sepsis [38]. To ensure consistent and

accurate sCRP measurement across different studies, it is important to address various challenges that may affect its accuracy. For instance, screening for oral trauma and controlling the salivary flow rate is necessary to account for the salivary dilution effect. However, there are no available reliable strategies to make salivary CRP an accurate quantitative measure of serum CRP, which limits the use of point-of-care systemic inflammation testing [39].

The current study found a significant positive correlation (r = 0.66, p < 0.001) between serum CRP and MPV in neonates with LOP. This means that when serum CRP levels increase, MPV also tends to increase. The positive correlation between MPV and serum CRP suggests that MPV is associated with the inflammatory response and may reflect the level of inflammation present in pneumonia. In addition to this positive correlation between CRP and MPV in the current study, the serum CRP/MPV ratio in neonates with LOP (3.86 ± 2.29) was significantly higher than in the control group (0.42 ± 0.39) with a p-value of less than 0.0001. The elevated Serum CRP/MPV ratio in neonates with LOP signifies a higher inflammatory state. CRP is a key acute-phase protein that increases during inflammation. Concurrently, as a marker of platelet activation, MPV is influenced by the inflammatory response. The higher ratio suggests a greater inflammatory burden and platelet activation in neonates with LOP [40]. Using MPV as a diagnostic marker in combination with serum CRP could provide a more comprehensive understanding of the inflammatory status in neonates with LOP. Omran et al found a significant increase of CRP/MPV in neonates with late-onset sepsis than in the control [12].

The current study reveals a statistically significant positive correlation between MPV and sCRP in neonates with LOP (r = 0.54, P = 0.01**). This correlation indicates that as sCRP levels increase, MPV also tends to increase. Our findings agree with the work of Omran et al, who found a significant positive correlation between sCRP and MPV (p <0.001) {27}. The positive correlation between MPV and sCRP suggests that both biomarkers are associated with the inflammatory response seen in late-onset pneumonia.

Elevated sCRP levels indicate systemic inflammation, and this correlation implies that higher levels of inflammation are associated with an increase in MPV. Understanding the correlation between sCRP and MPV is clinically valuable [41]. Monitoring both sCRP and MPV in neonates with LOP can provide complementary information about the severity of the inflammatory response [23,36]. If both sCRP and MPV are elevated, it could indicate a more pronounced inflammatory state, prompting close monitoring and possibly more aggressive treatment. The correlation between sCRP and MPV suggests that MPV, an easily measurable parameter, could serve as a supplementary diagnostic marker alongside sCRP. It may enhance the accuracy of diagnosing and monitoring the inflammatory status in neonates with LOP [42].

Moreover, the correlations between serum CRP, salivary CRP, and MPV underlined their interrelated nature, indicating the potential for a multi-marker approach in diagnosis and disease monitoring. The high AUC of sCRP in predicting serum CRP levels further advocates for its utility in disease assessment [38]. Understanding these correlations is critical for establishing the diagnostic potential of these markers. The strong correlations between serum CRP, salivary CRP, and MPV suggest their interlinked roles in reflecting the inflammatory response associated with LOP. Incorporating these markers collectively in diagnostic algorithms could enhance accuracy and offer a more holistic assessment of the inflammatory status in neonates with LOP, potentially leading to improved diagnostic and therapeutic approaches [43]. Further research should delve deeper into these correlations, exploring their clinical implications and potential for diagnostic integration.

Limitations of a study: It is crucial to provide possible limitation of the current study that limits the generalizability of its findings. The study's sample size is relatively modest, which may limit the generalizability of the findings to a broader neonatal population. The study being conducted in a single center may introduce institutional biases and limit the external validity of the results. The variability in the clinical presentation of LOP among neonates may introduce heterogeneity in the study population, potentially affecting the consistency and reliability of the observed clinical

and radiological features. The study's cross-sectional design limits the assessment of temporal relationships and trends over time, which could be valuable for understanding the progression and outcomes of LOP in neonates. While MPV, serum CRP, and sCRP were evaluated as potential diagnostic markers, other relevant biomarkers that could contribute to a more comprehensive assessment were omitted. We also should consider the factors that could affect the SCRP levels,[12] such as salivary flow rate, circadian rhythm, age, sex, type of salivary gland, salivary stimulation, feeding, and collection method. The study's exclusion of preterm neonates might limit the generalizability of the findings to the entire neonatal population, as preterm infants often have unique healthcare needs and susceptibilities. The study also employed specific radiological techniques (plain Chest X-rays); however, using advanced imaging modalities such as high-resolution computed tomography (HRCT) or other advanced imaging methods could have provided additional valuable insights into the lung pathology of neonates with LOP. In addition, the study's findings might be specific to a particular ethnic or geographical population. Caution should be exercised when generalizing the results to a more diverse or different population.

Suggestion for Future Research: Including a larger and more diverse cohort of neonates could provide a more comprehensive representation of LOP cases. Multicentre studies involving diverse healthcare settings could offer a more comprehensive view of LOP cases. We also must explore a broader array of biomarkers for a more accurate diagnosis.

CONCLUSION

This study offers a comprehensive understanding of the clinical, radiological, and biomarker profiles in neonates with LOP, aligning with the observations made by previous studies. The potential diagnostic utility of MPV, serum CRP, and sCRP was evident, opening new avenues for non-invasive diagnostic approaches. Integrating these biomarkers into clinical practice may enhance diagnostic accuracy and subsequently improve outcomes for neonates with LOP. Future research should focus

on validating these findings in larger cohorts and exploring the prognostic implications of these biomarkers in guiding therapeutic strategies.

ARTICLE HIGHLIGHTS

Research background

Neonatal sepsis is a significant cause of neonatal mortality, and late-onset pneumonia (LOP) is a challenging form of sepsis to diagnose. Saliva has been identified as a potential diagnostic fluid for neonates. C-reactive protein (CRP) and MPV are biomarkers that can indicate inflammation and are of interest in diagnosing neonatal infections.

Research motivation

The research is motivated by the need to improve the diagnosis of LOP in newborns, a serious condition that can lead to high mortality rates. Current diagnostic methods for neonatal infections, including pneumonia, can be challenging. The motivation is to find non-invasive and effective diagnostic tools that can aid in the early and accurate identification of LOP. Salivary C-reactive protein (sCRP) and mean platelet volume (MPV) are being investigated as potential biomarkers to enhance the diagnosis and management of LOP, aiming to improve clinical outcomes for affected newborns.

Research objectives

We aimed to assess the diagnostic accuracy of sCRP and MPV biomarkers, analyzing their temporal trends, considering demographic factors, and exploring their clinical implications in diagnosing late-onset pneumonia in newborns.

Research methods

The study involved 80 full-term neonates divided into a group with LOP and a control group. Clinical assessments, blood tests, and imaging were conducted to diagnose LOP.

Salivary and serum CRP levels, as well as MPV, were measured. Statistical analysis was performed to determine the diagnostic validity of these markers.

Research results

Neonates with LOP showed differences in weight, length, head circumference, serum CRP, MPV, and the CRP/MPV ratio compared to the control group. Clinical and radiological features of LOP were observed, including fever, cough, respiratory distress, and abnormal auscultatory findings. MPV, serum CRP, and sCRP exhibited good discriminative power for diagnosing LOP. Positive correlations were found between serum CRP, sCRP, and MPV.

Research conclusions

The study provides insights into the clinical, radiological, and biomarker profiles in neonates with LOP. MPV, serum CRP, and sCRP show potential for non-invasive diagnostic approaches. Integrating these biomarkers into clinical practice may enhance diagnostic accuracy and improve outcomes for neonates with LOP.

Research perspectives

Further research in neonatal late-onset pneumonia is needed to validate findings and assess generalizability across different populations and healthcare settings, investigate temporal trends and longitudinal studies, explore multi-marker approaches, assess ethnic and geographic variations, analyze the kinetics of sCRP and MPV, conduct studies on preterm neonates, compare diagnostic performance with other modalities, examine clinical implications, develop point-of-care testing methods, and investigate therapeutic implications. These research perspectives can lead to improved clinical practices and outcomes for affected neonates.

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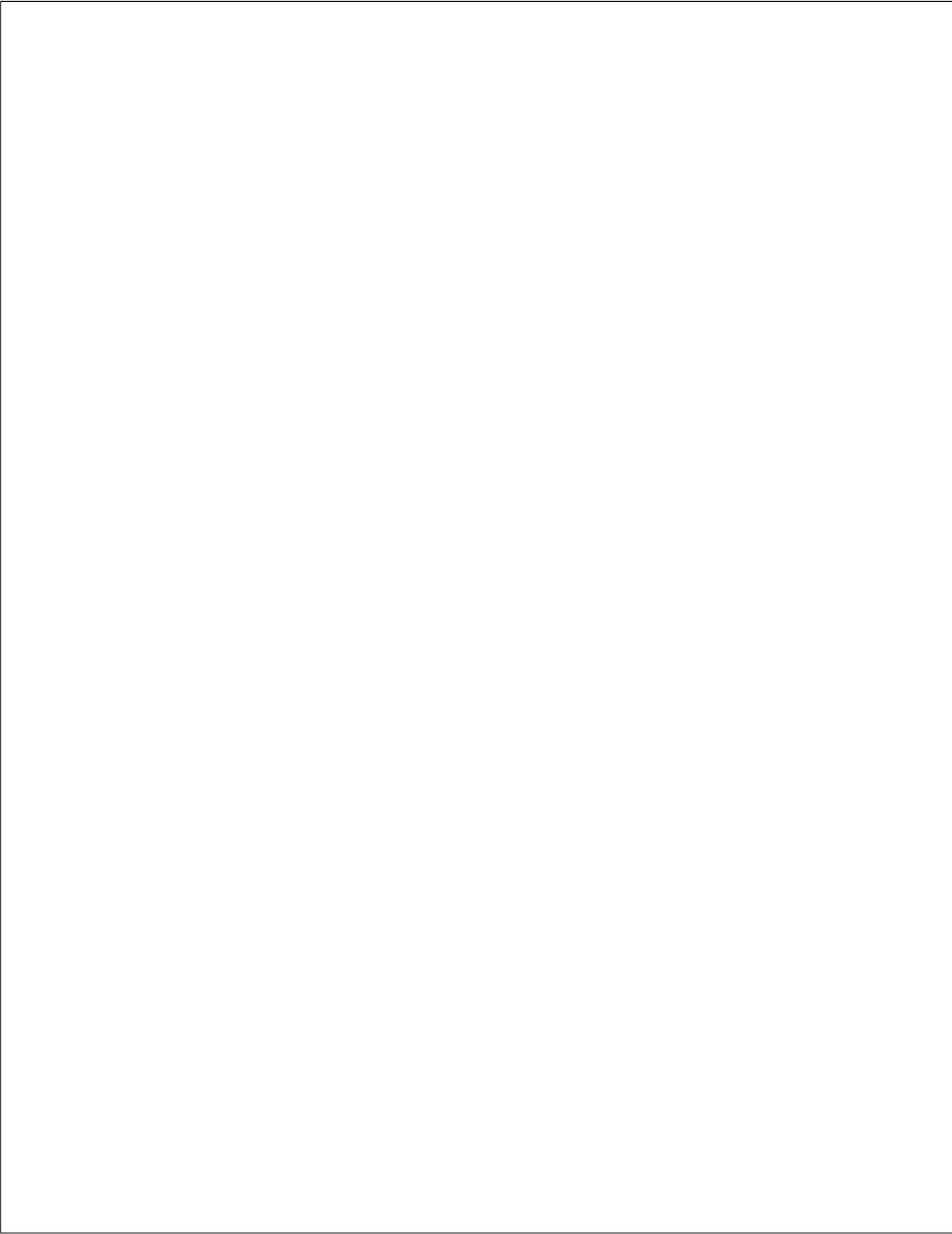
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PRIMARY SOURCES

1   Ahmed Omran, Mohammed Ali, Mai H. Saleh, Osama Zekry. "Salivary C-reactive protein and mean platelet volume in diagnosis of late-onset neonatal pneumonia", The Clinical Respiratory Journal, 2018
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11   "International Academy of Cardiology Annual
     Scientific Sessions 2017. 22nd World Congress on   12 words — < 1%
     Heart Disease. Vancouver, BC, Canada, July 14-16, 2017:
     Abstracts", Cardiology, 2017
     Crossref

12   Pei Wen Lim, Johan Garssen, Elena Sandalova.
     "Potential Use of Salivary Markers for   12 words — < 1%
     Longitudinal Monitoring of Inflammatory Immune Responses
     to Vaccination", Mediators of Inflammation, 2016
     Crossref

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