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Original Research Article

A Novel Combination of Serum Pct and Nitric Oxide for Early Diagnosis of Neonatal Sepsis

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Conflict of interest: Nil

Abstract

Introduction: Neonatal sepsis is general cause of mortality in new-born infants. It manifests either early (<7 days of birth) or late (>7 days). Despite of advanced neonatal treatment, sepsis still has significant effect on neonatal morbidity and survival rates.

Aim and objectives: The present study, aimed to investigate serum levels of PCT and NOx in neonates and to find out the correlation between PCT and NOx, in establishing the early diagnosis of neonatal sepsis. we included the following subjects in our study.

Materials and Methods: The clinical criteria taken as indicative of sepsis were: I. Mother suffering from fever or rupture of amniotic membrane >24 hr II. Low birth weight (< 2500 grams) and premature birth (<37 weeks). III. Clinical Signs and symptoms of sepsis which includes diarrhea, vomiting, poor sucking and abdominal distension. IV) Control group - Healthy neonates with no clinical sign and symptoms and negative for lab findings.

Results and Discussion: The present study was intended to estimate the levels of procalcitonin and nitric oxide levels in neonatal sepsis and to compare these values between controls and cases. As per the inclusion criteria we included 85 clinically proven cases neonatal sepsis and 35 controls. Even though the gold standard means for diagnosing sepsis is blood culture, the results of the blood culture are available only after minimum 12 hours and having high risk of contamination making it difficult to diagnose neonatal sepsis. Early diagnosis and early intervention is very important to save the life of the patient. Therefore, a rapid test with the best degree of sensitivity, reliability, and predictability is required for the early diagnosis and treatment of neonatal sepsis. We found significantly elevated levels of PCT and Nitric Oxide levels in subjects with neonatal sepsis as compared to healthy controls.

Conclusion: We found elevated levels of pro-calcitonin and nitric oxide levels in neonatal sepsis, by measuring these parameters early diagnosis and early therapeutic intervention can be taken.

Keywords: Neonatal Sepsis, Procalcitonin, Nitric Oxide, Neonatal Morbidity and Blood Culture.

Introduction

Neonatal sepsis is a general cause of mortality in new-born infants. It manifests either as early onset (<7 days of birth) or late onset (>7 days).[1]Despite of advanced neonatal treatment, sepsis still has an important effect on neonatal morbidity and survival rates. [2] Diagnosing sepsis is difficult due to its vague and nonspecific symptoms. The established test for diagnosing sepsis is a blood culture. However, chances of contamination or no culture growth leads to false-positive results. To reduce morbidity and mortality rates and increasing antibiotic resistance and hospital charges antibiotics are administered before culture results are obtained. Thus, an accurate and rapid diagnostic test is necessary. [3]

Procalcitonin (PCT) is secreted from C type cells of thyroid gland as a precursor of calcitonin. [4] It is composed of 116 amino acids. Nowadays, serum Procalcitonin (PCT) is used as a marker of sepsis. [5] Bacterial lipopolysaccharide (LPS) induces the release of PCT into the systemic circulation. Its concentration starts to rise from 3-4 hr after encountering endotoxin, peak about 6 hr, and remain increased for over 24 hr. [6] Its Half-life in serum is 20-24 hours, and it also distinguish bacterial infection from other types of inflammations which makes it suitable for daily monitoring. Therefore, quantitative determination of procalcitonin is appropriate for the diagnosis of infections. Nitric oxide (NO) is recognized as a mediator in a broad array of biologic systems. It is produced by enzyme Nitric oxide synthase (NOS; EC 1.14.13.39). NO Synthase has three isoforms namely Neuronal NOS, Endothelial NOS and Inducible NOS. [7]

Synthesis of NO: Arginine, O2, and NADPH are substrates for cytosolic NO synthase. Flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), heme, and tetrahydrobiopterin are coenzymes for the enzyme, and NO and citrulline are products of the reaction. [8]

Isoforms of NOS: Neuronal NOS is constitutively expressed in central and peripheral neurons. Endothelial NOS is mostly expressed in endothelial cells. It keeps blood vessels dilated, controls blood pressure, and has numerous other Vaso-protective and anti-atherosclerotic effects. [9] Inducible NOS is expressed in many cell types in response to lipopolysaccharide, cytokines, or other agents. Inducible NOS generates large amounts of NO that have cytostatic effects on parasitic target cells. Inducible NOS contributes to the pathophysiology of inflammatory diseases and septic shock. [10] NO play a key role in pathogenesis. In many experimental studies, endotoxin increases the constitutive release of NO by the endothelium and the activity of the iNOS enzyme. Mice lacking iNOS have been reported to be resistant to endotoxin-induced mortality and vascular hypo contractility, supporting a key role for iNOS in endotoxin shock. [11] In addition to endotoxin, cell wall components and enterotoxin from gram-positive organisms stimulates NO release, due to this NO will keep on producing over hours or even days. [12] Serum procalcitonin (PCT) is considered as valuable diagnostic marker for systemic bacterial infection and sepsis. Presently it's cellular sources and biological properties of PCT are unclear. During sepsis and septic shock, genetic expression of enzyme inducible nitric oxide synthase (iNOS) gets stimulated which leads to release of large amounts of nitric oxide (NO). PCT inhibits the iNOS effects cytokines TNF-α/IFN-γ in a systemic dose-dependent manner. This might be a reason of counter-regulatory mechanism which is directed against the production of NO and hypotension in severe sepsis and septic shock.[13]

Aim and Objectives:

The present study, aimed to estimate serum levels of PCT and NOx in neonates and to find out the correlation between PCT and NOx, in establishing the early diagnosis of neonatal sepsis.

Materials and Methods:

Permission from Institutional Ethics Committee and consent from participants were obtained before carrying out this study. This prospective cross-sectional study included 160 neonates who were admitted to the Neonatal Intensive Care Units (NICU) at JK Lone hospital.

Inclusion Criteria:

We included the following subjects in our study.

The clinical criteria taken as indicative of sepsis were:

I. Mother suffering from fever or rupture of amniotic membrane >24 hr.
II. Low birth weight (< 2500 grams) and premature birth (<37 weeks).
III. Clinical Signs and symptoms of sepsis which includes diarrhea, vomiting, poor sucking and abdominal distension.
IV. Control group - Healthy neonates with no clinical sign and symptoms and negative for lab findings.

Sample Collection:

Venous Blood samples were collected into empty red vacutainer and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 3,000 rpm for 10 minutes. Serum samples used for the measurement of serum PCT and Nitric oxide levels were stored at -80°C until they were used.

Microbiological examination:

2 ml of blood was added to blood culture media and incubated at 37 °C for 5-7 days. Bottles with positive results were subcultured on blood agar (Himedia, India) and EMB media. The isolated microbes were identified by standard bacteriological methods.

Measurement of serum nitric oxide: *Blood* nitric oxide levels were examined by nitrite/nitrate quantitation using the Nitric Oxide Colorimetric Assay Kit. (Biovision; Milpitas, California) according to the manufacturer's protocol. Briefly, Plasma was collected and filtered through a 10 KDa cutoff filter, then the Nitrate Reductase mixture and the enzyme cofactor were added to the plasma in the plates. The plates were incubated at room temperature to convert nitrate to nitrite. The enhancer and Griess Reagent were then added. After the color was developed for 10 min at room temperature, the absorbance was measured at 540 nm in a plate reader.[14]

Measurement of Procalcitonin: Procalcitonin was measured by CLIA method on fully automatic hormonal analyser Maglumi X3. Finally based on the lab results of blood culture, serum PCT, NO and clinical symptoms of sepsis, neonates were classified in to three groups:

1) Proven sepsis (n= 85): positive blood culture and clinical symptoms of sepsis.

2) Suspected sepsis (n= 45): with clinical symptoms but negative blood culture.

3) Control group(n=35): Healthy neonates with no clinical and biological data of infection.

Statistical analysis: The data analysis was done by using SPSS statistic analyser software version 19. Data was expressed as mean ± SD. To compare means of the variables, one-way ANOVA test was done. p-value< 0.05 was considered as significant.

Results

Table 1: Shows the comparison of Serum levels of PCT and Nitric oxide between cases and controls

Variable	Groups		
	Confirmed sepsis	Control	p value
PCT (µg/L)	10.5 ± 1.5	0.5 ± 0.2	0.05
Nitric Oxide (mmol/L)	125 ± 12.5	70 ± 12.5	0.05

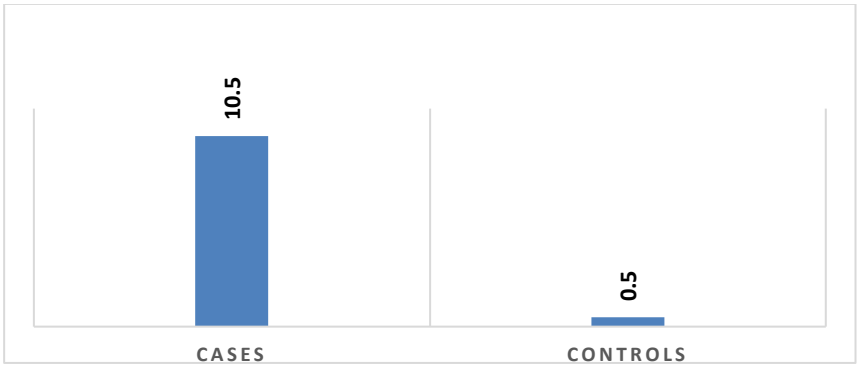

Figure 1: Shows the comparison of PCT values between cases and controls

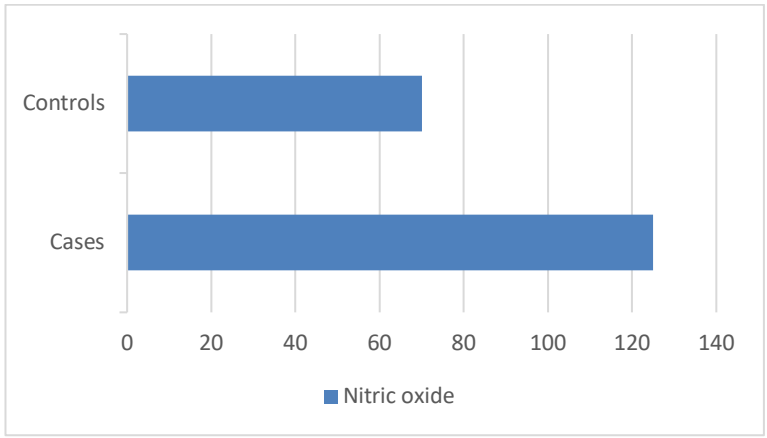

Figure 2: Shows the comparison of Nitric Oxide values between cases and controls

Discussion

The present study was intended to estimate the levels of procalcitonin and nitric oxide levels in neonatal sepsis and to compare these values between controls and cases. As per the inclusion criteria we included 85 clinically proven cases neonatal sepsis and 35 controls. We found significantly elevated levels of PCT and Nitric Oxide levels in subjects with neonatal sepsis as compared to healthy controls. Even though the gold standard means for diagnosing sepsis is blood culture, the results of the blood culture are available only after minimum 12 hours and having high risk of contamination making it difficult to diagnose neonatal sepsis. Early diagnosis and early intervention are very important to save the life of the patient. Therefore, a rapid test whose sensitivity, reliability, and predictability are accurate, is required for the early diagnosis and treatment of neonatal sepsis.

Conclusion

Blood tests such as CRP, Neutrophil, total white blood cell (WBC), absolute neutrophil count (ANC), platelet counts and blood culture are ordered to screen for suspected sepsis, these values are ineligible as infection markers due to insufficient sensitivity and specificity. Whereas utility of procalcitonin (PCT) as an early diagnostic tool for neonatal sepsis has been

reported. serum Procalcitonin (PCT) is used as a marker of sepsis. Bacterial lipopolysaccharide (LPS) induces the release of PCT into the systemic circulation. Its concentration starts to rise from 3-4 hr after encountering endotoxin, peak about 6 hr, and remain increased for over 24 hr. Its Half-life in serum is 20-24 hours, and it also distinguish bacterial infection from other types of inflammations. which makes it suitable for daily monitoring.

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