

## Original Research Article

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## Interplay between Inflammation, Coagulation And Redox Status in Early Onset Neonatal Sepsis: Insight into Prediction, Diagnosis and Management

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### ABSTRACT

Sepsis in newborn infants has a high risk of morbidity and mortality. Despite advances in medicine, diagnosis of neonatal sepsis remains as a major challenge. The aim of this study is to unravel the role played by new network of biomarkers namely high mobility group box 1 (HMGB1), thrombomodulin and heat shock protein (Hsp) 72 in addition to redox status in diagnosis of early onset neonatal sepsis before and after antibiotic therapy. The study enrolled 45 neonates, 30 of them had early onset sepsis (group 1), and 15 healthy neonates as a control group (group 2). Neonates with early onset sepsis were classified according to their culture status into culture-proven sepsis (n=19) (group 1a) and culture negative sepsis (n=11) (group 1b). Levels of HMGB1, thrombomodulin, Hsp72, nitric oxide (NO) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were estimated. Studied biomarkers were investigated before and 5 days after antibiotic therapy. Serum levels of HMGB1, NO and H<sub>2</sub>O<sub>2</sub> were significantly (p<0.05) higher in neonates with sepsis than in healthy neonates (65.7± 8.7 vs 38.2±9.9; 33.4±9.1 vs 13.6±1.8 and 59.6±7.9 vs 35.0±3.4 respectively) and in culture proven than culture negative neonates (70.4± 6.4 vs 57.5± 5.6; 36.4±8.8 vs 28.1±7.3 and 61.3±7.4 vs 55.3±7.0 respectively) before antibiotic therapy while thrombomodulin and Hsp72 were significantly (p<0.05) lower in neonates with sepsis than in healthy neonates (72.3±11.2 vs 144.2±15.6 and 14.6±1.6 vs 32.6±4.3 respectively) and in culture proven than culture negative neonates for thrombomodulin (67.8± 10.7 vs 80.1± 7.4) and non-significance for Hsp 72 showed p>0.05 (14.6±1.5 vs 14.6±1.9) before antibiotic therapy. Results of all the aforementioned biomarkers improved after antibiotic treatment in sepsis group. HMGB1, thrombomodulin and Hsp72 are considered promising biomarkers for early diagnosis of early onset neonatal sepsis.

#### Keywords

Neonatal sepsis, High mobility group box 1, Thrombomodulin, Heat shock protein 72, Redox status

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### Introduction

Neonatal sepsis is a serious problem associated with high degree of morbidity and mortality in both term and preterm infants (Camacho-Gonzalez *et al.*, 2013). Beyond being a life-threatening condition, sepsis can significantly impair neuron-developmental

outcome and can progress rapidly to septic shock and multiple organ dysfunction syndrome, early diagnosis is crucial to improve survival (Klinger *et al.*, 2010). Unfortunately, the diagnosis of sepsis may be difficult in the neonate. The earliest signs of the disease are often subtle and nonspecific, and can be easily confused with

signs of non-infectious diseases (Palazzi *et al.*, 2006). Blood culture is the gold standard for the diagnosis of sepsis, but at least 48–72 hours are needed for the results and the number of false negative results is not negligible, particularly in early-onset sepsis, for which the blood culture sensitivity is reported to be less than 10% (Hofer *et al.*, 2012). During sepsis, innate immune cells recognize pathogen-associated molecular patterns such, lipopolysaccharide (LPS), bacterial peptidoglycan and enterotoxins, or damage-associated molecular pattern, such as, heat shock proteins, uric acid, annexins, interleukin (IL)-1 $\beta$ , and high mobility group box 1 (HMGB1) (Pisetsky *et al.*, 2011). HMGB1 can actively affect the immune functions of inflammatory cells, once released, HMGB1 can bind to cell-surface receptors and mediate various cellular responses including chemotactic cell movement and release of pro-inflammatory cytokines (e.g., tumor necrosis factor alpha (TNF- $\alpha$ ) and IL-1 $\beta$ ), taken together, these observations characterized HMGB1 as a non-classical, pro-inflammatory cytokine (Huang *et al.*, 2012).

Inflammation and coagulation have a big participation in sepsis pathogenesis that makes them promising sepsis treatment targets. Thrombomodulin is an endogenous anticoagulant that helps in the maintenance of the anti-inflammatory and anticoagulant state of endothelial system (Okamoto *et al.*, 2016).

Heat shock proteins (Hsps) are proteins that play an important role in stressful conditions including infection (Evans *et al.*, 2010). The human Hsps family consists of at least 12 members, the best known members are the heat inducible form Hsp72, during time of infection, inducible Hsp72 is found extra-cellular to facilitate immunological defense responses (Evans *et al.*, 2010). Oxidative stress is defined as an imbalance between the

production of reactive oxygen species (ROS) and antioxidant defenses; mild oxidative stress has been demonstrated to promote cell survival, whereas severe oxidative stress has been demonstrated to cause oxidative injury and even death, additionally, sepsis promotes the unbalanced production of oxidant and anti-oxidant substances (Saugstad *et al.*, 2005).

This study was designed to highlight the role of HMGB1, thrombomodulin, Hsp72 and redox status as an intricate network for sepsis pathogenesis, diagnosis and treatment response in neonates.

## **Materials and Methods**

This case control study was conducted in Medical Biochemistry, Pediatrics, and Medical Microbiology Departments, Tanta University, Tanta, Egypt and was approved by the ethical committees of the University. Informed consents were obtained from parents to participate in this study.

## **Patients**

This study was performed on 30 (16 males and 14 females) newborns who were hospitalized at the neonatal intensive care unit at Tanta University Hospital for early onset neonatal sepsis (during the first 72 hours) (group 1). In the sepsis group (n=30), 19 infants had positive blood cultures and 11 infants had only clinical signs of sepsis with negative blood culture. A group of healthy matched controls (group 2, n=15) (9 males and 6 females) were enrolled for comparisons of biochemical assays and were selected from the well-baby outpatient clinic.

Infants were enrolled in this study if: a) their postnatal age was 60 breaths/min), chest retraction, cyanosis, respiratory distress-bradycardia (heart rate 180/min), poor skin color, capillary refilling time > 3 seconds,

hypotonia, irritability, lethargy, and seizures. Historical factors associated with increased risk for infections included premature rupture of the membranes (in term infants >18 hours), maternal fever during labor, intra-amniotic infection, and chorioamnionitis. Two or more abnormal values of the sepsis screen (white blood cell count  $10.000/mm^3$ , immature-to-total neutrophil ratio higher than 0.2 and positive C-reactive protein (CRP)) were considered as supportive for diagnosis of infection) Johnson *et al.*, 1997).

Infants were excluded if they had congenital malformations, congenital infections associated with the TORCH complex, and refusal of parental consent. The following routine laboratory assessments of sepsis were done for all participants included blood culture, complete blood count (CBC), total and differential white cell count, platelet count and CRP.

### **Blood sampling**

Venous blood samples were aseptically collected in sterile plain and EDTA tubes, centrifuged at 3000 rpm for 10 min at 4°C to obtain serum and plasma which were stored at -20°C till analysis.

First blood samples were obtained from patients at day 0 immediately at the time of sepsis diagnosis during first 72 hours after birth. Another sample was obtained from each patient at day 5 after antibiotic therapy. Only one blood sample was obtained from each healthy control subject.

### **Biochemical assessments**

The serum HMGB1 and Hsp72 as well as plasma thrombomodulin concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits (Sunred

Company, City, State, USA; Chongqing Biospes Co., Ltd, City, China; R&D Systems, City, State, USA respectively). All ELISA techniques were done according to the manufacturer's protocol and read on microplate reader (Stat Fax®2100, Fisher Bioblock Scientific, France), at 450 nm with correction wavelength set at 570 nm. In addition serum nitric oxide (NO) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) concentrations were assessed in serum using commercial kits supplied by Biodiagnostic Company, Egypt.

### **Statistical analysis**

Analysis was performed by Statistical Package for Social Sciences, version 14.0 for windows (SPSS, Chicago, IL, USA). Data were expressed as mean + standard deviation.

Paired and unpaired t-test for comparison between means of two groups and one way analysis of variance (ANOVA) for comparison between means of three groups were used. The level of significance was set at  $p < 0.05$ . Receiver operating characteristics (ROC) analysis was used to identify the optimal diagnostic values of the studied parameters.

## **Results and Discussion**

### **Demographic data, clinical characteristics and routine laboratory data of the studied neonates**

Table 1 shows that there were non-significant differences between group 1 and 2 regarding gestational age, birth weight, hemoglobin level, polymorph nuclear leucocytic count (PMN) and total leucocytic count (TLC) ( $p > 0.05$ ) moreover, platelet count showed a significant decrease in group 1 when compared with group 2 ( $p < 0.05$ ). CRP showed significant increase in group 1 when compared to group 2 ( $p < 0.05$ ).

### **Blood culture results**

Table 2 shows the blood culture results of those 19 infants with culture positive results.

### **Inflammatory biomarkers associated sepsis**

Table 3 shows a significant increase in HMGB1 serum level in sepsis group patients (group 1 at 0 day and 5 days after antibiotic therapy) when compared to group 2. Meanwhile, Hsp72 and thrombomodulin showed a significant decrease in sepsis group patients (group 1 at 0 day and 5 days after antibiotic therapy) when compared to group 2 ( $P < 0.05$ ). There was a significant decrease in HMGB1 in group 1 at day 5 after antibiotic therapy when compared to the same group at day 0 while Hsp72 and thrombomodulin showed the reverse ( $P < 0.05$ ).

Table 4 shows that HMGB1 concentrations at day 0 were significantly higher in group 1a compared to group 1b ( $P < 0.05$ ) with a non-significant decrease at day 5 ( $P > 0.05$ ), also there was a significant decrease in HMGB1 in group 1a at day 5 compared to day 0 levels ( $P < 0.05$ ), moreover, similar finding was not found in group 1b ( $P > 0.05$ ).

Plasma thrombomodulin level was significantly lower in group 1a compared to group 1b at day 0 ( $P < 0.05$ ) however, it showed a non-significant difference between groups 1a and 1b at day 5 after antibiotic therapy ( $P > 0.05$ ), moreover, thrombomodulin level was significantly increased in groups 1a and 1b after antibiotic therapy compared to day 0 concentrations ( $P < 0.05$ ). Serum Hsp72 levels showed non-significant difference between group 1a and group 1b at days 0 and 5 ( $P > 0.05$ ), however, they significantly increased after antibiotic therapy in both group 1a and group 1b compared to their levels prior to antibiotic therapy ( $P > 0.05$ ). In table 4 CRP concentrations were non-significantly higher

in group 1a compared to group 1b at day 0 and at day 5 after antibiotic treatment ( $P > 0.05$ ), moreover, CRP significantly decreased after antibiotic therapy in both group 1a and group 1b compared to their concentrations prior to antibiotic therapy compared to day 0 concentrations ( $P < 0.05$ ).

### **Results of redox status biomarkers**

Table 3 shows a significant increase of both NO and  $H_2O_2$  serum levels in group 1 at 0 day and 5 days after antibiotic therapy when compared to group 2 ( $P < 0.05$ ). In addition, NO and  $H_2O_2$  showed a significant decrease in group 1 at day 5 when compared to the same group at day 0 ( $P < 0.05$ ). In table 4 serum NO and  $H_2O_2$  were significantly higher in group 1a compared to group 1b at day 0 ( $P < 0.05$ ), however, there was a non-significant difference between groups 1a and 1b at day 5 after antibiotic therapy ( $P > 0.05$ ).

Moreover, after antibiotic therapy serum NO and  $H_2O_2$  levels showed significant decrease in groups 1a and 1b ( $P < 0.05$ ).

### **Results obtained from ROC curve**

Table 5 summarizes the results obtained from ROC curve analysis for diagnostic values of all studied biomarkers.

Blood culture is the gold standard for the diagnosis of sepsis, but at least 48–72 hours are needed for the results and the number of false negative results is not negligible, particularly in early-onset sepsis) Hisamuddin *et al.*, 2015). In the last decades, a great deal of efforts has been focused on biochemical markers for early diagnosis of sepsis and link those biomarkers to the results of blood culture. CRP has been considered as a biomarker for sepsis for many years, but it is considered as a non-specific biomarker) Hisamuddin *et al.*, 2015).

**Table.1** Comparison between the studied groups regarding descriptive data

| Groups/Parameters                             | Group 1 (sepsis group<br>n=30) | Group 2 (control group<br>n=15) |
|---|--------------------------------|---------------------------------|
| Gestational age (weeks)                       | 36.9±1.8                       | 37.1±1.1                        |
| Birth weight (gm)                             | 3040±269                       | 3192±135                        |
| Mode of delivery,<br>Cesarean delivery, n (%) | 14(47%)                        | 10(67%)                         |
| Chorioamnionitis, n (%)                       | 20(67%)                        | 3(20%)                          |
| Premature rupture of<br>membrane, n (%)       | 18(60%)                        | 2(13%)                          |
| Mechanical ventilation, n<br>(%)              | 10(33%)                        | -                               |
| Disseminated<br>intravascular coagulation     | 12(40%)                        | -                               |
| Apgar score (first<br>minute) (range/median)  | 4-8(4.5)                       | 5-8(6)                          |
| Hb (g/dl)                                     | 13.5±1.9                       | 14.1±1.1                        |
| TLC (x10 <sup>3</sup> /uL)                    | 12.1±3.1                       | 11.4 ±5.2                       |
| PMN count (x10 <sup>3</sup> /uL)              | 5.9±3.1                        | 5.7±2.9                         |
| Platelet count (x10 <sup>3</sup> /uL)         | 161±82*                        | 301±48                          |
| CRP (mg/L)                                    | 22.1±6.2*                      | 4.2±1.5                         |

Data are mean ± standard deviation of each group except range and median for Apgar score. Student t- test was used to compare significance between the groups. \*Significant at p value < 0.05. Hb; Hemoglobin, TLC; Total leucocytic count, PMN; polymorph nuclear leucocytic, CRP; C reactive proteins.

**Table.2** Bacterial isolates ratio in patients with culture-proven sepsis (group 1a) (n = 19)

|   |            |
|---|------------|
| culture proven sepsis (group 1a) n<br>and % | 19(63.33%) |
| Coagulase-negative staphylococci            | 6(31.58%)  |
| Escherichia coli                            | 5(26.32%)  |
| Staphylococcus aureus                       | 3(15.78%)  |
| Pseudomonas aeruginosa                      | 2(10.53%)  |
| Streptococcus viridans                      | 2(10.53%)  |
| Klebsiella pneumoniae                       | 1(5.26%)   |

**Table.3** Comparison between the studied groups regarding inflammatory Biomarkers and redox status

| Groups/Parameters                    | Group 1 (sepsis group n=30) |                        | Group 2 (control group n=15) <sup>c</sup> |
|--------------------------------------|-----------------------------|------------------------|---|
|                                      | At day 0 <sup>a</sup>       | At day 5 <sup>b</sup>  |   |
| HMGB1 (pg/ml)                        | 65.7± 8.7 <sup>bc</sup>     | 54.8±5.8 <sup>ac</sup> | 38.2±9.9 <sup>ab</sup>                    |
| Thrombomodulin (pg/ml)               | 72.3±11.2 <sup>bc</sup>     | 94.3±7.6 <sup>ac</sup> | 144.2±15.6 <sup>ab</sup>                  |
| Hsp 72 (pg/ml)                       | 14.6±1.6 <sup>bc</sup>      | 21.5±2.7 <sup>ac</sup> | 32.6±4.3 <sup>ab</sup>                    |
| NO (µmol/L)                          | 33.4±9.1 <sup>bc</sup>      | 17.7±2.4 <sup>ac</sup> | 13.6±1.8 <sup>ab</sup>                    |
| H <sub>2</sub> O <sub>2</sub> (µM/L) | 59.6±7.9 <sup>bc</sup>      | 48.6±4.9 <sup>ac</sup> | 35.0±3.4 <sup>ab</sup>                    |

Data are mean ± standard deviation of each group. One-way ANOVA with Tukey’s post hoc test were used to compare significance between groups. \*Significant at p value < 0.05. HMGB1;high mobility group box 1, Hsp 72; heat shock protein 72, NO; Nitric oxide, H<sub>2</sub>O<sub>2</sub>; hydrogen peroxide, CRP. <sup>a,b,c</sup> significance between groups, <sup>a</sup> significance from group 1 at day 0, <sup>b</sup> significance from group 1 at day 5, <sup>c</sup> significance from group 2.

**Table.4** Comparison between studied biomarkers in groups 1a and 1b before and 5 days after antibiotic therapy

| Parameter                              |        | Group 1a<br>n=19 | Group 1b<br>n=11 | unpaired t test<br>p |
|--|--------|------------------|------------------|----------------------|
| HMGB1 (pg/ml)                          | Before | 70.4± 6.4        | 57.5± 5.6        | 0.001 *              |
|  | After  | 54.1 ± 6.9       | 55.6± 2.9        | 0.9                  |
| Paired t-test                          | p      | 0.001 *          | 0.8              |                      |
| Thrombomodulin (pg/ml)                 | Before | 67.8± 10.7       | 80.1± 7.4        | 0.002 *              |
|  | After  | 92.6± 6.4        | 97.2 ± 8.9       | 0.4                  |
| Paired t-test                          | p      | 0.002 *          | 0.03 *           |                      |
| Hsps 72 (pg/ml)                        | Before | 14.6±1.5         | 14.6±1. 9        | 0.9                  |
|  | After  | 21.6±3.2         | 21.9±1.7         | 0.5                  |
| Paired t-test                          | p      | 0.002 *          | 0.001 *          |                      |
| NO (µmol/L)                            | Before | 36.4±8.8         | 28.1±7.3         | 0.01 *               |
|  | After  | 17.9±2.0         | 17.5±3.1         | 0.6                  |
| Paired t-test                          | p      | 0.001 *          | 0.001 *          |                      |
| H <sub>2</sub> O <sub>2</sub> (µmol/L) | Before | 61.3±7.4         | 55.3±7.0         | 0.04 *               |
|  | After  | 46.3±4.3         | 47.0±5.5         | 0.7                  |
| Paired t-test                          | p      | 0.001 *          | 0.001 *          |                      |
| CRP(mg/L)                              | Before | 22.2±7.4         | 21.9±3.6         | 0.07                 |
|  | After  | 7.0±1.3          | 6.3±1.6          | 0.23                 |
| Paired t-test                          | p      | 0.001 *          | 0.001 *          |                      |

Data are mean ± standard deviation of each group. To compare significance between the two groups paired and unpaired t-test were used. \*Significant at p value < 0.05. HMGB1;high mobility group box 1, Hsp 72; heat shock protein 72, NO; Nitric oxide, H<sub>2</sub>O<sub>2</sub>; hydrogen peroxide; CRP; C reactive protein.

**Table.5** Diagnostic value of the inflammatory mediators and redox status

| Parameter                            | Cut-off value | Sensitivity (%) | Specificity (%) | Area under the curve | P value |
|--------------------------------------|---------------|-----------------|-----------------|----------------------|---------|
| HMGB1 (pg/ml)                        | >49.5         | 97              | 94              | 0.99                 | 0.001*  |
| CRP (mg/L)                           | >15.5         | 96              | 93              | 0.98                 | 0.001*  |
| H <sub>2</sub> O <sub>2</sub> (µM/L) | >46.5         | 96              | 67              | 0.93                 | 0.001*  |
| Thrombomodulin (pg/ml)               | >63.5         | 87              | 99              | 0.92                 | 0.001*  |
| NO (µmol/L)                          | >20.5         | 96              | 89              | 0.90                 | 0.001*  |
| Hsp 72 (pg/ml)                       | >17           | 90              | 80              | 0.21                 | 0.001*  |

HMGB1;high mobility group box 1, Hsp 72; heat shock protein 72, NO; Nitric oxide, H<sub>2</sub>O<sub>2</sub>; hydrogen peroxide, CRP;C reactive protein.

Recent evidence indicates that HMGB1, a ubiquitous nuclear protein, is an important pro-inflammatory cytokine that mediates the response to infection, injury, and inflammation, although residing predominantly in the nucleus of quiescent macrophages/monocytes (Vande Walle *et al.*, 2011), results of current study revealed a significant increase of HMGB1 in sepsis group when compared to healthy control group moreover there was a significant decrease of HMGB1 at day 5 after antibiotic therapy only in group 1a patients that explained its important role in the diagnosis and treatment of sepsis. Moreover, HMGB1 showed a significant increase in group 1a when compared to group 1b that reflected its correlation with blood culture results and established it as an important target for sepsis diagnostic and therapy (Buhimschi *et al.*, 2009).

HSPs are highly-conserved and present in cells under normal conditions, but are over-expressed under some specific stress conditions such as sepsis (Tsai *et al.*, 2015) moreover, Hsp72 over-expression can reduce mortality in experimental models of septic shock and can down-regulate expression of inflammatory genes (Struzik *et al.*, 2015). Results of the current study revealed a significant decrease of Hsp72 in group 1

when compared to group 2 moreover, there was a significant increase of Hsp72 at day 5 after antibiotic therapy reflect its role in the diagnosis and treatment response of sepsis. Moreover, culture positive and culture negative groups showed a non-significant difference as regard to Hsp72 level that made it as un-suitable biomarker for blood culture results.

Nuclear factor kappa B (NF-κB) signaling pathways regulate the transcription of many genes involved in immunity and inflammation one of them is HMGB1.

Hsp72 modulatory effects on inflammation can be attributed to its regulation of the NFκB pathway by its potent inhibitory effects on HMGB1-induced activation of NF-κB pathways (Song *et al.*, 2008), these findings identify an essential role for HSP72 in blocking inflammation and preventing HMGB1 release, making them cornerstones for better understanding and management of neonatal sepsis and greatly support the aim of our results and giving a new promising hope for better therapeutic approaches of neonatal sepsis.

The activated NFκB pathway leads to the production of ROS that cause tissue damage during sepsis (Marshall and Reinhart, 2009).

Results revealed that both NO and H<sub>2</sub>O<sub>2</sub> showed a significant increase in sepsis group when compared to control groups in addition, there was a significant decrease of both biomarkers at day 5 after antibiotic therapy that highlighted their role in sepsis diagnosis and treatment.

In addition, both NO and H<sub>2</sub>O<sub>2</sub> correlate positively with blood culture results as it showed a significant increase in group 1a when compared to group 1b that reflect their importance as blood culture related biomarker for early onset neonatal sepsis. Valerio *et al.*, 2012 who did a study on neonatal sepsis reported that sepsis promotes production of an excess of ROS and these molecules are meant to destroy the infectious agent but are not specific substances and may instead harm the patient. Meanwhile, Sirisinha (2011) reported that induction of Hsp72 can suppress lipopolysaccharide-induced HMGB1 release as well as inhibition of oxidative stress-induced HMGB1 release by direct protein-to-protein interaction in macrophages, this support greatly the beneficial effect of targeting the aforementioned studied biomarkers in the therapy of neonatal sepsis. At physiological conditions, the anticoagulant system comprising antithrombin (AT), thrombomodulin/activated protein C (APC), and tissue factor pathway inhibitor (TFPI) is activated in response to hypercoagulability; however, this anticoagulant system is severely compromised in individuals with sepsis (Okamoto *et al.*, 2012). Results of the current study revealed a significant decrease of thrombomodulin in group 1 when compared to group 2 moreover; there was a significant increase of it at day 5 after antibiotic therapy reflects its role in the diagnosis and treatment response of sepsis. In addition, thrombomodulin showed a significant decrease in group 1a when compared to group 1b that reflect its role as suitable blood culture

related biomarker for early onset neonatal sepsis. These results run hand in hand with the results of Hao and Wang 2013 who reported that determination of thrombomodulin levels may be helpful in evaluating severity, prognosis and drug response in neonates with early onset sepsis. Well, in line another mechanism for thrombomodulin in sepsis is its effect on HMGB1. During inflammation HMGB1 is released and bind to the receptor for advanced glycation end products (RAGE), HMGB1-RAGE signaling has been implicated in the pathogenesis and/or progression of sepsis, thrombomodulin interferes with HMGB1 binding to RAGE, thereby impairing HMGB1-RAGE signaling, alternatively, thrombomodulin may antagonize HMGB1 by enhancing thrombin-mediated proteolytic degradation of it to less a pro-inflammatory form (Kashiwadate *et al.*, 2016), that confirms their patho-physiological mechanism in neonatal sepsis making them promising candidates for therapy that may help in reducing the period and the dose of antibiotic treatment of sepsis to reduce patient suffering. Diagnostic values obtained from the ROC curve showed that HMGB1 had the best accuracy, followed by CRP which is a nonspecific biomarker for sepsis diagnosis. To our knowledge, the combination between current studied biomarkers together in the pathogenesis of early onset neonatal sepsis has not been studied before. HMGB1, thrombomodulin, Hsp72 and redox status as an intricate biomarkerS network can be used for diagnosis and treatment response of early onset neonatal sepsis, which makes them as promising targets for diagnosis, management and treatment of early onset neonatal sepsis.

#### **Authors' contributions**

This work was carried out in collaboration between all authors. Author NAS reached literature and conceived the study, contributed



to biochemical performed data analysis and wrote the first draft of the paper. Author MSF was involved in protocol development, gaining ethical approval, patient recruitment and data analysis. Author WHA as involved in protocol development, gaining ethical approval and tissue culture assay. All authors reviewed, edited the manuscript and approved the final manuscript.

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### **Competing of interest**

Authors have declared that no competing interests exist.

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