**ORIGINAL ARTICLE**

# A Study of Prothrombin Time, Thrombin Time, Fibrinogen and Vitamin K Levels in Sudanese Neonates with Proven Sepsis

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## ABSTRACT

**Aim:** This study aims to assess Prothrombin Time (PT), Thrombin Time (TT), fibrinogen and Vitamin K levels in Sudanese neonates with sepsis. It also aims to correlate those parameters with outcome, sepsis onset, and etiologic agent in the case group.

**Methods:** The study was done in Omdurman maternity hospital from June 2013 to April 2015 on a total of 100 neonates divided equally into case (neonates with proven sepsis) and control (healthy neonates). PT was assessed by measuring the clotting time needed by the plasma after calcified thromboplastin was added, expressed in seconds. TT was also assessed by the clotting procedure after thrombin was added to plasma, also expressed in seconds. Fibrinogen was assessed using the Clauss method, using 1/20 diluted plasma then counting clotting time after thrombin was added, with results obtained in mg/dl. Vitamin K was assessed using high-performance liquid chromatography (HPLC) after plasma was prepared, extracted, injected into HPLC system, separated using C-18, then reduced by the zinc in the post-column reduction reactor then assessed by fluorescent detector, the result expressed in ng/ml.

**Results**: Among the case group; neonates who died constituted 10 (20%), with 40 recovered (80%). PT was significantly prolonged in septic neonates compared to the healthy ones (p value 0.01). Early sepsis neonates showed significant prolongation in both PT and TT compared to the late onset neonates (p value 0.02 and 0.00). Fibrinogen showed significant increase in septic neonates compared to healthy one (p value 0.00). Vitamin K didn't show significant decrease in septic neonates compared to the control. None of the outcome, onset of sepsis and etiologic agent of sepsis showed significant correlation with fibrinogen or Vitamin K.

**Conclusion:** In concordance with our findings, sepsis leads to coagulopathy and thus the morbidity and mortality rates could increase among neonates with sepsis. Involvement of coagulation evaluation in sepsis can be beneficial in diagnosis and management of septic cases

**Keywords:** Neonatal sepsis, Fibrinogen, Prothrombin time, Thrombin time, Vitamin K, Mortality, Sudan

## INTRODUCTION

Neonatal sepsis is bacterial bloodstream infection in neonates (<28 days)[1]. It remains a worldwide cause of neonatal morbidity and mortality[2-4], due to nonspecific presentation and high risk of negative outcome if untreated[5]. While the rates of mortality have started to decline, recovery time for most infants is prolonged[6], and some infants experience residual deficits in mental and physical development[7].

Systemic neonatal bacterial infection creates a significant burden. In spite of ongoing efforts in early diagnosis, treatment and prevention, neonatal sepsis remains an enigmatic field for consultants. The urgent need to find a new, highly accurate and reliable biomarker is paramount to guide physicians in assessing risk of infection and choice of antibiotic therapy. Studies are ongoing in the search for a novel marker for neonatal sepsis[8].

Changes in coagulation remain the main feature of the sepsis, along with that the Pathophysiological mechanism of sepsis isn't yet completely clarified[9]. Both hemostasis and inflammation are linked to pathophysiologic processes that significantly interact with each other. Inflammation triggers activation of the hemostatic system, which induces significant inflammatory activity. As such, the T hemostatic system acts reciprocally with the inflammatory cascade, creating a hemostasis-inflammation cycle where each process activates the other, the two systems entering a positive feedback loop. Strong links between the immune and hemostatic systems occur at the level of whole hemostatic system components. During inflammatory response, inflammatory mediators (especially proinflammatory cytokines) play a crucial role in affecting the hemostatic system through stimulating disturbance in several mechanisms[10].

Disseminated Intravascular Coagulation (DIC) can result from excessive coagulation activation[11,12]. DIC functions as a mortality predictor in critical septic patients[13, 14]. Coagulation activation, fibrinolytic function inhibition, and high risk organ dysfunction characterize sepsis-induced DIC[15-17], these changes usually developing at the late stage of sepsis induced DIC[11,18,19].

Most of the evidence for the anti-inflammatory mechanisms of Vitamin K suggests a role in cell-signaling complex nuclear factor kappa-B inhibition[20]. Investigating coagulation in the whole body can produce more useful clinical information than classical tests. Correct timing of anticoagulant therapy may ultimately cause decreased multisystem organ dysfunction incidence in sepsis[21]. The

aim of this study is to assess PT (Prothrombin time), TT (Thrombin time), and levels of fibrinogen and Vitamin K in the case group (Sudanese neonates with sepsis) compared with the control (healthy neonates). We also correlated sex, mode of delivery and gestational age with fibrinogen and Vitamin K levels of both groups. It also aims to correlate these factors with outcome, onset of sepsis, and etiologic sepsis agent in the case group.

## MATERIALS AND METHODS

The study prospectively, organized in Omdurman Maternity hospital in Sudan, on a cohort of 100 neonates, divided into two groups. We selected septic patients with confirmed blood culture positivity and healthy ones without any indication of sepsis as controls.

The study population was categorized according to gender, delivery mode, and gestational age, case group categorized additionally according to outcome, sepsis onset, and causative bacterial agents.

Blood culture, Gram stain, culture and sensitivity, plus biochemical tests for microorganism identification were performed initially, and then culture-positive samples were taken as the study specimens. Neonatal blood was withdrawn and plasma prepared for PT, TT, Fibrinogen and Vitamin K.

**Prothrombin Time (PT) procedure:** PT was assessed using 50 microliters of pre-warmed plasma, a piece of metal ball (Stago) then added, and finally 100 microliters of pre-warmed calcified thromboplastin was added, the clotting time was counted immediately by the semi-automated pre-calibrated coagulometer (Stago stat-4. France), a calibrator (Uri-calibrator, Stago) was used as a control with the test.

**Thrombin Time (TT) procedure:** TT was assessed using 50 microliters of pre-warmed plasma, a piece of metal ball (Stago) then added, and finally 50 microliters of pre-warmed thrombin was added, the clotting time was counted immediately by the semi-automated pre-calibrated coagulometer (Stago stat4. France), a calibrator (Uri-calibrator, Stago) was used as a control with the test.

**Fibrinogen procedure:** Fibrinogen was assessed by the Clauss method using a 1/20 dilution of plasma prepared by the addition of 10 microliters of plasma to 190 microliter of Owren-Koller buffer (Stago. France), and mixed. The reagent was prepared to reach 37+/-0.2C. A lyophilized reagent was left for about 15 minutes at room temperature before use. Into clean new disposable cuvette, 100 microliter of 1/20 diluted pre-warmed plasma was added, then a piece of metal ball was obtained, incubated to reach (37+/- 0.2C), then 50 microliter of fibrinogen reagent (FIBRI-PREST AUTOMATE. Stago. France) was added, the clotting time was counted in a similar way as mentioned in TT. The result was interpreted in mg/dl.

**Vitamin K procedure:** Vitamin K was assessed using High-Performance Liquid Chromatography (HPLC), then 1ml of plasma added to solid-phase extraction using SPE-cartridge (Immundiagnostik. Germany) pre-rinsed with methanol (Immundiagnostik. Germany) and bi-distilled water (3 times), 10 microliters form internal standard (Immundiagnostik. Germany) as an internal control to ensure high accuracy during test preparation and analysis), then plasma was precipitated. The supernatant is then extracted with an organic solvent and evaporated. After re-suspension, the sample is measured in an isocratic High Performance Liquid Chromatography (HPLC) system using column C-18 (MERCK Manu-Kart. Germany). A post column reduction reactor filled with zinc (Bischoff. Germany) applied after Vitamin K was separated in C-18 column (post column reduction reactor reduces Vitamin K to be assessable by the fluorescent detector) (HPLC 10 ADVP with Fluorescent detector. Schimatzu Japan) was used. Result expressed in ng/ml through the chromatogram using control of known Vitamin K concentration (Immundiagnostik. Germany).

**Data analysis:** Data analyzed by IBM SPSS Statistics 20; means were compared, then one sample T-test was used to compare means and variables among different tests, P values were calculated to test significance.

## RESULTS

A cohort of hundred neonatal venous blood specimens (50 from each group) was included in the study. The sex distribution of the neonates included in the study was 26 (52%) female and 24 (48%) male patients. An equal number of control (healthy neonates) included in the study comprised 27 (54%) female and 23 (46%) males, as shown in Table 1.

Distribution of study population (N=100) by gender. Groups were divided into two subgroups according to delivery mode, namely caesarean section and normal vaginal delivery. In the case group, 17 neonates (34%) were delivered normally, and 33 (66%) by cesarean section. In the control, 7 neonates (14%) were delivered normally, and 43 (86%) by cesarean section (Table 2).

Distribution of study population (N=100) regarding their mode of delivery. Case group was classified according to their outcome into; dead constituted 10 (20%) and recovered neonates 40 (80%), Figure 1.

The patients identified based on the sepsis they presented. There were 17 (34%) patients presented with early-onset (0-7 days), and 33 (66%) late-onset (7-28 days) as shown in Figure 2.

The etiologic agent of sepsis classified into Gram-positive represented 9 (18%) and Gram-negative 41 (82%), Figure 3.

Case group causative bacterial agent was distributed as follows: Pseudomonas 23 (46%), Salmonella 9 (18%), Klebsiella 7(14%), Staph epidermidis 3(6%), Strepococcus fecailis 3(6%), E.coli 2 (4%), 2 Staph. aureus (4%), and 1 with Streptococci (Non group B) (2%).

Figure 4 shows the distribution of causative bacterial agents among the case group (n=50). Causative agent mortality among the case group was distributed as; Pseudomonas 3 (30%), Salmonella 3 (30%), S. epidermidis (20%), Klebsiella 1 (10%), and E. coli 1(10%), as shown in Figure 5. Sepsis onset mortality among case group distributed into; early-onset 4 (40%) and late-onset 6 (60%), as shown in Figure 6.

PT significantly prolonged in the case group in comparison to control (mean; 16.6 and 13.9 sec). p value 0.01, whereas TT showed a significant decrease in case group compared to control (18.7 and 20.5 sec) p value 0.00. Fibrinogen showed significant increase in case group compared to control (482.2 and 393.7 mg/dl), p value =

0.00. Vitamin K showed an insignificant decrease in case compared to control (0.86 and 1.23 ng/ml), p value >0.10), (Table 3).

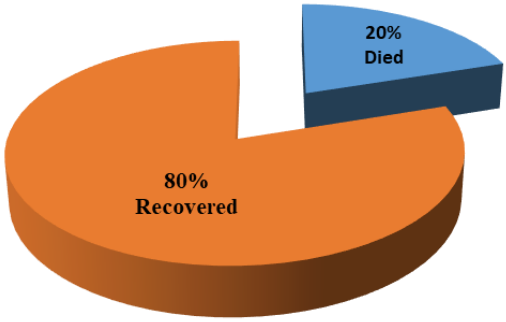


Figure 1. Outcome distribution (percentage) among case group.

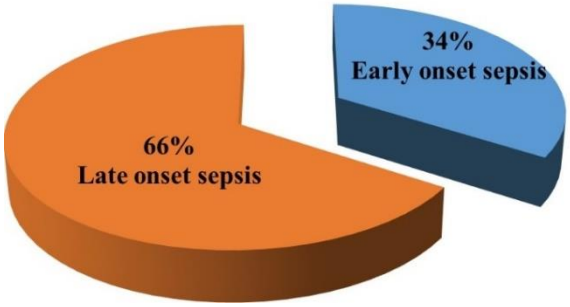


Figure 2. The mode of sepsis distribution among case group.

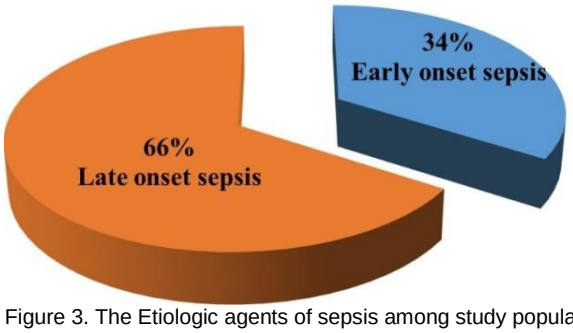


Figure 3. The Etiologic agents of sepsis among study population.

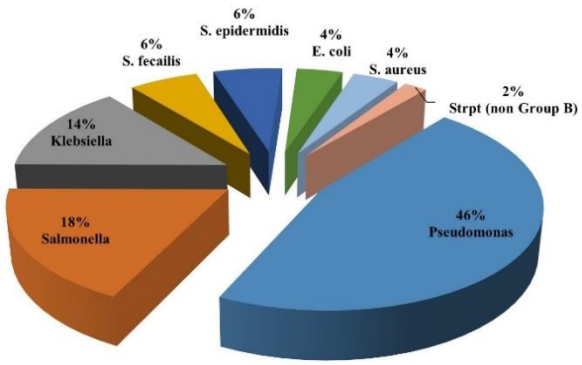


Figure 4. Distribution of causative bacterial agent among case group

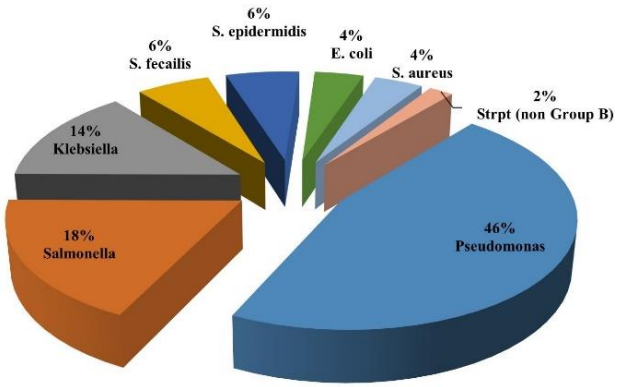


Figure 5. Causative agent mortality among the study group.

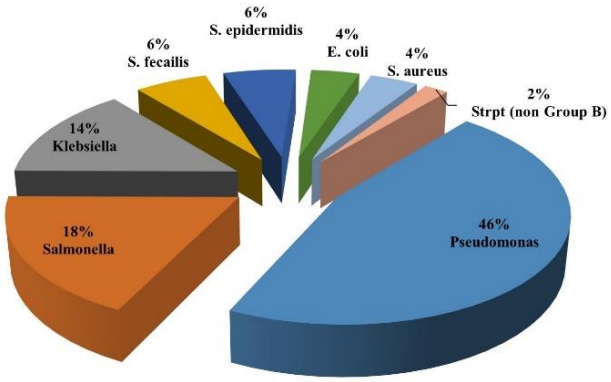


Figure 6. Sepsis onset mortality among case group

PT didn't show significant correlation with gender, delivery mode between groups (p value; 0.84, 0.59 respectively) and also with sepsis outcome, and etiologic of sepsis in case group (p values > 0.13 and >0. 46 respectively). But showed significant correlation with sepsis onset (p value <0.02), (See Table 4).

TT didn't show significant correlation with gender, delivery mode between groups (p value; 0.92, 0.77 respectively) and also with sepsis outcome and etiologic of sepsis in case group (P value; 0.90 and 0. 54). But showed significant correlation with sepsis onset (p value 0.00), (Table 4).

Fibrinogen didn't show significant correlation with gender, delivery mode between groups (P value; 0.23, 0.65 respectively) and also with sepsis outcome, sepsis onset and etiologic of sepsis in case group (P value; 0.49, 0.80 and 0. 59).

Table1. Shows gender distributions

| Gender | Case group | Control group | Total |
|---|---|---|---|
| Female | 26 (52%) | 27 (54%) | 53 |
| Male | 24 (48%) | 23 (46%) | 47 |
| Total | 50 | 50 | 100 |

Table 2. Distribution of study population regarding their mode of delivery

| Mode of delivery | Case group | Control group | Total |
|---|---|---|---|
| Normal vaginal delivery | 33 (66%) | 43 (86%) | 76 |
| Caesarean section | 17 (34%) | 7 (14%) | 24 |
| Total | 50 | 50 | 100 |

Vitamin K didn't show significant difference between both groups (p value 0.10) (Table 1).

Vitamin K didn't show significant correlation with gender, delivery mode between groups (p values; 0.24, 0.06 respectively) and also with sepsis outcome, sepsis onset and etiologic of sepsis in case group (p value; 0.46, 0.71, and 0.15).

Table 3. Shows Fibrinogen and vitamin k in the study population

| Parameter | Mean | P. value |
|---|---|---|
| PT (case)<br>PT (control) | 16.6 sec<br>13.9 sec | 0.01 |
| TT (case)<br>TT (control) | 18.7 sec<br>20.5 sec | 0.00 |
| Fibrinogen (case)<br>Fibrinogen (control) | 482.2 mg/dl<br>393.7 mg/dl | 0.00 |
| Vitamin k (case)<br>Vitamin k (control) | 0.86 ng/ml<br>1.23 ng/ml | 0.10 |

Table 4. Sepsis onset in PT and TT among case group.

| Parameter | Mean | P. value |
|---|---|---|
| PT (Early onset sepsis )<br>PT (Late onset sepsis) | 16.6 sec<br>13.9 sec | 0.01 |
| TT (Early onset sepsis )<br>TT (Late onset sepsis) | 18.7 sec<br>20.5 sec | 0.00 |

## DISCUSSION

Tight links between haemostasis and inflammation help explaining the procoagulant tendency in sepsis[20]. Both systems are tightly interrelated physiologically and significantly affect other, haemostatic system activation caused by sepsis also significantly induces inflammatory activity[21].

In our study; significant prolonged PT is detected in neonates with sepsis, which can be used as a marker for sepsis. The coagulation alteration is one of the major hallmark of sepsis[9], recent paragraph we discussed the tight interrelation between haemostatsis and inflammation, and sepsis is one of the best example that show this tight relation[21.], activation of intrinsic coagulation pathway explains significant prolonged PT in case population than control, These finding was consistent with studies of Mihajlovic D and co-workers[9], I Krishna et al13 and Peker E et al14 who concluded PT was significantly higher in septic neonates. These finding was revealed to the activation of coagulation system in generalized inflammation in sepsis. In contrary, Iba, T and co-workers reported that the PT ratio is found to be less effective marker for assessing sepsis-triggered coagulopathy compared to fibrin-related markers[22].

The study concluded that there was significant decrease in TT and increased fibrinogen, this result was in line with the result of with Levi M et al[24] and another study of Charan and co-workers who found that fibrinogen level in septicemic patients was significantly higher[23]. Mihajlovic D and co-workers also described increased level of fibrinogen among same population[9]. These results are most likely related to the fact that fibrinogen is an acute phase protein increased in inflammatory status; therefore an increased fibrinogen is not unexpected in sepsis due to generalized inflammation, TT shorted as a result of increased fibrinogen.

So far, assessment of Vitamin K has not been used for diagnostic purposes. Alternatively, other tests are used for its estimation such as uncarboxylated prothrombin (PIVKA-II). However, Levi M et al detected that repeated evaluation of PT might be beneficial in follow up the coagulopathy. This is essential and required for cases with vitamin K deficiency.[24] Other studies measures vitamin k indirectly by measuring Osteocalcin, uncarboxylated prothrombin and vitamin K related parameter[25,26]. Therefore, this study aimed to measure vitamin K in neonates with sepsis which is substantial point of morbidity[27-32].

In any case, no significant difference reported between septic neonates and healthy neonates (p value 0.10), further studies in these areas, especially that with intervention to study Vitamin K levels and its role in sepsis, currently its used in neonatal management of bleeding in sepsis and in DIC, this area remain enigmatic area for researchers.

## CONCLUSION

From this study, it has been concluded that: Sepsis causes elevation in PT, indicating FVII deficiency. TT elevation above the reference may indicate one or more of the following: Hypofibrinogenemi, Dysfibrinogenemia or Hypoalbunaemia.

### LIMITATION

First limitation; the study is hospital based, so newborns with similar related symptoms of sepsis that didn't bring them to the hospital may have been neglected or missed from the study. Another limitation: lack of availability of enough samples (the number collected in more than one year). Also; small resources locally is one of the limitations of the study, in term of Facility (financial), logistics, no enough HPLC systems ready for Vitamin K assessment and lack of trained experts in HPLC with fluorescent detection analysis and interpretation, lack of resources especially Vitamin K kits, and personnel (nurses, phlebotomists, and other health professions) compared to the whole admitted neonates in case group and the equipment (the study is self-funded).

Another limitation is the poor community health awareness, low educational level, and socio-economic status of study population families. One of the limitations is the limited local data of previous studies with similar parameters among Sudanese populations especially for Vitamin K.

**Declaration:** Ethics Approval and Participation Consent: Clearance obtained from Omdurman Maternity Hospital, Research Ethical Committee. Informed consent obtained by principal investigator from neonate mothers before proceeding.

**Funding:** No funding or financial aid received.

**Acknowledgment:** We would like to thank the staff of Hematology and Microbiology, Omdurman Maternity Hospital, for their assistance in specimen's analysis. We also thanks the staff of the Neonatal ICU team for help in offering all initial data of all cases

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