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Comparative Analysis of Some Coagulation Parameters in Umbilical Cord and Maternal Venous Blood Samples

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ABSTRACT

Introduction: The umbilical cord blood is known to contain potentially life-saving cells called stem cells capable of self-renewal and differentiation, offering therapeutic benefits for patients worldwide. The Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT), and International Normalized Ratio (INR) tests are used clinically to assess the integrity of the intrinsic, extrinsic, and common pathways as well as monitor anticoagulant therapy and diagnose coagulation disorder. The difference in the level of APTT, PT, and INR in cord blood and venous blood are yet to be accessed; thus, this study aimed to determine the difference between these parameters in cord blood and venous blood samples from mothers.

Materials and Methods: The study utilized eighty (80) blood samples comprising 40 cord blood obtained directly from the umbilical cord immediately after delivery and 40 venous blood obtained from the same pregnant women before delivery. 3ml of blood each was obtained aseptically from the vein and umbilical cord using a syringe and immediately dispensed into a sample containing 333mls of tri-sodium citrate anticoagulant with proper mixing and centrifugation to obtain platelet-poor plasma for analysis. The APTT, PT, and INR values were analyzed and calculated manually using the spectrum reagent test kit following the manufacturer's instructions. The data obtained were analysed using Graph-Pad Prism version 8. Descriptive Analyses were done using Mean and Standard Deviation, and the p-value was set at $p<0.05$.

Results: The value of APTT, PT, and INR was 30.5 ± 9.5s, 14.6 ± 2.9s, and 1.18 ± 0.41, respectively, for cord blood samples and 38.6 ± 9.9s, 17.8 ± 2.3s, 1.39 ± 0.49 for venous blood samples respectively. A comparison of the values of APTT, PT, and INR between cord blood and venous blood samples showed that APTT, PT, and INR were higher in venous blood samples compared to cord blood samples ($p=0.0004$, $p=0.0047$, $p=0.0142$), respectively. The reference ranges for APTT, PT, and INR for cord blood were established in this study to be within 27–34 seconds, 14–16 seconds, and 1.1–1.3, respectively, and found to be within already established normal reference ranges.

Conclusion: APTT, PT, and INR values are higher in venous blood than in cord blood samples. In cord blood samples, APTT, PT, and INR are within the normal reference range. The elevated APTT, PT, and INR in venous blood indicate that bleeding is inevitable during parturition. APTT, PT, and INR baseline values should be accessed and incorporated as an integral part of routine antenatal tests for proper management of spontaneous or excessive bleeding complications during delivery.

Keywords: Umbilical Cord Blood, Maternal Venous Blood, Prothrombin time, and Activated Partial Thromboplastin time, coagulation

INTRODUCTION

The umbilical cord blood is known to contain potentially life-saving cells called stem cells capable of self-renewal and differentiation, offering therapeutic benefits for patients worldwide. Cord blood, also known as umbilical cord blood, is the blood that remains in the placenta attached to the umbilical cord after childbirth. They contain potentially lifesaving haematopoietic stem cells (different from embryonic stem cells) with the potential to develop into blood vessels, organs, and tissues (1). They provide essential therapeutic values in cancer management and treatment due to their rich haematopoietic stem cells content (2-4).

Haematological and haemostatic changes occur during pregnancy to ensure the efficient delivery of oxygen to the fetus, with varying degrees of response to the magnitude of the haematological change (5-7). The principal changes that occur during pregnancy may include mild thrombocytopenia, elevation of procoagulant factors with diminished fibrinolysis, physiologic anaemia, and neutrophilia (7, 8,).

Haemostatic parameters constitute measurable indices in the haemostatic system used to assess the functionality of the coagulation system of an individual to establish a state of health or disorder (9,10). In clinical practice, prothrombin time is a blood test used for the assessment of a patient's coagulation status. It evaluates both the extrinsic and common pathways of coagulation and helps identify deficiencies in factors II, V, VII, and X and low fibrinogen concentration (11,12). Prothrombin time measures the time (in seconds) for plasma to clot after adding thromboplastin (a mixture of tissue factor, phospholipid, and calcium) to the patient's plasma sample (12). The INR represents the patient's prothrombin time ratio divided by a control prothrombin time value obtained using an international reference thromboplastin reagent developed by the WHO (13,14). Activated Partial Thromboplastin Time is also used together with prothrombin time to assess a patient's coagulation status (9,10). The APTT is based on the principle that in citrated plasma, adding a platelet substitute, factor XII activator, and CaCl2 allows for clot formation. The absence of tissue factor from this reaction mixture has led to using the term 'partial'. The test checks for factors such as factors VIII, IX, XI, and XII (9,10,15). A Paucity of information exists that compares the activated partial thromboplastin time (APTT) and prothrombin time (PT) levels in cord blood and normal venous blood from mothers in Port Harcourt, Nigeria. The study assessed the levels of activated partial thromboplastin time (APTT), prothrombin time (PT), and international normalized ratio (INR) in cord blood samples derived from the umbilical cord and compared with venous blood samples obtained from pregnant women in Port Harcourt, Nigeria. The relevance of assessing the activated partial thromboplastin time (APTT), prothrombin time (PT), and international normalized ratio (INR) in cord blood and venous blood from mothers is essential for evaluating the risk of bleeding during and after delivery.

MATERIALS AND METHODS

Study Design/ Population

A cross-sectional study design was adopted for this study, which used 80 blood samples, forty (40) cord blood samples obtained from the mother immediately after delivery, and forty (40) venous blood samples obtained from the same pregnant women before delivery.

Eligibility Criteria

The inclusion criteria for this study included Pregnant women in the labour ward/ delivery room of Rivers State University Teaching Hospital who provided informed consent to obtain both venous before delivery and cord blood samples from the umbilical cord of their newborn for this study and who underwent vaginal delivery and/or cesarean section with live birth. The study excluded Nonpregnant women, Pregnant women who were unwilling to provide informed consent, and Cord blood from premature and stillborn babies.

Ethical Approval/ Informed Consent

Ethical approval was obtained from the Research and Ethics Committee of the Rivers State University Teaching Hospital. Oral informed consent was obtained from the participants before blood collection. Participants were made to understand the nature of the study and the fact that participation in the study is voluntary, with confidentiality of personal data maintained at all times during and after the study.

Blood Collection and Handling

Three milliliters (3mls) of cord blood was obtained aseptically with a syringe from the umbilical cord attached to the placenta during delivery. The blood was dispensed immediately into a red-capped sample bottle containing 380mls of tri-sodium citrate anticoagulant, and 3mls of venous blood was also collected aseptically from a prominent vein of the participant into another red-capped sample bottle containing tri-sodium citrate anticoagulant. Both sample bottles were inverted gently to mix the blood and anticoagulant. The samples were centrifuged for fifteen minutes at 3,000 rpm to obtain platelet-poor plasma; the supernatant plasma was used for the test.

Statistical Analysis

Data obtained were analysed using Graph-Pad Prism version 8. Descriptive Analysis were done using Mean and Standard Deviation and a p-value set at $p<0.05$. Student t-test to check for statistical significance for inferential statistics. Kolmogorov Smirnov test for data normality. The parametric Test (unpaired t-test) was used to determine statistical significance for Gaussian distributions, while the Non-Parametric (Mann-Whitney test) was used to determine statistical significance for Non-Gaussian distributions. The reference Range at a 95% confidence interval is set at 2.5th and 97.5th percentiles for the lower limit and higher limit, respectively.

MATERIALS AND METHODS

The samples for APTT and PT were analyzed using the spectrum reagent test kit manufactured by Spectrum Diagnostics following the manufacturer's instructions. The INR is calculated by dividing the sample prothrombin time by the mean of the normal reference range and multiplied by the reagents International Sensitivity Index (ISI) value. The ISI for the PT reagent used for this study is 1.0.

INR = [patient PT/ control PT] ISI

Where INR = International Normalized Ratio, ISI = International Sensitivity Index, PT = Prothrombin time.

RESULTS

Demographic Details of Study Participants

A total of 80 blood samples were collected from 40 pregnant women (40 venous blood before delivery and 40 cord blood during delivery), all aged between 19-40 years with Parity between 0-5, Weight (43-58kg), Systolic and Diastolic blood pressure range were between (110/80- 124/85). 20% (8) of the pregnant women were prime, 25% (10) had 1 (G2P1) children, 16.3% (13) had 2 (G2P2) Children while 33% (31) had >3 (G3P1 and above) children. The demographic details of the participants are shown in Table 1.

Table 1: Demographic Details of Study Participants

Parameters	Number or Range
Number of samples (n)	80 (40 Cord blood and 40 Venous blood of mothers)
Sex	All pregnant female due delivery
Age (years)	
<20	3 (7.5%)
21-25	13 (32.5%)
26-30	17 (42.5%)
>30	7 (17.5%)
Parity	0-5
Prime	8 (20%)
G2P1	10 (25%)
G2P2	7 (17.5%)

G3P1 and above	15 (37.5%)
Weight	43-58kg
Diastolic blood pressure (mmHg)	80-85
Systolic blood pressure (mmHg)	110-125

Comparison of Mean ± SD of the studied parameters between cord blood and venous blood.

The comparison of activated partial thromboplastin time (APTT), prothrombin time (PT), and international normalized ratio (INR) between cord blood and venous blood showed a statistically significant difference, with APTT, PT, and INR having p-values of p=0.0004, p=0.0047, and p=0.0142, respectively.

Table 2: Comparison of Activated Partial Thromboplastin Time, Prothrombin time, and International Normalized Ration in Cord Blood and Venous Blood of mothers.

Sample/ Parameters	Cord Blood Mean+SD	Venous Blood Mean+SD	P-Value	Remarks
n	40	40		
PT (seconds)	14.6 ± 2.9	17.8 ± 2.3	0.0047	S
INR	1.18 ± 0.41	1.39 ± 0.49.	0.0142	S
APTT (seconds)	30.5 ± 9.5	38.6 ± 9.9	0.0004	S

Key: $\bar{x}$ = Mean; SD = Standard Deviation; S = Significant, PT = Prothrombin Time; APTT = Activated Partial Thromboplastin Time; n = Number of Subjects.

Mean ± SD, Median, and Range of the studied parameters in cord blood.

Table 3 shows the mean ($\bar{x}$), standard deviation (SD), median, range, distribution, minimum and maximum levels of activated partial thromboplastin time (APTT), prothrombin time (PT), and international normalized ratio (INR) in cord blood. The mean ± SD of APTT, PT, and INR were 30.5 ± 9.5, 15 ± 3, 1.18 ± 0.41, respectively, as shown in Table 3.

Table 3: Establishment of Reference Range of Activated Partial Thromboplastin Time, Prothrombin Time and International Normalized Ratio in Cord Blood.

Parameters	N	Min	Max	Median	Mean	Distribution	Reference Range (2.5^{th}-97.5 percentile)
PT (sec)	40	10	24	14	15	N-G	14–16
INR	40	0.7	3.3	1.1	1.18	N-G	1.1–1.3
APTT (sec)	40	18	51	28.5	30.5	N-G	27–34

Key: N-G = Non-Gaussian Distribution; Min = Minimum Value; Max = Maximum Value.

DISCUSSION

The activated partial thromboplastin time, prothrombin time, and international normalized ratio test are relevant in assessing the risk of development of coagulation disorders in apparently healthy individuals (10), monitoring of anticoagulant therapy as well as diagnosis of coagulation disorders (12) and as such serve as a preventive intervention in the management of patients. This study was set out to investigate the levels of APTT, PT, and INR in cord blood collected immediately after delivery from the umbilical cord of newborns with venous blood samples collected from the same pregnant patients shortly before delivery.

Findings from the study indicate that the APTT, PT, and INR values are elevated in venous blood from mothers compared to cord blood, implying that there is a higher tendency for a hypercoagulable state to exist in cord blood than venous blood, particularly during pregnancy or immediately after vaginal or cesarean section delivery. This finding is in consonant with the findings of Bremme (16), who noted that during pregnancy, the overall balance of coagulation and fibrinolytic system shift towards the hypercoagulable state with a marked increase in same during the term and postpartum periods with subsequent return to a nonpregnant state approximately four weeks after delivery.

Findings in the study also agree with the work of Franchini (17), who suggested that normal pregnancy is associated with alterations in a haemostatic system with increased clotting factors in the blood, resulting in shortened clotting time that can predispose pregnant women to a hypercoagulable state. The higher values of APTT, PT, and INR in this study will not be unconnected with the high pregnancy hormone in the venous circulation and this is in agreement with the suggestion of Durotoye et al., (18), who postulated that during pregnancy, there is high release of hormones such as estrogen and progesterone necessary for the maintenance of pregnancy. An increase in this hormone, especially estrogen, has the ability to stimulate liver cells (hepatocytes) for the production and release of coagulation factors, and thus, the increase in the coagulation parameters in venous blood is seen in this study.

Furthermore, this study revealed that the reference ranges for APTT, PT, and INR for cord blood were found to be within the established normal reference range of venous blood samples. This implies that the hypercoagulable state experienced during pregnancy and the postpartum period does reflect in the blood circulating in the umbilical blood, although it is a rich source of thromboplastin.

Also, the fact that clotting factors increase as pregnancy draw near to term, the values of APTT, PT, and INR in cord blood, which were reduced when compared to the venous blood during pregnancy as shown in this study, is probably a result of an increase in the clotting factors which further increases during the process of childbirth thereby shortening the time for the blood to clot as the body tends to prevent excessive bleeding during childbirth.

CONCLUSION

APTT, PT, and INR values are higher in venous blood samples than in cord blood samples. In cord blood samples, APTT, PT, and INR are within the normal reference range. The elevated APTT, PT, and INR in venous blood indicate that bleeding is inevitable during parturition.

RECOMMENDATION

The activated partial thromboplastin time, prothrombin time, and international normalized ratio values are essential in monitoring a patient's coagulation system and identifying any underlying coagulation defects. This is necessary and should be taken into consideration, especially for an issue like pregnancy, where bleeding is inevitable. Based on the findings in this study, it is recommended that APTT, PT, and INR baseline values be accessed and incorporated as an integral part of routine antenatal tests for proper management of spontaneous or excessive bleeding complications during delivery.

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