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# Coagulation Parameters And Immune Cell Counts In Pregnant Women of Advanced Maternal Age

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## ABSTRACT

### Introduction
Haemostasis and immunity are vital aspects of health greatly affected in pregnancy. Pregnancy-induced hypercoagulability and upregulated immune response are believed to be beneficial at some level, but are capable of triggering immunothrombosis. Advanced maternal age is considered a risk factor for several health complications associated with pregnancy. To this end, a comparative investigation of cellular immune response and coagulation are warranted.

### Methods
This study was conducted among pregnant women attending antenatal clinic at University of Calabar Teaching Hospital. Pregnant women of advanced maternal age were recruited alongside equal number of younger pregnant women. Blood sample was collected from each participant into appropriate sample bottles. Standard manual methods were also employed for prothrombin time, activated partial thromboplastin time and relative plasma viscosity tests. Platelet and total white blood cell counts were carried out by haemocytometry, while differential white blood cell counts were carried out by microscopy. Results are expressed as Mean ±SD following student t-test analysis of data on SPSS version 22.0. Statistical significance was drawn at a $p \leq 0.05$.

### Results
Pregnant women of advanced maternal age had significantly shortened (p=0.006) PT (11.56±1.01secs) and lower (p=0.009) INR (0.95±0.07) compared to the younger pregnant women (12.52±1.33secs and 1.02±0.10 respectively). Pregnant women of advanced age had significantly higher (p=0.001) mean values than younger pregnant women in the following parameters; platelet count (258.40±38.35x$10^9$/l vs 180.76±37.74x$10^9$/l), total WBC count (11.44±2.98x$10^9$/l vs 8.03±2.21x$10^9$/l), neutrophils count (6.44±1.69x$10^9$/l vs 4.53±1.33x$10^9$/l), lymphocyte count (4.59±1.30x$10^9$/l vs 3.19±0.95x$10^9$/l).

### Conclusion
The present study observed higher hypercoagulability and immune response in older pregnant women compared to the younger ones.

### Key words
Pregnancy, maternal age, haemostasis, immune response.

# INTRODUCTION

Physiological changes are known to accompany pregnancy which results in altered values for some of the basic health biomarkers. Haemostasis and immunity are vital aspects of health greatly affected in pregnancy (1-3). At one end, immune response in pregnancy oscillates between the need for tolerance of the foetus and the ability to control infection. At the other end, there is significant surge in the concentration of clotting factors alongside lowered concentration of anticoagulants in the system (4,5). Together with downregulated activities of the fibrinolytic system, these changes tip the haemostatic balance in favour of a hypercoagulability state. While these interrelated changes are thought to be beneficial as the body's protective mechanism from excessive obstetric haemorrhage and infection, pregnancy has also been identified as a risk factor for thrombosis (6,7). There is growing concern to identify conditions with increased risk of venous thrombosis because of the associated high morbidity and mortality burden. Maternal health, in general, is often complicated by vulnerability to several health challenges, while pregnancy can be quite precarious in some cases (8-11).

Reproductive age accommodates a wide range of more than 30 years (15-49 years), particularly for women (12). Howbeit, increasing maternal age has been associated with several health risks. This remains a valid source of dilemma in the subject of women empowerment as more women delay child-bearing at present compared to earlier years (13,14). The cutoff for advanced maternal age with regards to pregnancy is considered between 35 and 40 years of age. Identifying and mitigating possible health risks peculiar to this category of pregnant women are necessary to effectively support maternal health in contemporary times. The female gender is already a recognized risk factor for venous thrombosis, while the risk is heightened in pregnancy compared to a non-pregnant state (15-18). Emerging evidence with regards to venous thrombosis supports the interrelationship between activated immune response and coagulation (19-21). While pregnancy is known to drive changes in this direction, the impact of advanced maternal age remains to be fully investigated, particularly within the study area. For the assessment of the effect of maternal age on coagulation and cellular immune response, prothrombin time (PT), activated partial thromboplastin time (APTT), relative plasma viscosity, platelet and white cell counts were considered in the present study.

## MATERIALS AND METHODS

This study was conducted among pregnant women attending antenatal clinic at University of Calabar Teaching Hospital. Pregnant women of advanced maternal age (from 35 years of age) were recruited alongside equal number of younger pregnant women. All the pregnant women were within the second trimester of pregnancy as at the time of the study. Age-matched non-pregnant women were also recruited as control subjects. These consenting study participants were apparently healthy and not on interfering medication/ anticoagulant therapy within the preceding one year to the study period. Ethical considerations including confidentiality were maintained. A structured questionnaire was administered to obtain biodata and medical history.

Blood sample was appropriately obtained from each subject into dipotassium ethylene diamine tetra-acetic acid bottle at a concentration of 2mg/ml of blood for assessment of platelet count, total white blood cell count and differential white blood cell count. Additional volume of blood was dispensed into 3.13% trisodium citrate bottle at a ratio of 9:1. Haemocytometry and microscopic blood film reading were employed for blood cell counts. Quick's one-stage method was used for prothrombin time assay and modified kaolin method for activated partial thromboplastin time measurement. Relative plasma viscosity was assessed using a capillary viscometer. Data generated were entered into Microsoft excel spreadsheet and analysed using Statistical Package for Social Sciences (SPSS) software version 22.0. Results are expressed as Frequencies and Mean±SD, while Student t-test was used for comparison. Statistical significance was drawn at a $p \leq 0.05$.

# RESULTS

In Table 1, it is shown that pregnant women had significantly (p=0.001) shortened PT (12.24±1.27secs) and lower INR (0.99±0.09) compared to the non-pregnant women (13.14±1.20secs and 1.08±0.11 respectively). The RPV was significantly higher (p=0.009) in pregnant women than in non-pregnant women (1.69±0.18mPa/s vs 1.61±0.11mPa/s). in comparison to controls, pregnant women also significantly had higher (p=0.001) mean values of platelet count (219.58±54.37x$10^9$/l vs 182.64±45.00x$10^9$/l), total WBC count (9.74±3.12x$10^9$/l vs 7.05±2.29x$10^9$/l), neutrophils count (5.58±1.78x$10^9$/l vs 4.01±1.41x$10^9$/l), lymphocyte count (3.89±1.33x$10^9$/l

vs 2.80±1.08x$10^9$/l), monocyte count (0.16±0.13x$10^9$/l vs 0.03±0.01x$10^9$/l).

Table 2 shows that pregnant women of advanced maternal age had significantly shortened (p=0.006) PT (11.56±1.01secs) and lower (p=0.009) INR (0.95±0.07) compared to the younger pregnant women (12.52±1.33secs and 1.02±0.10 respectively). Pregnant women of advanced age had significantly higher (p=0.001) mean values than younger pregnant women in the following parameters; platelet count (258.40±38.35x$10^9$/l vs 180.76±37.74x$10^9$/l), total WBC count (11.44±2.98x$10^9$/l vs 8.03±2.21x$10^9$/l), neutrophils count (6.44±1.69x$10^9$/l vs 4.53±1.33x$10^9$/l), lymphocyte count and (4.59±1.30x$10^9$/l vs 3.19±0.95x$10^9$/l).

Table 1. Prothrombin time, International normalized ratio, activated partial thromboplastin time, relative plasma viscosity, platelets count, total blood white cell count and differential white blood cell counts of pregnant and non-pregnant women

| **Parameters** | **Pregnant Women n = 50** | **Non-pregnant Women n = 50** | **P-Value** |
|---|---|---|---|
| PT (Secs) | 12.24±1.27 | 13.14±1.20 | 0.001 |
| INR | 0.99±0.09 | 1.08±0.11 | 0.001 |
| APTT (Secs) | 32.00±5.54 | 30.94±5.85 | 0.354 |
| RPV (mPa/s) | 1.69±0.18 | 1.61±0.11 | 0.009 |
| Platelets (x $10^9$/l) | 219.58 ± 54.37 | 182.64 ± 45.00 | 0.001 |
| TWBC (x $10^9$/l) | 9.74 ± 3.12 | 7.05 ± 2.29 | 0.001 |
| Neutrophils (x $10^9$/l) | 5.48 ± 1.78 | 4.01 ± 1.41 | 0.001 |
| Lymphocytes (x $10^9$/l) | 3.89 ± 1.33 | 2.80 ± 1.08 | 0.001 |
| Eosinophils (x $10^9$/l) | 0.19 ± 0.12 | 0.16 ± 0.11 | 0.155 |
| Monocytes (x $10^9$/l) | 0.16 ± 0.13 | 0.03 ± 0.01 | 0.001 |

Key: **PT** = prothrombin time, **INR** = international normalized ratio, **APTT** = activated partial thromboplastin time, **RPV** = relative plasma viscosity, **TWBC** = total white blood cell count.

Table 2. Prothrombin time, International normalized ratio, activated partial thromboplastin time, relative plasma viscosity, platelets count, total blood white cell count and differential white blood cell counts of pregnant women based on maternal age

| **Parameters** | **Pregnant Women of advanced age n = 25** | **Pregnant Women of young age n = 25** | **P-Value** |
|---|---|---|---|
| PT (Secs) | 11.56±1.01 | 12.52±1.33 | 0.006 |
| INR | 0.95±0.07 | 1.02±0.10 | 0.009 |
| APTT (Secs) | 31.24±4.33 | 32.56±6.53 | 0.337 |
| RPV (mPa/s) | 1.70±0.18 | 1.68±0.18 | 0.779 |
| Platelets (x $10^9$/l) | 258.40 ± 38.35 | 180.76 ± 37.74 | 0.001 |
| TWBC (x $10^9$/l) | 11.44 ± 2.98 | 8.03 ± 2.21 | 0.001 |
| Neutrophils (x $10^9$/l) | 6.44 ± 1.69 | 4.53 ± 1.33 | 0.001 |
| Lymphocytes (x $10^9$/l) | 4.59 ± 1.30 | 3.19 ± 0.95 | 0.001 |
| Eosinophils (x $10^9$/l) | 0.22 ± 0.14 | 0.17 ± 0.09 | 0.109 |
| Monocytes (x $10^9$/l) | 0.20 ± 0.15 | 0.13 ± 0.10 | 0.057 |

Key: **PT** = prothrombin time, **INR** = international normalized ratio, **APTT** = activated partial thromboplastin time, **RPV** = relative plasma viscosity, **TWBC** = total white blood cell count.

# DISCUSSION

Healthcare delivery in Nigeria carries the burden of inadequate infrastructure in the face of serious health challenges (8, 22,23). it is, therefore, beneficial to explore preventive healthcare that is evidence-based. In the present study, pregnant women were observed to have shortened PT with a consequent lowered INR compared to the non-pregnant controls. In addition, the RPV and platelet count mean values were seen to be higher in participants who were pregnant. Increased blood volume and changes in the vasculature such as vasodilation to accommodate more blood flow occur in pregnancy. These changes alongside hormonal fluctuations that are upregulated in pregnancy activate various aspects of haemostasis including platelet and coagulation factors involvements. Consequently, hypercoagulability has been identified as part of the physiological adaptations occasioned by hormonal changes in pregnancy that exists to ensure minimal obstetric haemorrhage (17,18). Other observable alterations associated with pregnancy that were recorded in this study pertain to the white blood cell populations where significantly higher counts were recorded for total white blood cells, neutrophils, lymphocytes and monocytes. Instinctive upregulation of immunity is thought to be a pregnancy-induced protective measure for both maternal and foetal wellbeing. Beyond the necessity to combat infections during pregnancy, stimulation of adaptive immunity is crucial for optimal pregnancy outcome in relation to tissue remodeling and placental development (24-27).

The findings of significantly shortened PT and lower INR in pregnant women of advanced maternal age suggests pronounced hypercoagulable state in older pregnant women. This group of pregnant women also had significantly higher platelet count, total WBC count, neutrophils count and lymphocyte count. Age on its own is thought to be associated with some level of inflammation as well as endothelial dysfunction which is capable of activating platelets and other components of haemostasis (28-30) Meanwhile, activated coagulation triggers further immune response thereby sustaining inflammation. Possibly, baseline immunothrombosis in older women within the reproductive age bracket may be exacerbated in pregnancy giving rise to the observed pattern of changes in markers of inflammation and thrombosis. Moreover, the placenta is believed to be an important regulator of immune responses during pregnancy. The possibility of sub-optimal placentation in women of advanced maternal age may exist and is capable of driving heightened immune response in such subjects (27,31).

# CONCLUSION

The present study observed higher hypercoagulability and immune response in older pregnant women compared to the younger ones.

### Conflict of Interest

The authors declare no conflict of interest.

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