Cover page for Clinical Trials Document

Study title	Establishment of Reference Intervals of Complete Blood Count and Coagulation Tests in Pregnant Women at Hung Vuong Hospital
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I. STUDY PROTOCOL

Reference intervals are defined as the 2.5th percentile to 97.5th percentile of a normal population. Therefore, it is common to interpret results as abnormality based on normal reference intervals in pregnant women due to their biophysiological changes [1]. As a result, it is crucial to define normal reference intervals for pregnant women. Complete blood count and coagulation tests such as activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen are of a routine hematology panel. There have been many studies conducted on Asian pregnant women [2, 3], but none was conducted in Vietnam. Therefore, we conduct this study to determine trimester specific reference intervals for healthy pregnant women.

Patient selection

From June 2023 to August 2023, pregnant women visiting obstetrics clinics at Hung Vuong Hospital are selected if eligible after history taking and clinical examination. Inclusion criteria was singleton pregnancy. Exclusion criteria were hypertension, diabetes, pre-eclampsia, gestational diabetes, hemoglobinopathy, current infection, positive screening for *treponema pallidum*, hepatitis B virus, HIV, usage of anticoagulant drug. Each patient will have their blood drawn 4 millimeters (mL), 2 mL into citrate anticoagulated tube to test for APTT, PT, fibrinogen, and 2 mL into Ethylenediaminetetraacetic acid (EDTA) tube to test for complete blood count.

To establish reference intervals using non-parametric method, the minimum sample size needed is 120, according to CLSI EP28-A3c [4], because of the 90% confidence interval reported for the 2.5th percentile. However, Sample Sizes for Clinical, Laboratory and Epidemiology Studies [5] suggested a calculation for larger minimum required sample size $N_{Rank} = \eta\sqrt{3} \times \left[\frac{Z_{1-\gamma/2}}{\rho_{plan} \times Z_{1-\gamma/2}}\right]^2$ where $\eta = \frac{\sqrt{(\gamma/2)[1-(\gamma/2)]}}{\phi_{1-\gamma/2}} = 2.11$ $(\phi_{1-\gamma/2} = \frac{1}{\sqrt{2\pi}} e^{\frac{Z^2_{1-\gamma/2}}{2}})$ with $\alpha = 0.05$, $\gamma = 0.1$. ρ_{plan} is margin of error, which is the estimate of the percentage that the width of the confidence interval of the reference limits is of the width of the reference interval. The recommended ρ_{plan} is 10%. From the formula above, the calculated sample size required is 258 patients. Because we want to establish trimester specific reference intervals, the total combined number of each trimester is at least 774 patients.

Ethical approval

The study was approved by the Ethical committee of Hung Vuong Hospital, Vietnam. Written consent was obtained before study participation.

Samples collection and instrument

Venous blood samples were collected in trisodium citrate 3.2% and K_2EDTA plastic whole blood tube and then immediately analyzed.

Instrument and analysis

Coagulation assays, which comprises of Activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen (FIB), was performed on ACL TOP 550 automated coagulation analyzer (Werfen, Barcelona, Spain) and complete blood count, including White blood cell (WBC) counts and percentage and absolute count of different

leukocytes (neutrophils, lymphocytes, monocytes, eosinophils and basophils), red blood cells (RBC), hemoglobin (Hgb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC distribution width (RDW), platelet counts (PLT), mean platelet volume (MPV), was performed on DxH900 automated hematology analyzer (Beckman Coulter, CA, USA).

All instruments were maintained and calibrated according to the manufacturer's instruction. Two levels of controls were done on ACL TOP 550 analyzer every eight-hour shift and three levels of controls were run DxH900 once a day.

II. STATISTICAL ANALYSIS PLAN

Patients are divided into three groups, based on their gestational age. The three groups are the first, second and third trimester. The non-parametric method, recommended to establish reference intervals by CLSI EP28-A3c, is used to determine the lower reference limit 2.5th percentile and upper reference limit 97.5th percentile for each group. Each reference limit was reported along with its 90^{th} confidence interval. Groups are then compared using Mann-Whitney U and Kruskal-Wallis tests. A two-tailed P-value < 0.05 was considered significant.

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