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## OBSTETRICS

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# Evaluation of Repeated Antenatal Blood Testing for Anemia, Human Immunodeficiency Virus, and Syphilitic Infection Screening during the Third Trimester: A single-center university hospital setting

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### ABSTRACT

**Objectives:** To compare first and repeated antenatal blood tests for screening anemia, human immunodeficiency virus (HIV), and syphilis serology status. Factors influencing maternal anemia were also evaluated utilizing multivariate analysis.

**Materials and Methods:** A prospective descriptive study involved 1,089 pregnant women who attended an antenatal care unit in a university hospital setting. Participants were asked to fill in a questionnaire. Blood tests at the first antenatal visit and again during the third trimester, approximately 12 weeks apart, were performed routinely (in all women). An analysis was performed to compare the results from both blood sampling periods.

**Results:** Hemoglobin and hematocrit levels were found to be significantly lower in the third trimester (first vs. third trimester; hemoglobin  $12.2 \pm 1.2$  vs.  $11.9 \pm 1.2$  g/dL, hematocrit  $36.6 \pm 3.5\%$  vs.  $36.1 \pm 3.3\%$ ,  $p < 0.001$ ). The incidence of anemia was 14.9% and 23.9% in the first and second laboratory tests, respectively ( $p < 0.001$ ). Anemia diagnosed in the first trimester (odds ratio (OR) 5.46, 95% confidence interval (CI) 3.74–7.57), maternal underweight (OR 1.59, 95%CI 1.02–2.49), and poor compliance (OR 2.56, 95%CI 1.25–5.21) with ferrous supplementation were considered significant risk factors for anemic status being observed in the third trimester. The prevalence of HIV and syphilis infection were 3.6/1,000 and 1.8/1,000, respectively. Four syphilis seroconversions were observed in which 2 of these 4 were subsequently confirmed as syphilis infection by specific Treponemal test (0.2%). There was no HIV seroconversion in the study population.

**Conclusion:** A significant decrease in hemoglobin and hematocrit levels warrants the need for a repeated complete blood count in the late trimester. Patients with risk factors, including i) first trimester diagnosis with anemia, ii) low body weight, and iii) poor compliance with taking antenatal supplements, require close monitoring to alleviate the severity of anemia at delivery. Due to a 0.2% seroconversion rate of syphilitic infection, the authors recommend repeat syphilitic serologic testing regardless of the sexual transmitted infection risks. Despite a high prevalence of HIV

infection, absent of seroconversion in the study population warrants re-consideration of universal repeated screening of HIV infection in the third trimester. Further cost-utilization studies are required to draw conclusion regarding repeated serologic screening blood tests.

**Keywords:** anemia, HIV, repeated blood test, seroconversion, syphilis, pregnancy.

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## การวิเคราะห์ผลของการตรวจเลือดฝากครรภ์ซ้ำในไตรมาสที่ 2 หรือ 3, งานวิจัยสถาบันเดียวในโรงพยาบาลมหาวิทยาลัย

วิภาดา เหล่าสุขสถิตย์, เกษม เรืองรองมรกต, ภาวิน พัวพรพงษ์, อรสา เหมะจันทร์, เมลิตา สุขสมานวงศ์, ธารารัตน์ หาญประเสริฐพงษ์

### บทคัดย่อ

**วัตถุประสงค์:** การวิจัยศึกษาเปรียบเทียบการตรวจเลือดในการฝากครรภ์ครั้งแรก และการตรวจเลือดซ้ำในไตรมาสที่สาม ได้แก่ ตรวจประเมินภาวะซีด ตรวจการติดเชื้อเอชไอวี และการติดเชื้อซิฟิลิส ร่วมกับเก็บข้อมูลปัจจัยที่มีผลให้เกิดภาวะซีดในหญิงตั้งครรภ์ **วัสดุและวิธีการ:** หญิงตั้งครรภ์จำนวน 1,089 คน ได้รับการฝากครรภ์ที่โรงพยาบาล เข้าร่วมวิจัยโดยตอบแบบสอบถาม และ ทบทวนเวชระเบียน

**ผลการศึกษา:** หญิงตั้งครรภ์ในงานวิจัย 1,089 คน พบว่ามีระดับฮีโมโกลบิน และฮีมาโตคริตลดลงอย่างมีนัยสำคัญในไตรมาสที่สาม โดยเข้าเกณฑ์วินิจฉัยภาวะซีดในหญิงตั้งครรภ์จากการตรวจเลือดในการฝากครรภ์ครั้งแรก และจากการตรวจเลือดในไตรมาสที่สามคิดเป็นร้อยละ 14.9 และ 23.9 ตามลำดับ ปัจจัยที่มีผลต่อภาวะซีดในไตรมาสที่สามคือ ตรวจพบภาวะซีดในการตรวจเลือดครั้งแรก, ภาวะน้ำหนักน้อยกว่าเกณฑ์, และการรับประทานธาตุเหล็กเสริมอย่างไม่สม่ำเสมอ นอกจากนี้พบการติดเชื้อเอชไอวีคิดเป็นร้อยละ 0.36 โดยไม่พบการติดเชื้อเพิ่มในการตรวจเลือดในไตรมาสที่สาม ส่วนการติดเชื้อซิฟิลิสพบร้อยละ 0.18 และพบการติดเชื้อเพิ่มในการตรวจเลือดในไตรมาสที่สามจำนวน 2 ราย

**สรุป:** ผู้มีพันธสนับสนุนการตรวจเลือดเพื่อประเมินภาวะซีดซ้ำในไตรมาสที่สาม โดยเฉพาะหญิงตั้งครรภ์ที่มีปัจจัยเสี่ยงดังผลการวิจัยข้างต้น และควรได้รับการเฝ้าระวังภาวะแทรกซ้อนจากภาวะซีดในช่วงคลอด นอกจากนี้ พบผู้ติดเชื้อซิฟิลิสเพิ่มในการตรวจเลือดซ้ำร้อยละ 0.2 ผู้มีพันธสนับสนุนการตรวจเลือดซ้ำเพื่อวินิจฉัยการติดเชื้อซิฟิลิส หญิงตั้งครรภ์ที่ติดเชื้อเอชไอวีในงานวิจัยมีอุบัติการณ์สูงในประชากร แต่ไม่พบการติดเชื้อเพิ่มในการตรวจเลือดซ้ำ ผู้มีพันธเห็นว่าควรพิจารณาอย่างถี่ถ้วนในการส่งตรวจการติดเชื้อเอชไอวีซ้ำในหญิงตั้งครรภ์ทุกราย

**คำสำคัญ:** โลหิตจาง, เอชไอวี, การตรวจเลือดซ้ำ, ผลเลือดเปลี่ยนแปลง, ซิฟิลิส, หญิงตั้งครรภ์

## Introduction

The ultimate goal of pregnancy care is health and safety of both the mother and the newborn baby. In order to achieve this goal, antenatal care plays an important role. The risks to individual woman can be recognized by taking their history, physical examination, and performing certain investigations, including blood tests, to guide an obstetrician and healthcare team for proper patient management both during the antenatal and the intrapartum period. Prenatal blood screening for common diseases, such as anemia, thalassemia, and serious sexual transmitted infections (STIs), is universally performed during the patient's first visit. Abnormal results obtained can subsequently allow the physician to provide proper counseling and further management to prevent maternal and fetal morbidity/mortality.

The Royal Thai Collage of Obstetricians and Gynecologists (RTCOCG) guideline for the first prenatal blood test is a modified version of the international guideline. The World Health Organization (WHO) recommendation for prenatal blood tests includes: i) either hemoglobin/hematocrit level or complete blood count (CBC), ii) Rh blood typing, and iii) syphilis, human immunodeficiency virus (HIV), and viral serologic screening relevant to the individual setting of each hospital<sup>(1)</sup>. Thalassemia screening, ABO blood typing, and hepatitis B serologic test were added into the RTCOCG version according to the considered high prevalence of thalassemia diseases and hepatitis B carriers among the Thai population. Nonetheless, there is growing evidence suggesting that a single blood investigation may be insufficient. Anemia is common during the late trimester. Patients can also contract HIV and syphilis at any time during pregnancy if they remain sexually active. Evidence suggests that repeated serologic screening for both HIV and syphilis should be offered but only for women in high-risk populations<sup>(2-3)</sup>. The detection of anemia as well as HIV and syphilitic seroconversion allows a proper care plan and patient management to be implemented to prevent future complications.

The RTCOCG also published a guideline in 2011

suggesting that a repeated blood test for assessing the hemoglobin or hematocrit level, HIV, and syphilis serology should be done in all Thai pregnant women. Despite both evidence and the RTCOCG recommendation, a significant number of hospitals and antenatal clinics still only conduct a single antenatal blood test policy. HRH Maha Chakri Sirindhorn Medical Centre (MSMC) is a university hospital situated in a suburban area of Thailand, 70 km from the capital Bangkok. The hospital antenatal clinic serves approximately 1,400 antenatal care per month. Before 2015, a blood test was performed once only in the first prenatal visit, and thus it was unable to estimate the rates of seroconversion and new cases of anemia that arose in the second half of pregnancy. This protocol has been cautiously revised according to the RTCOCG guideline since 2015 to include performing a repeated blood test in the third trimester. In previous years, the proportion of teenage pregnancies in our area had been reported to be slightly high at 7.7%, with these considered a high risk group, and so the number of malnourished and anemic patients was expected to increase in our population, especially in the late trimester. In 2015, the hospital prevalence of HIV and syphilis in pregnancy were reported to be approximately 5/1,000 and 1/1,000, respectively. Such data define this as an endemic area for HIV infection<sup>(4)</sup>. It has been demonstrated that repeated HIV screening is a cost-effective strategy for the prevention of the perinatal transmission of HIV, even in a resource-limited country<sup>(5)</sup>. Hence, it would be worthwhile to evaluate whether repeated serology screening in the third trimester is necessary in our local setting.

The primary objective of the present study was to evaluate the utility of repeated antenatal blood tests in regards to detection of anemic status, through CBC values, and seroconversion of both syphilis and HIV in the third trimester as compared to the first prenatal visit. The factors influencing these changes were also determined utilizing multivariate analysis. The knowledge obtained from this study can facilitate policy modification in order to tackle these common antenatal care problems.

## Materials and Methods

This study was a prospective descriptive study conducted to evaluate the current antenatal intervention, i.e., the repeated blood sampling scheme, during the period between January 2016 and December 2016. All women having antenatal care at the MSMC were asked to participate the study when they visited the MSMC for the repeated blood test at the clinic. Only singleton pregnant women with neither fetal anomaly nor maternal hematologic diseases affecting either their red cell count or platelet concentration other than thalassemia (and hemoglobinopathy) were included in the study. All participants received standard dose of iron supplement, 200 mg of ferrous fumarate (approximately 66 mg of elemental iron) daily starting at 16-20 weeks once the patients were clear from morning sickness symptoms. In addition, women with a history of blood and other components' transfusion were not eligible for the study. All the participants had received their first antenatal blood test at either the MSMC or at other clinics according to the standard practice. The repeated blood test was performed at either 28-32 weeks gestation or at least 8 weeks following the first blood sampling if they had started their booking late. Nonetheless, if the first blood test had been done in the third trimester and it was not possible to achieve an 8-week interval for the blood sampling, the repeated blood test was performed in the labor ward just before delivery. All the pregnant women received pre-test counseling and had to give their voluntarily consent prior to the test according to the standard practice guideline. A questionnaire regarding the patient's demographic data and the factors associated with anemia and seroconversion was given to the patient following their written informed consent.

The repeated blood test included CBC, Anti-HIV, and Venereal Disease Research Laboratory test (VDRL) according to the RTCOG recommendation. Participants who had their first blood test positive for either Anti-HIV or VDRL were not tested again for the respective positive test, but their data were still included in the other analyses. Anemia was defined, according

to the Centers for Disease Control and Prevention (1998), as when the hemoglobin level falls below 11 g/dL (hematocrit level falls below 33%) in the first and third trimesters<sup>(6)</sup>. The study received ethical approval from the Institutional Review Board (IRB) and was funded by the Faculty's Routine to Research (R2R) funding scheme.

### Statistical analysis

According to the primary outcome, in order to detect statistical significance with expected a 20% shift from a positive outcome (anemia) to negative and a 10% shift from negative to positive, with type I and II errors of 0.05 and 0.2, respectively; a total of 244 participants with both first and repeated blood test results were required. Nonetheless, the author wanted to evaluate the seroconversion incidence, especially for Anti-HIV, in which at least an incidence of 1/1,000 newly diagnosed Anti-HIV positive is considered an endemic area. Therefore, such a study would need to enroll at least 1,000 participants.

The demographic and outcome data were reported herein as either the mean  $\pm$  standard deviation (SD) or proportion as appropriate. A significant difference in the hematocrit level, hemoglobin level, and platelet concentration between the first and repeated blood sampling tests was determined using the paired t test. The proportions of patients diagnosed with anemia were compared using McNemar's chi square test. Multivariate logistic regression analysis was used to identify the factors affecting the patient anemic status during the third trimester as a dependent variable, while the patient's age, body weight, educational background, income, parity, iron intake, iron supplementation, and a previous diagnosis of anemia as independent variables were fitted into the model using a backward elimination approach. The odds ratio (OR) with a 95% confidence interval (CI) was calculated. It was expected that the seroconversion rate of either the Anti-HIV or VDRL result would not be common, and therefore, only descriptive analysis would be used. A p value  $< 0.05$  was determined to be statistically significant.

## Results

During a one-year period between January 2016 and December 2016, a total of 1,089 women with singleton pregnancy who were having antenatal care at the MSMC were enrolled into the study. Mean  $\pm$  SD age and BMI of the participants were  $28 \pm 6.3$  years old and  $26.7 \pm 4.8$  kg/m<sup>2</sup>, respectively. Approximately half (48.6%) were primigravida participants. The prevalence of teenage pregnancy (aged under 20 years old) and advanced maternal age (aged over 35 years

old) were 10% and 17%, respectively. Only 1% of the participants were underweight (BMI < 18.5 kg/m<sup>2</sup>), while approximately 40% of participants were overweight (BMI = 25.0 - 29.9 kg/m<sup>2</sup>) and 20% were obese (BMI  $\geq$  30 kg/m<sup>2</sup>) among the total number of women participating in the study. More than 75% had commenced their antenatal care earlier than 14 weeks of gestation (considered early antenatal care). The participants' occupational backgrounds are summarized in Table 1.

**Table 1.** Demographic data of the study population.

	Demographic data	Proportion
Age group	Teenage	10.0%
	20 – 35 years	72.9%
	Elderly	17.1%
Weight	Underweight	1.0%
	Normal	39.2%
	Overweight	39.3%
	Obese	20.5%
Education	Lower than bachelor's degree	67.6%
	Bachelor's degree	32.4%
Income	Low	5.6%
	Normal	68.9%
	High	25.5%
Occupation	Unemployed/housewife	28.8%
	In agriculture	2.9%
	Office worker	11.6%
	Privately owned business	34.7%
	Others or unclassified	22.0%
Gravidity	Nulliparous	48.9%
	Multiparous	50.5%
	Grand multiparous (> 4)	0.6%
Antenatal care	Late first antenatal care (> 14 weeks)	23.7%
	First antenatal care < 14 weeks	76.3%
Thalassemia screen	Normal	64.8%
	Positive screening for thalassemia trait/disease	17.1%
	Doubtful results	18.1%

Over one quarter of the participants were either unemployed or a housewife, while the majority were currently working in either the commercial or industrial sector, with only 3% employed in the agricultural sector. Approximately two-thirds of the participants had an educational background lower than a bachelor's degree. Nonetheless, almost 95% had an annual income of more than 60,000 baht (25.5% had a considered high annual income of more than 240,000 baht), leaving only 5.6% who had an income of less than 60,000 baht/year.

All the participants underwent a repeated blood test at the MSMC, comprising 43.6% who underwent their first blood test in an external clinic/health center and the other 56.4% who initially started their antenatal care at the MSMC. The majority (64.8%) of the participants were found to be negative (normal) for

thalassemia in the screening performed either by the MSMC or as part of the national (Ministry of Public Health) screening program. Approximately 17.1% of the study population were diagnosed with a thalassemia trait, primarily the hemoglobin E trait/disease and beta-thalassemia trait, respectively. Another 18.2% were suspected to have a thalassemia trait without diagnostic confirmation. This group of participants were couples without a risk of severe fetal thalassemia diseases according to the couple screening program. Overall, both hemoglobin and hematocrit levels were significant lower in the third trimester testing when compared to the first antenatal care visit, as shown in the Table 2 (first vs. repeated blood test; hemoglobin  $12.2 \pm 1.2$  vs.  $11.9 \pm 1.2$  g/dL, hematocrit  $36.6 \pm 3.5$  vs.  $36.1 \pm 3.3\%$ ,  $p < 0.001$ ).

**Table 2.** Comparison of the hemoglobin level, hematocrit level, platelet count and anemic status between the first and repeated antenatal test.

Demographic data	First blood test <sup>†</sup>	Repeated blood test	p value*
Hemoglobin (g/dl)	12.2 (1.2)	11.9 (1.2)	< 0.001
Hematocrit (%)	36.6 (3.5)	36.1 (3.3)	< 0.001
Women diagnosed with anemia <sup>‡</sup> , n (%)	162 (14.9%)	260 (23.9%)	< 0.001
Platelet count	301,425 (62,314.7)	268,955 (62,781.4)	< 0.001
Positive Anti-HIV serologic status, n (%)	4/1,088 (0.36%) <sup>¶</sup>	0/1,084 (0%) <sup>§</sup>	-
Positive syphilitic infection, n (%)	2/1,088 (0.18%) <sup>¶</sup>	2/1,086 (0.18%) <sup>§</sup>	-

Data are presented as mean (SD)

<sup>†</sup> Mean (SD) gestational age at first ANC = 11 (5.2) weeks.

\* Student t-test for mean differences & chi-square test for proportional data.

<sup>‡</sup> Defined by the WHO criteria<sup>(6)</sup> - see text

<sup>¶</sup> Prevalence at first antenatal blood test

<sup>§</sup> Incidence of sero-conversion at the second blood test

The incidences of anemia, according to the WHO criteria, were significantly higher at the second laboratory test (third trimester) time-point (14.9% vs. 23.9%,  $p < 0.001$ ). Similarly, the mean platelet count in the repeated blood test was significantly lower than the first ANC result (mean difference,  $-31,816 \pm 52,332$ ,  $p < 0.001$ ).

In order to identify the key risk factors influencing the diagnosis of anemia in the third trimester, log

regression analysis (both univariate and multivariate) was performed. Body composure, compliance with taking ferrous supplementation, and diagnosis of anemia during the first trimester were considered to be the determining factors for anemia in the third trimester. Patients diagnosed with anemia in the first trimester were approximately 5.5 times more likely to be anemic in the third trimester (multivariate analysis: adjusted OR 5.46, 95%CI 3.74-7.97,  $p < 0.001$ ). Underweight patients

had a 1.6 times higher chance to be diagnosed with anemia during the third trimester (adjusted OR 1.59, 95%CI 1.02-2.49,  $p < 0.05$ ). Also, they were approximately 1.5-2.5 times more likely to be diagnosed with anemia in the late trimester if the patients did not take ferrous

supplementation regularly (adjusted OR 1.54 and 2.56 in less and poor compliance patients, respectively,  $p < 0.05$ ). Other factors collected from the study questionnaire that were included in the multivariate analysis are shown in Table 3.

**Table 3.** Multivariate logistic regression analysis<sup>§</sup> regarding the risk factors influencing maternal anemia in the third trimester (repeated blood test).

Factor	Adjusted OR	95%CI	p value
Age group			0.06
Teenage pregnancy	1.04	0.60-1.81	0.88
Elderly gravidarum	0.66	0.42-1.05	0.08
Body weight			< 0.01
Underweight	1.59	1.02-2.49	< 0.05
Overweight	0.70	0.13-3.76	0.68
Obesity	1.22	0.78-1.92	0.38
Lower educational background	0.92	0.64-1.32	0.65
Income			0.13
Low income	1.59	0.84-3.03	0.16
High income	0.94	0.64-1.39	0.76
Parity			0.59
Multiparous	1.26	0.90-1.76	0.18
Grand multiparous	1.11	0.17-7.15	0.91
Reduced-ferrous diet <sup>†</sup>	0.72	0.51-1.03	0.07
Ferrous supplementation			< 0.01
Less compliance (sometimes take)	1.54	1.07-2.22	< 0.05
Poor compliance (seldom take)	2.56	1.25-5.21	< 0.05
No compliance (never take) <sup>‡</sup>	1.61	0.37-7.03	0.53
Previous diagnosed anemia (first visit)	5.46	3.74-7.97	< 0.001

OR: odds ratio, CI: confidence interval

<sup>§</sup> Factors included in the model were age, body mass index, educational background, income, and parity as fixed variables, while reduced ferrous diet, compliance with ferrous supplementation, abnormal glucose tolerance, aspirin prescription, hometown region, history of postpartum hemorrhage, and history of low birth weight were the tested variables.

<sup>†</sup> Reduced ferrous diets include i) vegetarian diet, ii) meat, internals, green leaf, or egg abstention.

<sup>‡</sup> n = 10.

Concerning the serologic results, the prevalence of HIV and syphilis infections were 3.6/1,000 and 1.8/1,000, respectively. Overall, proportions of women with risk factors of HIV, syphilis and STIs (including

cervicitis, pelvic inflammatory diseases, condyloma) in the study population were multiple partners 20.8%, history of STIs 1.5% and history of illicit drugs used 5.8%, respectively. There were 4 new positive syphilis

screening results in the second laboratory tests, in which 2 of these 4 were confirmed as syphilis infection by Treponemal and non-Treponemal specific tests. The first patient, 24 years old and nulliparous, had no syphilis screening during the first trimester. The second patient, 35 years old and nulliparous, was a housewife and had no history of either multi-partners or a previous STD. She only had a poor income of less than 60,000 baht annually. The other 2 women were confirmed with false-positive syphilis screening by showing negative Treponemal test. Regarding the HIV blood test, there was no seroconversion observed in the study population. We did not repeat the test for hepatitis B surface antigen (HBsAg) in the third trimester, while the prevalence from the first antenatal blood test was 2.8% (30/1,089).

## Discussion

Overall, the study demonstrated a statistically significant decrease in both hemoglobin (mean difference  $0.4 \pm 1.1$  mg/dL, 95%CI 0.3-0.5,  $p < 0.001$ ) and hematocrit (mean difference  $0.5 \pm 3.3\%$ , 95%CI 0.3-0.7,  $p < 0.001$ ) levels in the third trimester when compared to the first visit antenatal blood test. Anemia diagnosed at the first visit was the strongest determinant factor influencing maternal anemia in the late trimester, while a patient's BMI and compliance with taking ferrous supplementation were also associated with the diagnosis of anemia in the late trimester. The prevalence of HIV infection in pregnancy was 3.6/1,000 without new seroconversion observed in the study population. The prevalence of syphilis infection was observed to be 1.8/1,000, but there were 2 cases of seroconversion found in the repeated blood tests.

Hypervolemia is a physiologic change that occurs from the beginning of pregnancy and progresses until delivery. For example, the plasma volume expansion is around 14% at 12 weeks gestation<sup>(7)</sup> and increases to a maximum of around 40–45% after 32–34 weeks<sup>(8)</sup>. However, there is a discrepancy between the increases in both components in which the plasma volume extension is more than the increase in the red cell volume. Consequently, hemoglobin and hematocrit concentrations slowly decline, but the hemoglobin should not drop lower than 10.5 g/dL (hematocrit  $< 32\%$ )

in the mid-trimester and 11.0 g/dL (hematocrit  $< 33\%$ ) in the first and third trimester. These numbers are used as cut-off values for diagnosing anemia in pregnancy<sup>(6)</sup>. In our study population, a decrease in both hemoglobin ( $-0.4 \pm 1.1$  mg/dL, 95%CI 0.3-0.5,  $p < 0.001$ ) and hematocrit ( $-0.5 \pm 3.3\%$ , 95%CI 0.3-0.7,  $p < 0.001$ ) levels, and platelets concentration in the late trimester could be theoretically explained by a dilution effect. However, the dilution effect cannot cause adverse outcomes if anemia is not diagnosed.

In 2011, the World Health Organization (WHO) reported that the prevalence of anemia in women of reproductive age (15-49 years old), who are eligible to get pregnant, and in pregnant women were 19.9% and 24.5%, respectively<sup>(9)</sup>. Compared to the WHO study, this study found the prevalence of anemia in pregnancy at 14.9% and 23.9% as observed at the first and repeated antenatal blood tests, respectively. The prevalence of anemia varies among regions and countries. A study in China by Zhao and colleagues demonstrated the prevalence of anemia in pregnancy during 2012-2016 were 10.1%, 26.7%, and 28.1% in the first, second, and third trimester, respectively<sup>(10)</sup>. Whereas a systematic review performed in Nigeria observed 25-45.6% of iron deficiency anemia in the pregnant population<sup>(11)</sup>. Policies regarding blood tests for anemia are, therefore, different from country to country. For example, a retrospective study in Austria observed proportions of anemia diagnosed during the first ( $< 16$  weeks) and second (third trimester) to be 2.2% and 13%, respectively. The Austrian authority then concluded that the first examination is not mandatory for antenatal care<sup>(12)</sup>. Our finding regarding anemia prevalence and its increase during the late trimester did not against implementation of the repeated blood tests scheme. The authors suggest that each institution should evaluate own population and adjust the policy accordingly.

Previous diagnosis with anemia detected from the first prenatal visit was the greatest predictive factor for a diagnosis of anemia in the late trimester. Moreover, pregnant women who were malnourished as indicated by a low pre-pregnancy BMI ( $< 18.5$  kg/m<sup>2</sup>) had a higher chance to be anemic in the third trimester. Poor compliance with taking ferrous supplementation

regularly also increased the risk of anemia later during the gestational period. This can imply that in the study population a patient's nutritional status was a major determining factor of anemia in pregnancy, and this was similar to what has been previously described in the literature. In Pakistan, as another Asian population, specific types of food consumption, such as pica, tea, and a low intake of eggs and red meat were associated with anemia in an urban area<sup>(13)</sup>. Another study involving a Portuguese population reported that being a teenager was the only risk factor of iron storage depletion<sup>(14)</sup>. A systematic review from the Nigerian group observed that multiparity and low socioeconomic status, or just being in the third trimester were risk factors for iron deficiency anemia. Moreover, factors associated poor compliance to routine iron therapy included poor utilization of antenatal services, low educational attainment, long distance to healthcare facility, being single, and teenage pregnancy<sup>(15)</sup>. A multivariate analysis study performed in Ghana, which observed a high prevalence of over 50% with anemia in pregnancy, demonstrated that poor dietary intake was associated with a 2.7-time increase of anemia (adjusted OR 2.73, 95%CI 1.35-5.50). Other associate risk factors were young maternal age, fewer antenatal contacts, and low BMI<sup>(16)</sup>. Therefore, pregnant women who are at risk for undernourished dietary status, i.e., low BMI observed in our study, teenage pregnant and lesser socioeconomic status found in other reports, should be identified during the antenatal care. Nutritional support - enriched ferrous diet, and iron supplement therapy should then be provided with scheduled repeated blood tests in the late trimester. However, we could not demonstrate the effect of teenage pregnancy in our study. This may be because our institution has a robust health education program for teenager (to be) mothers during the antenatal care. Henceforth, the nutritional status was observed to be a distinctive factor irrespective of the maternal age group in our study.

Anemia in pregnancy can subsequently cause a number of adverse outcomes, such as i) a small for gestational age and low birth weight baby, especially if the anemia is experienced in the first trimester<sup>(17)</sup>, ii) preterm delivery, and iii) perinatal and neonatal

mortality<sup>(6, 18)</sup>. An anemic pregnant woman is less tolerable to peripartum bleeding than a non-anemic one, especially in patients at risk of postpartum hemorrhage. Anemic pregnant women are generally asymptomatic; thus, a healthy-looking, no underlying illness woman can still have the disease. The healthcare personnel responsible for the antenatal care should be cautious with pregnant women who are at risk, for example underweight or previously diagnosed with anemia, and should provide prompt treatment and monitor the patient's compliance in taking the proper amount of ferrous supplementation. If necessary, anemic patients who cannot tolerate oral iron supplementation/treatment may be provided with the parenteral form which has been proven to be more effective<sup>(19)</sup>. However, whether this anemia prevention method can improve perinatal outcomes is still unclear. Our limitation here was that our study did not originally plan to collect maternal and neonatal outcomes. Consequently, either a prospective or experimental study should be performed in the future to evaluate the effect of anemia and the efficacy of ferrous supplementation on the obstetrics and neonatal outcomes.

Determining the prevalence and rates of serological conversion concerning HIV and syphilis were also among the study objectives. HIV infection in pregnancy unfavorably affects both the mother and fetus. Early screening at the first prenatal visit and prompt treatment is advisable. Though, as mentioned elsewhere, it is still controversial to recommend repeated HIV screening in the third trimester. One study conducted in a high-prevalence area showed a 3% seroconversion rate<sup>(20)</sup>. We did not observe any cases of HIV seroconversion in our study population; nonetheless, concerns were raised considering the high prevalence observed (>1/1,000). The authors suggest a careful consideration to the RCOG recommendation to repeat HIV serology in each institution's population during the third trimester.

Regarding syphilitic infection, the prevalence has been reported to broadly range between 0.7-400 per 100,000 pregnant population, as referenced by various reports worldwide, including one from Thailand<sup>(21-24)</sup>. It

is important to note that even in populations with an expected high prevalence of STIs, such as migrants and refugees, the prevalence of syphilis infection can be low<sup>(24)</sup>. Albright and colleagues reported that repeated syphilitic screening in the late trimester is not cost-effective in low prevalence populations<sup>(25)</sup>. Nevertheless, an increased incidence of congenital syphilis observed in a United States population between 2012 and 2014<sup>(26)</sup> demonstrated a contrary situation for considering implementing a stringent protocol for syphilis screening regardless of the background prevalence. The detection of seroconversion is notable because the only approach to prevent 98.2% of congenital syphilis is to provide treatment to the mother<sup>(27)</sup>. From our study, we anticipated a higher prevalence (1.8/1,000) and incidence of seroconversion than what was earlier expected (2 participants, incidence of 1.8/1,000). Moreover, both the seroconverting participants' history did not demonstrate much concern for STI infection (single partner, housewife, and no previous history of STI infection). As a result, the authors recommend repeated syphilitic serologic testing regardless of the STI risks. A cost-effectiveness study enrolling a larger population is also encouraged in the future.

## Conclusion

In conclusion, due to the dilution effect of the blood volume during pregnancy, a significant decrease in hemoglobin and hematocrit levels in the study warrants a repeated CBC test in the late trimester. Patients at a high risk of developing anemia in the second/third trimester included i) patients diagnosed with anemia at the first antenatal visit, ii) underweight patients, and iii) patients who have not complied with their ferrous supplementation/medication. These groups of patients require close monitoring to prevent or alleviate the severity of anemia during delivery. The authors also support repeated screening for syphilis owing to the present of syphilis seroconversion though further cost-effectiveness study should be performed in the future. Nonetheless, despite a considered high prevalence of HIV serology status, absent of HIV seroconversion warrants re-consideration of universal

repeated screening of HIV infection in the third trimester. Each institution policy regarding the repeated antenatal blood tests should be based on diseases' prevalence and health setting.

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## Potential conflicts of interest

The authors declare no conflicts of interest.

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