Normal Serum Cystatin C Level during the First Trimester of Thai Pregnant Women

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Background: *Cystatin C is a biomarker of renal function and other diseases. Physiologic changes during pregnancy can alter it level and lead to misinterpretation of result.*
Objective: *To determine reference values for serum cystatin C in the first trimester of pregnancy.*
Material and Method: *The Thai singleton uncomplicated pregnant women who attend antenatal clinic at Maha Chakri Sirindhorn Medical Center during August 2013 to December 2014 were included. Gestational age of individual was calculated by last menstrual period or ultrasound scan. Demographic data and obstetrics outcomes were recorded. Peripheral blood was taken on the first prenatal visit. Serum cystatin C was measured by particle-enhanced turbidimetric immunoassay (PETIA) with ARCHITECT c Systems® (ABBOTT, Germany).*
Results: *A total of 48 pregnant women were eligible for the study. The overall mean and standard deviation of serum cystatin C was 0.58±0.08 mg/L. There was no significant correlation between serum cystatin C and creatinine level (r = 0.22, p-value = 0.13).*
Conclusion: *The reference value of serum cystatin C was established for Thai pregnant women in the first trimester. Further studies of its correlation to specific obstetrics conditions should be considered.*

Keywords: *Serum cystatin C, Pregnancy, First trimester*

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Cystatin C is a protein in the Cystatin superfamily which is produced from nucleated cells. It is a cysteine protease inhibitor that has a significant role in normal cellular metabolism and in the collagen degradation process[1]. For clinicians, it is a sensitive marker of renal function and it is used to estimate the glomerular filtration rate in different groups of patients[2-4]. Cystatin C is also used as a biomarker for predicting some disorders such as cardiovascular disease, diabetic nephropathy, pregnancy induced hypertension[5], and fetal growth restrictions[6]. Serum cystatin C detection is popular use in clinical practice, because it is easy to test by an automated machine, and is also available for testing in many hospitals.

During pregnancy, cysteine protease is necessary for trophoblastic invasion in normal placental development. The maternal decidua limits the placentation process by expression of cystatin C[7]. High expression of cystatin C in the placenta will result in abnormal vessel formation and microvascular endotheliosis, which is a pathognomonic pathology found in preeclampsia[8,9]. Thilaganathan B et al, reported that serum cystatin C in early pregnancy was significantly higher in those who subsequently developed pregnancy induced hypertension as compare to control group (0.65 vs. 0.57 mg/L, p = 0.001)[10]. Saleh S et al, also found raise serum cystatin C in the second trimester of pregnancy in women who subsequently develop preeclampsia when compare to control group (0.76 vs. 0.53 mg/L, p = 0.008)[11]. These findings may constitute a way to screen for preeclampsia in the future.

Physiologic changes during pregnancy can affect the serum cystatin C levels, so the normal reference value in pregnant women is needed to determine its clinically significant level. There are a few reports of the serum cystatin C level in normal pregnancy[12,13]. However, it may not represent the state of Thai pregnant women.

Currently, there is no report of a normal value of serum cystatin C in Thai pregnant women. The aim

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of this study is then to determine reference value for serum cystatin C in the first trimester of Thai pregnant woman.

Material and Method

An observational study was conducted at the antenatal clinic, Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University, between August 2013 to December 2014. The study protocol was approved by the institute ethical review board (SWUEC/EX 22/2556, SWUEC/X-019/2557). The participants in this study were Thai pregnant women who had a first visit to the antenatal clinic during 8-12 weeks of gestation as calculated by the first day of the last menstrual period or by crow-rump length measurement.

Inclusion and exclusion criteria

A total of 100 Thai pregnant women age between 18-40 years old were recruited in each gestational age by the simple sampling method. The inclusion criteria were as follows: singleton, healthy mothers without underlying diseases, and consent to participate in the study. The exclusion criteria were any antenatal obstetrics complications, history of smoking or drug abuse, high dose steroid user, suspected fetal anomaly, or fetal death.

Each pregnant woman was interviewed for demographic data, menstrual history and medical history. Additionally, 2 mL of blood samples were collected at the same time of the antenatal blood test. All blood samples were allowed to clot and centrifuged. The maternal serum samples were stored at -80°C for up to 1 year until analysis with only 1 freeze thaw cycle.

Assay of cystatin C

Each serum sample was measured in duplicate for cystatin C level using a particle-enhanced turbidimetric immunoassay (PETIA) with an automated machine (ARCHITECT c System, ABBOTT, Germany). Twenty samples were used for machine calibration at the lower and the upper levels of 0.84 mg/L and 4.1 mg/L, respectively.

Statistical analysis

The sample size was calculated on the basis of standard deviation 0.12 mg/L of cystatin C from a previous report[12]. Assuming a two-sided alpha level of 0.05, the number needed in each group of participant was 20 women. Demographic data is presented as percentages, mean and standard deviation (SD) as appropriate. The Pearson's correlation coefficient was calculated.

Results

A total of 100 pregnant women met the inclusion criteria. Fifty-two cases were excluded as follows: 2 cases of an-embryonic pregnancy, 2 cases of embryonic death, 3 cases of miscarriage, 3 case of twin pregnancy, 3 cases of obstetrics complication, 5 cases of history of smoking, 10 cases of medical problems, 10 cases losing follow-ups, 12 cases of changing their gestational age, and 2 cases of serum missing. The demographic data are presented in Table 1. The participants' age range was 18-38 years old. Thirty-seven percent of them were primigravida. All the participants had normal blood pressure and did not have known renal disease.

The overall mean serum cystatin C during the first trimester in this study was 0.58$\pm$0.08 mg/L. The number of pregnant women in each group of gestation was similar as shown in Table 2. The level of serum cystatin C was stable during the study period and is presented in Table 2. The distribution of serum cystatin C levels and serum creatinine levels in each gestation is shown in Fig. 1. The cystatin C level was not correlated with serum creatinine (r = 0.22, *p*-value = 0.13).

Discussion

Currently, serum cystatin C is used as a marker of renal function in different population, because it does not interfere with age, sex, muscle mass, and nutritional status. In obstetrics, there is evidence of it use in first and second trimester of pregnancy to predict preeclampsia[14], gestational diabetes[15] and fetal growth restriction[6].

The overall mean serum cystatin C in this study was 0.58$\pm$0.08 mg/L which is comparable to a previous report by Thilaganathan B et al, from the United Kingdom[10]. Obrenovic R et al, reported serum cystatin C level of 0.69$\pm$0.16 mg/L in 38 uncomplicated Serbian pregnant women[16]. While, Babay Z et al, studies in healthy Saudi Arabian pregnant women and found serum cystatin C levels were high 0.89$\pm$0.12 mg/L during the first trimester[12]. Discrepancy in outcome between Babay's finding and those of the present study are due to the different study population, different period of time, and the strong correlation between serum cystatin C and serum creatinine level which differ from our results. Most researchers focused on serum cystatin C in the second and third trimester as it is a common

period for obstetrics complications to occur. While, the authors look for early diagnosis and intervention with common obstetrics conditions so we try to find out the normal value of cystatin C in the first trimester.

There is no change of this serum level during 8-12 weeks of gestation. So, physiologic changes during first trimester of pregnancy do not alter cystatin C level. We cannot know however its effects on functional alteration in early pregnancy. However, this value can be used as reference values for further study in Thai pregnant women.

There was no correlation between serum cystatin C and serum creatinine during first trimester of pregnancy in this study. This confirms that serum cystatin C is not affected by the creatinine levels.

Since cystatin C is involved in implantation and placental development, it is possible to use it as a marker of some common obstetrics conditions[15,17]. Early intervention and treatment of these diseases can reduce morbidity to patients and get more favorable obstetrics outcomes.

Limitations of this study are the descriptive design, large percentage of exclusion, small sample size, data from single center, and lack of clinical correlation. Further clinical studies in specific group of patients such as recurrent miscarriage, ectopic pregnancy and multiple pregnancy need to be carried out.

Table 1. Demographic data of participants (n = 48)

Parameters	Mean ± SD	Range
Age (year)	26.92±5.75	18-38
Primigravida (percent)	18 (37.5)	-
Multigravida (percent)	30 (62.5)	-
Body weight (kilogram)	56.78±13.56	37.3-103
Height (centimeter)	158.63±5.56	150-170
Systolic pressure (mmHg)	111.46±11.99	84-138
Diastolic pressure (mmHg)	68.42±9.86	45-88

Conclusion

The specific reference value of serum cystatin C in the first trimester of Thai pregnant woman is established. Further research with specific conditions during pregnancy is needed.

What is already known on this topic?

Serum cystatin C is a marker of renal function. Cystatin C has a significant role in placental formation and may involve in pathogenesis of some diseases. Abnormal rising of serum cystatin C is found in preeclampsia and fetal growth restriction. There is

Table 2. The mean serum cystatin C and creatinine level in each gestational age

	8 week (n = 9)	9 week (n = 10)	10 week (n = 10)	11 week (n = 11)	12 week (n = 8)
Cystatin C (mg/L)	0.59±0.04	0.61±0.09	0.57±0.08	0.58±0.09	0.57±0.06
Creatinine (mg/dL)	0.60±0.06	0.55±0.07	0.54±0.06	0.55±0.07	0.57±0.06

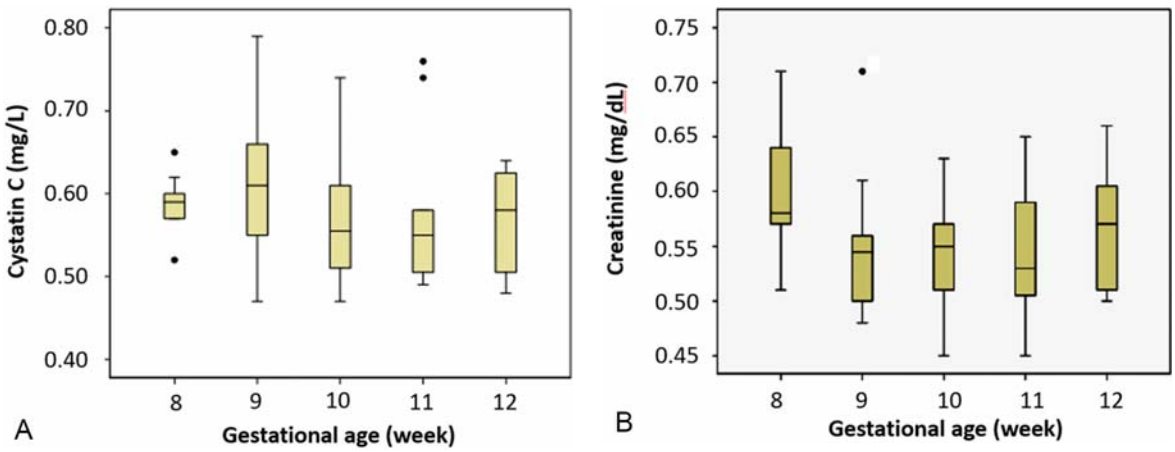

Fig. 1 Serum cystatin C (A) and serum creatinine (B) levels in each gestational age.

possibility to use serum cystatin C for prediction of certain obstetrics conditions.

What this study adds?

This study shows the normal value of serum cystatin C during first trimester in Thai pregnant women. Serum cystatin C does not differ significantly when divided into each group according to gestational age.

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Potential conflicts of interest

None.

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ระดับปกติของซีรัมซิสเตตินซีระหว่างไตรมาสแรกของสตรีตั้งครรภ์ไทย

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ภูมิหลัง: ซิสเตตินซีเป็นตัววัดชีวภาพในการทำงานของไตและโรคอื่นๆ การเปลี่ยนแปลงทางสรีรวิทยาระหว่างตั้งครรภ์อาจมีผลต่อระดับของซิสเตตินซี และทำให้แปลผลผิดพลาดได้

วัตถุประสงค์: เพื่อกำหนดค่าอ้างอิงของระดับซีรัมซิสเตตินซีในไตรมาสแรกของการตั้งครรภ์

วัสดุและวิธีการ: สตรีไทยตั้งครรภ์เดี่ยวที่ปกติไม่พบภาวะแทรกซ้อนซึ่งมาฝากครรภ์ ณ โรงพยาบาลศูนย์การแพทย์สมเด็จพระเทพรัตนราชสุดาฯ สยามบรมราชกุมารี ระหว่างเดือนสิงหาคม พ.ศ. 2556 ถึง เดือนธันวาคม พ.ศ. 2557 ที่ได้รับคัดเลือกเข้าร่วมการศึกษา ให้กำหนดอายุครรภ์โดยคำนวนจากประจำเดือนครั้งสุดท้ายหรือตรวจคลื่นเสียงความถี่สูง มีการบันทึกข้อมูลพื้นฐานและผลลัพธ์ของการตั้งครรภ์ และเจาะเลือดเก็บไว้ตั้งแต่มาฝากครรภ์ครั้งแรก แล้วทำการตรวจระดับซีรัมซิสเตตินซีด้วยวิธี particle-enhanced turbidimetric immunoassay (PETIA) ด้วยเครื่อง ARCHITECT c Systems ของบริษัท ABBOTT ประเทศเยอรมัน

ผลการศึกษา: มีสตรีตั้งครรภ์ทั้งสิ้น 48 ราย ที่ครบตามเกณฑ์คัดเลือก ได้ค่าเฉลี่ยและค่าเบี่ยงเบนมาตรฐานของซีรัมซิสเตตินซี โดยรวมเท่ากับ 0.58±0.08 มิลลิกรัมต่อลิตร ไม่พบว่ามีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติระหว่างระดับซีรัมซิสเตตินซี และครีเอตินิน ($r = 0.22$, p-value = 0.13)

สรุป: ค่าอ้างอิงของระดับซิสเตตินซี ในสตรีไทยตั้งครรภ์ปกติในช่วงไตรมาสแรก ควรที่จะพิจารณาทำการศึกษาความสัมพันธ์ของสารนี้กับโรคทางสูติศาสตร์อื่นต่อไป