Original Article

Reference Intervals for Serum Cystatin C in the Second and Third Trimester of Thai Pregnant Women

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Background: Cystatin C is a specific protein which is used to evaluate renal function. Few obstetrics conditions involve in its high level during pregnancy.

Objective: To determine reference values for serum cystatin C in the second and third trimester of pregnancy.

Materials and Methods: The Thai singleton uncomplicated pregnant women who had antenatal care at HRH Maha Chakri Sirindhorn Medical Center during August 2016 to July 2017 were included. The participants were allocated in two groups by their gestational age. Demographic data, antenatal care, and obstetrics outcomes were collected. Venous blood was collected additionally from routine antenatal blood test. The frozen-thawed serum was measured for cystatin C by particle-enhanced turbidimetric immunoassay (PETIA) with ARCHITECT c Systems® automate machine (ABBOTT, Germany).

Results: A total of 132 pregnant women were eligible for the study. The reference intervals for serum cystatin C in second and third trimester of normal individual pregnant women were 0.41 to 0.94 mg/L and 0.43 to 1.33 mg/L respectively. The mean and standard deviation of serum cystatin C in second and third trimester were 0.62±0.13 mg/L and 0.80±0.18 mg/L respectively. There was no significant correlation between serum cystatin C and creatinine level (r = 0.04, *p*-value = 0.61).

Conclusion: The reference value of serum cystatin C in the second and third trimester was established for Thai pregnant women.

Keywords: Serum cystatin C, Pregnancy, Second/third trimester

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Cystatin C is an endogenous cysteine protease inhibitors belonging to cystatin superfamily protein which produced at a stable rate in all nucleated cell[1]. It plays a role in renal function measurement by estimation of glomerular filtration rate in adults including pregnant women[2]. For obstetrics, it also was used as a serum marker for predicting specific conditions in late pregnancy such as preeclampsia[3], gestational diabetes[4], and fetal growth restrictions[5]. Furthermore, few researchers investigated the cut off level of serum cystatin C during first trimester to predict subsequent obstetrics risks, but still had conflicting results and needed for further study[6,7].

Cysteine protease is necessary for trophoblastic invasion in placental development while the maternal decidua limits the placentation process by expression of cystatin C[8]. High cystatin C expression in the placenta was found in preeclampsia[9]. Thilaganathan B, et al[10], reported that serum cystatin C in early pregnancy was significantly higher in those who subsequently developed pregnancy induced hypertension as compared to control group (0.65 vs. 0.57 mg/L, $p = 0.001$). Saleh S, et al[11], also found raise serum cystatin C in the second trimester of pregnancy in women who subsequently developed preeclampsia when compared to control group (0.76 vs. 0.53 mg/L, $p = 0.008$). These findings suggested that the high level of serum cystatin C might be useful to identify patients at risk for preeclampsia[12].

Serum cystatin C reference ranges were published for both men and non-pregnant women which had a common reference range between the ages of 1 to 50 years[13]. During pregnancy, the renal blood flow progressively increases especially in late gestation which may affect with the level of serum cystatin C. So the normal references in each trimester is needed to determine its clinically significant level. A previous study of the normal serum cystatin C in the first trimester of 48 Thai pregnant women was reported[14]. There were few reports of the normal serum cystatin C level in the second and third trimester that mostly focus on Caucasian population[15,16]. However, it might not suitable to imply this reference values for Thai pregnant women.

The aim of the present study was then to define reference values for serum cystatin C in the second and third

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trimester of uncomplicated Thai pregnant woman.

Materials and Methods

An observational study was conducted at the antenatal clinic, HRH Maha Chakri Sirindhorn Medical Center, Thailand, during August 2016 to July 2017. The study protocol was approved by the local institute ethical review board (SWUEC/E-169/2559) and registered to Thai Clinical Trials Registry (TCTR20161121001). The participants in the present study were Thai pregnant women who attended antenatal care during 14 to 40 weeks of gestation as calculated by the first day of the last menstrual period or by ultrasound dating scan.

Inclusion and exclusion criteria

A total of 140 Thai pregnant women age between 18 to 40 years old was recruited (70 women in each trimester; during 14 to 27 weeks of gestation and 28 to 40 weeks of gestation respectively) 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 second antenatal blood test. All blood samples were allowed to clot and centrifuged. The serum samples were frozen at -80 degree Celsius until analysis.

Assay of cystatin C

Each serum sample was measured 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 0.14 mg/L of cystatin C in the second trimester from a previous report[17]. Assuming a two-sided alpha level of 0.05, the number needed in each group of participant was 62 women. Demographic data is presented as percentages, mean and standard deviation (SD) as appropriate. The independent t-test was used to compare continuous variables. The Pearson's correlation coefficient was calculated.

Results

A total of 140 pregnant women met the inclusion criteria. Sixty-two cases in second trimester group and seventy cases in third trimester group were noted. Eight cases were excluded as follows: 3 cases of late obstetrics problem, 3 cases of discover medical problem, and 2 cases of missing data. The demographic data were presented in Table 1. The participants' age range was 19 to 40 years old. Thirty-seven percent of second trimester group and fifty-one percent of third trimester group were primigravida. All the participants had normal blood pressure and did not have known renal disease.

The reference intervals for serum cystatin C in second and third trimester of normal individual pregnant women were 0.41 to 0.94 mg/L and 0.43 to 1.33 mg/L respectively. The overall mean and standard deviation of serum cystatin C in second and third trimester were 0.62±0.13 mg/L and 0.80±0.18 mg/L respectively. The mean serum cystatin C had a trend to increase significantly during the study period as presented in Table 2. The mean and standard deviation of serum creatinine level in second and third trimester were 0.48±0.10 mg/dL and 0.40±0.09 mg/dL

Table 1. Demographic data of participants

Parameters	Second trimester (n = 62)	Third trimester (n = 70)
Age (years)	29.4±5.6	27.3±5.9
Primigravida (percent)	23 (37.1)	36 (51.4)
Multigravida (percent)	39 (62.9)	34 (48.6)
Body weight (kilograms)	60.9±12.1	57.6±13.3
Height (centimeters)	160.1±5.8	157.9±5.2

Table 2. The mean (±SD) serum cystatin C and creatinine level in each trimester

	Second trimester (n = 62)	Third trimester (n = 70)	*p*-value*
Serum cystatin C (mg/L)	0.62±0.13	0.80±0.18	<0.001
Serum creatinine (mg/dL)	0.48±0.10	0.40±0.09	<0.001

* Independent t-test

respectively. The distribution of serum cystatin C levels and serum creatinine levels in each trimester was shown in Figure 1. There was no significant correlation between serum cystatin C level and serum creatinine (r = 0.04, *p*-value = 0.61).

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[18]. Previous studied showed evidences of it usages to predict preeclampsia[10,19], gestational diabetes[4,20] and fetal growth restriction[5] in second trimester of pregnancy.

The measurement of serum cystatin C in this study was 0.62±0.13 mg/L in second trimester and increased to 0.80±0.18 mg/L in third trimester, which was comparable to a previous report by Babay Z, et al[17]. They studied in 197 healthy Saudi Arabian pregnant women and found serum cystatin C levels in second and third trimester were 0.65±0.14 mg/L and 0.82±0.19 mg/L respectively. While other researchers reported higher normal values of cystatin C than our results. For example, Obrenovic R, et al[15], reported serum cystatin C level of 0.78±0.26 mg/L for second trimester and 1.21±0.30 mg/L for third trimester in 109 uncomplicated Serbian pregnant women. Jia L et al[21], studied in 623 Chinese pregnant women and found serum cystatin C level of 0.75±0.16 and 1.19±0.23 mg/L in second and third trimester, respectively. Discrepancy in outcome between previous findings and those of the present study were due to the different population, different inclusion criteria especially gestational age, and using different method of measurement (latex particle enhanced immune turbidity assay). However, it was shown that mean serum cystatin C raised progressively from the second to the third trimester. Physiological changes during pregnancy and/or inconstant cystatin C production might play a role in this findings.

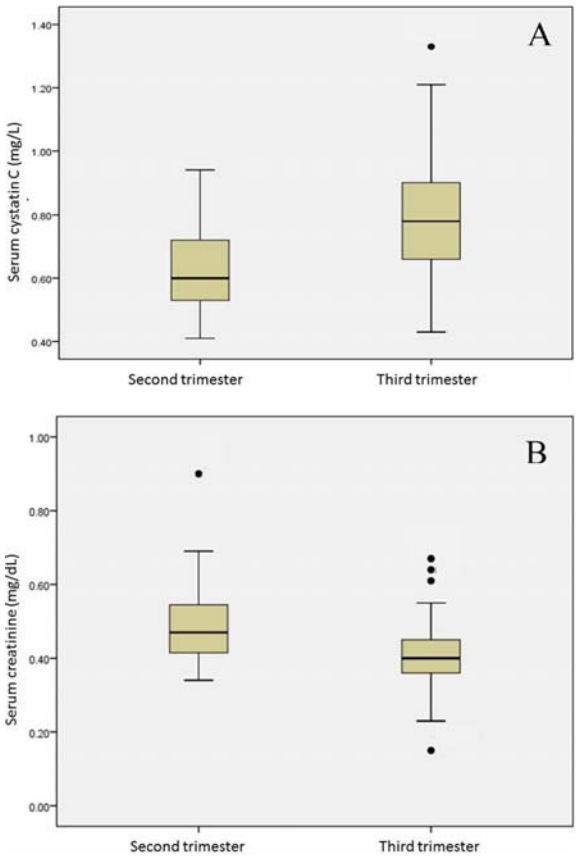

Figure 1. Graphs show box plot of serum cystatin C (A) and serum creatinine (B) levels in each trimester.

Most of obstetrics risks commonly occur during second and third trimester which early diagnosis and treatment can decrease its severity or morbidity. Our results also showed that serum cystatin C had a trend to increase in late pregnancy. So we need to know the normal reference values of cystatin C during this period then we can identify the patients who need special antenatal care. 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 pregnancy. This confirmed that serum cystatin C was not affected by the creatinine levels.

Limitations of this study were the descriptive design, small number of participants, lack of pre-pregnancy cystatin C level for comparison, and lack of clinical correlation. Further study in larger number of participants and specific obstetrics conditions should be considered.

Conclusion

The normal reference values of serum cystatin C in the second and third trimester of Thai pregnant woman were established.

What is already known in this topic?

Serum cystatin C is a marker of renal function in pregnancy. Cystatin C has a significant role in placental formation and may be affected by certain obstetrics condition. There is a possibility to use serum cystatin C for prediction of preeclampsia.

What this study adds?

The present study showed the normal reference value of serum cystatin C during second and third trimester in uncomplicated Thai pregnant women. Serum cystatin C differed significantly when divided into each trimester.

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Potential conflicts of interest

The authors declared no conflicts of interests.

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