doi.org/10.36721/PJPS.2020.33.5.SP.2445-2448.1

Reference intervals for glomerular filtration function markers among pregnant women of Shandong Province, east China

Yanqiu Xu[1#], Shiqing Cheng[1#], Yan Su[1], Bin Liu[2] and Fang Luan[1]*

[1]Department of Clinical Laboratory, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Shandong, China
[2]Department of Biomedical Engineering, Shandong Provincial Hospital affiliated to Shandong First Medical University, Shandong, China

Abstract: The aim of the study was to establish reference intervals (RIs) for glomerular filtration function markers among pregnant women of Shandong Province, east China. From Janunary 2017 to December 2018, we retrospectively analyzed serum samples from 360 pregnant women and a control cohort of 60-non-pregnant women. The glomerular filtration function markers included Cystatin C (CysC), Creatinine (Cr) and Estimated Glomerular Filtration Rate (eGFR). BeckmanAU5800 detection system was used to determine the serological level of CysC by immunonephelometry method and Cr by enzyme method, eGFR was calculated according to age, gender and Cr results. We calculated the RIs according to the guidelines in C28-A3 published by the Clinical and Laboratory Standards Institute (CLSI). The calculated RIs for serum CysC were (0.40-0.67) mg/L, (0.5-0.85) mg/L, (0.77-1.49) mg/L in 1st, 2nd, and 3rd trimester respectively. Cr were (37.26-57.47) μmol/L, (33.70-54.82) μmol/L, (33.66-62.69) μmol/L in each cohort. eGFR based on Cr were (115.24-140.05) ml/min per 1.73m^2, (117.42-141.88) ml/min per 1.73m^2, (109.00-146.00) ml/min per 1.73m^2. The results show the necessity to establish special RIs for glomerular filtration function markers during pregnancy, even in each trimester. CysC levels increase obviously, so we also should cautiously treat it in the three trimesters.

Keywords: Reference intervals, pregnancy, cystatin C, glomerular filtration rate, creatinine.

INTRODUCTION

Plasma volumes of pregnant women increase from the early stage of pregnancy (6~8 weeks) to the late stage of pregnancy (32~34 weeks), with an increase of 30%-45% and an average increase of 1500 ml during pregnancy (Belzile M 2019). The high level of plasma volume is maintained until delivery (Soma-Pillay P, 2016). The progressive expansion in plasma volume throughout normal pregnancy functionally impact on kidney physiology. More seriouly, preeclampsia (the hypertensive disorder unique to pregnancy) (Paauw ND, 2016), pregestational diabetes (Gonzalez Suarez ML, 2019), and other complications of pregnancy also directly or indirectly lead to the increase of renal burden of pregnant women (Sugrue R, 2018).

The ability to estimate glomerular filtration function is critical in the care of the pregnant patient. How to correctly evaluate the glomerular filtration function of pregnant women and how to correctly diagnose and treat the kidney disease of pregnancy are the problems that clinicians must consider. The aim of this study is to determin(e) reference intervals (RIs) for specific glomerular filtration function indexes including Cystatin C (CysC), creatinine (Cr), and Estimated Glomerular Filtration Rate (eGFR) in 1st, 2nd and 3rd trimester that are suitable for clinical use, by sampling normal pregnancy women in Shandong Province, and by following specific rules for establishing RIs (CLSI, 2008).

MATERIALS AND METHODS

Study design and patients selection

Retrospectively collected 30,807 pregnant women seen for prenatal care during January 2017 to December 2018 from Shandong Provincial Hospital, which has east and west sections. Three-hundred sixty normal pregnant women were screened and the exclusion criteria included familial hypertension or diabetes, renal disease or on current regular medications other than vitamin supplements, hyperemesis of pregnancy, gestational hypertension or diabetes, prematurity, threatened abortion. The pregnant women were divided into 1st trimester (1~12 weeks, n=120, 20-39 years old), 2nd trimester (13~27 weeks, n=120, 22-40 years old) and 3rd trimester (28~40 weeks, n=120, 21-39 years old). Sixty healthy non-pregnant women were recruited from the health examination center. Selection criteria of healthy non-pregnant women included no history of infertility, normal menstrual cycle, no familial hypertension or diabetes, no past history of kidney or gynecological disease. All the participants were Asian and belong to the Han population. This work was done with the approval (ethical approval number is NSFC: No.2020-226) of the Medical Ethics Committee of Shandong First Medical University in accordance with the Declaration of Helsinki.

*Corresponding author: e-mail: luanfang2004@126.com

#These two authors contributed equally to this work

Laboratory evaluation

Peripheral blood and urine were collected from pregnant and non-pregnant women on an empty stomach. The levels of Cr, glucose and alanine amino transferase (ALT) were assayed with enzymatic method, and CysC was measured with latex particle enhanced immune turbidity assay using the Beckman AU5800 system original matching reagent (Beckman, U.S.A.). The eGFR based on creatinine was calculated using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation (Levey AS, 2009). The Cr calibration can be traced to Isotopic dilution mass spectrometry (IDMS) and CysC calibration can be traced to referencematerials ERM-DA471/IFCC. The total imprecision of Cr and CysC was 5.00% and 8.33% respectively. Hemoglobin was assayed by full-automatic blood analyzer (Mindray, China). Serum was separated to measure the expression of ferritin with electrochemical luminescence method using the Beckman immuno-assay system (Beckman, U.S.A.). Urine protein level was tested using dry chemical test paper (FUJIFILM-7000, Japan). ALL the testing items passed the quality assessment of National Clinical Laboratory. Cr also passed RIQAS (Randox, UK).

STATISTICAL ANALYSIS

Statistical analyses were performed using GraphPad Prism Software (Version 5.0) (La Jolla, CA, USA). The required number of inclusions in 1st, 2nd and 3rd trimester was respectively 120 pregnant women. Shapiro-Wilk normality test was used for normality test. Because not all of the data were normally distributed, we present all data as median (Q1, Q3) and use Mann-Whitney U test for comparison between groups. 95% confidence interval (P 2.5 ~P 97.5) was used as RIs (CLSI, 2008). All *p*-values were two-tailed and *p*-values less than 0.05 were considered significant.

RESULTS

Description of the subjects

One-hundred twenty pregnant women (respectively in 1st, 2nd, and 3rd trimester) and 60 non-pregnant women (control cohort) met our inclusion criteria and were eligible for the final analysis. The clinical characteristics of all the participants are presented in table 1. The levels of hemoglobin (U=1550, 667.0, 1563 respectively, $p<0.0001$) and ferritin (U=2184, 403.0, 315.0 respectively, $p<0.0001$) are significantly lower in 1st 2nd and 3rd trimester respectively compared with control cohort.

Reference intervals for eGFR, Cr and CysC in 1st, 2nd, and 3rd trimester

The mean or medians and RIs for CysC, Cr and eGFR are shown in table 2. CysC levels in 1st trimester were significantly lower than normal cohort (U=584.0 $p<0.0001$), while the levels in 3rd trimester were higher than normal cohort (U=1259 $p<0.0001$). The levels of Cr in 1st, 2nd, and 3rd trimester were lower than normal cohort (U=815.5, 408.5, 2243 respectively, $p<0.0001$), while eGFR levels were significantly higher than control cohort (U=1656, 1163, 246.5 respectively, $p<0.0001$). The statistical results revealed significantly difference of CysC, Cr and eGFR levels between 1st and 2nd (U=1068 $p<0.0001$, U=4923 $p<0.0001$, U= 5793 $p=0.0088$), 2nd and 3rd trimesters (U=57.5 $p<0.0001$, U=4966 $p<0.0001$, U=5175 $p=0.0002$). However, there is only significantly difference of CysC level between 1st and 3rd trimesters (U=1 $p<0.0001$).

Reference intervals comparison with data from other studies

RIs for pregnant women from our study compared with other studies are shown in table 3. The similar trends were observed, but there were differences in specific reference interval values.

DISCUSSION

Serum creatinine, produced at a constant rate, is dependent on muscle mass and varies little from day to day (Cockcroft DW, 1976). The proteinase inhibitor CysC, produced at a constant rate by nucleated cells, is freely filtered and almost entirely reabsorpted by the tubules (Massey D, 2004). Cr and CysC appear to be accurate markers of glomerular filtration in nonpregnant women and have been proposed to accurately reflect glomerular filtration in pregnant women. In this study, we establisehed new RIs for serum CysC Cr and eGFR levels based on 120 blood samples respectively in 1st, 2nd and 3rd trimester among pregnant women in Shandong Province (table 1), and compared the levels with non-pregnant women (table 2).

A previous study based on 623 pregnant women has already aimed to define reference ranges for Cr and CysC in West China (Jia L, 2017). Another study from Canada measured serum creatinine and CysC concentrations on normal pregnant women in the first trimester (n=5), second trimester (n=68) and third trimester (n=64) (Kreepala C, 2019). Although we improved the accuracy of the measurement, mainly because of our matching detection system and larger sample size, the standard deviation still remained high, due to the physiological variation in serum CysC and Cr concentrations after pregnacy. We made comparions of these data in table 3 and the different detecting system between the three centres could have partially explained differences. The source of difference maybe also come from human races and populations. For instance, in China where people eat a very healthy mostly wheat and vegetable-based diet, cholesterol values are significantly lower than that in the Western World. And the diet is usually rich in seafood in east China compared with west China.

Table 1: Clinical characteristics of pregnant and non-pregnant women included in the investigation

Parameter		Pregnant women		Non-pregnant women
				Control cohort (n=60)
	T1 (n=120)	T2 (n=120)	T3 (n=120)	
Age (years)				
Median (Q1, Q3)	29 (27, 33.75)	30 (28, 34)	29.5 (28, 34)	29 （26,32）
Urine protein	negative	negative	negative	negative
Hemoglobin(g/L)				
Median (Q1, Q3)	124 (115, 131)*	117 (112, 124)*	122.5 (115, 131)*	134 (127, 139)
Ferritin (ng/mL)				
Median (Q1, Q3)	23.36 (8.83, 44.32)*	9.49 (6.24, 16.50)*	11.05 (7.01, 17.90)*	40.17 (33.17, 45.17)*
Glucose(mmol/L)				
Median (Q1, Q3)	4.89 (4.71, 5.13)	4.82 (4.58, 5.05)	4.63 (4.42, 4.83)*	4.94 (4.67, 5.25)
ALT(U/L)				
Median (Q1, Q3)	11 (8, 17)	13.5 (10, 20)*	9 (8, 12)	10 (8, 14)

Statistically significant difference of 1st, 2nd, or 3rd trimester in comparison to control cohort, * p<0.0001

Table 2: Medians and reference intervals for eGFR, Cr, and CysC in T1, T2 and T3 group

Parameters		Median (reference intervals)		
	T1	T2	T3	control cohort
Cysc(mg/L)	0.51 (0.40-0.67)$^{*,\#\#}$	0.67 (0.50-0.85)$^{\$\$}$	1.12 (0.77-1.49)$^{*,\&}$	0.70
Cr（μmol/L）	47 (37.26-57.47)$^{*,\#\#}$	44 (33.70-54.82)$^{*,\$\$}$	48 (33.66-62.69)*	57
eGFR (ml/min per 1.73 m^2)	127 (115.24-140.05)$^{*,\#}$	130 (117.42-141.88)$^{*,\$}$	126 (109.00-146.00)*	120.00

1st, 2nd, or 3rd trimester versus control cohort: *, p<0.0001. 1st trimester group versus 2nd trimester group: #, p<0.01. ##, p<0.0001.
1st trimester group versus 3rd trimester group: &, p<0.0001.
2nd trimester group versus 3rd trimester group: $, p<0.001. $$, p<0.0001.

Different areas showed the different intervals, indicating we need calculate RIs for each region. And the new established Cysc and Cr RIs for pregnent women were different from current RIs in use for healthy female adults. However, decision limits for effective management should be determined by further studies which specify the diagnostic values of these indexes.

We demonstrate increasing CysC concentrations throughout pregnancy. The CysC levels in the 2nd trimesterare very are very close to thoes of normal non-pregnant women, while CysC levels in the 3rd trimester are significantly higher. But the increased CysC levels in the 3rd trimester are still lower than CysC levels [1.4±0.2 mg/L (0.99-1.86)] reported in preeclampsia (Krittanont Wattanavaekin, 2018). The change of CysC is in contrast to the changes of serum Cr and eGFR in this study. In the process of pregnancy, eGFR and serum Cr increase and decrease in a corresponding manner. CysC is reported to be superior to Cr for reflecting renal function (Wei L, 2015). The increased production of CysC in the 3rd trimester may be due to the alteration of glomerular barrier charge, but the mechanism requires further exploration. CysC seems very sensitive as a marker of glomerular filtration function in pregnancy. Therefore, we should critically consider its increase in 3rd trimester. Nonetheless, measurement of CysC may prove to be a useful clinical tool in preeclampsia (Krittanont Wattanavaekin, 2018).

Multi-center reference intervals research is the most important progress in developing RIs. Although the samples were collected from east and west sections of Shandong Provincal Hospital. The limitations of the study is lack of coastal and Mountain population.

Pregnancy affects essentially all aspects of kidney physiology (Katharine L. Cheung, 2013). Obstetric nephrology is truly an interdisciplinary effort, and good outcomes are contingent on collaboration among obstetricians, nephrologists, rheumatologists, and endocrinologists. Our study could be helpful for clinicians to monitor renal function throughout normal pregnancy.

CONCLUSION

In this study, we established reference intervals for serum CysC, Cr and eGFR of pregnant women in 1st, 2nd and 3rd trimester respectively in Shandong Province, east China. More attentions should be paid to glomerular filtration

Table 3: Reference intervals of Cr and CysC compared with data reported in the literature

Parameters	Method	Reagent	Detecting system		Reference Intervals	
				T1	T2	T3
Cysc (mg/L)	immune turbidity assay	Beckman USA	Beckman CoulterAU5800	0.40-0.67	0.5-0.85	0.77-1.49
Cysc (mg/L) Current RI				0.54-1.15		
Cysc (mg/L) published Jia L,2017	immune turbidity assay	Siemens, Germany	Siemens Centaur 2400	0.56-0.84	0.59-0.91	0.96-1.42
Cysc (mg/L) published Ayub Akbari,2005	immune turbidity assay	Dade Behring, Mississauga, ON, Canada	Not noted	-	0.48-0.89	0.46-1.35
Cr (μmol/L)	enzymatic assay	Beckman USA	Beckman CoulterAU5800	37.26-57.47	33.7-54.82	33.66-62.69
Cr (μmol/L) Current RI				40-135		
Cr (μmol/L) published Jia L,2017	enzymatic assay	Dade Behring, Germany	Siemens Centaur 2400	37.35-54.45	35.91-53.21	41.02-64.54
Cr (μmol/L) published Ayub Akbari,2005	Not noted	Not noted	Not noted	-	39-69	40-78

RI: Reference Interval

function in consider of the RIs differences between pregnant women and non-pregnant women. Meanwhile, a greater emphasis needs to be placed on CysC levels in the three trimesters.

ACKNOWLEDGEMENT

This work was financially supported by grants from Key Development Program of Shandong Province (Grant No. 2018GSF118116), National Natural Science Foundation of China (Grant No. 81101484) and Medical, Health Science and Technology, Development Plan of Shandong Province 2017WS030, China.

REFERENCES

Belzile M, Pouliot A, Cumyn A and Cote AM (2019). Renal physiology and fluid and electrolyte disorders in pregnancy. *Best Pract. Res. Clin. Obstet. Gynaecol.,* **57**: 1-14.

Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A (2016). Physiological changes in pregnancy. *Cardiovasc J. Afr.,* **27**(2): 89-94.

Paauw ND, Joles JA, Drost JT, Verhaar MC, Franx A, Navis G, Maas AH and Lely AT (2016). High-normal 10 years women rate in early-onset preeclamptic filtration glomerular estimated postpartum. *Hypertension,* **68**(6): 1407-1414.

Gonzalez Suarez ML, Kattah A, Grande JP and Garovic V (2019). Disorders renal pregnancy: Core curriculum 2019. *Am. J. Kidney Dis.,* **73**(1): 119-130.

Sugrue R, Zera C (2018). Pregestational diabetes in pregnancy. *Obstet. Gynecol. Clin. North. Am.,* **45**(2): 315-331.

CLSI. EP28-A3C. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. Clinical and Laboratory Standards Institute. Wayne, PA, 2008 (77pp. CLSI document)

Levey AS, Stevens LA, Schmid CH, Zhang YL,Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T and Coresh J (2009). CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann. Intern Med.,* **150**(9): 604-612.

Cockcroft DW and Gault MH (1976). Prediction of creatinine clearance from serum creatinine. *Nephron,* **16**(1): 31-41.

Massey D (2004). Commentary: Clinical diagnostic use of cystatin C. *J. Clin. Lab Anal,* **18**(1): 55-60.

Jia L, Yongmei J, Leiwen P and Yi Y (2017). The reference intervals for renal function indexes in chinese pregnant women. *Pak. J. Pharm. Sci.,* **30**(3(Special)): 1133-1138.

Kreepala C, Srila-On A, Kitporntheranunt M, Anakkamatee W, Lawtongkum P and Wattanavaekin K (2019). The pregnancy and proteinuria during cystatin serum association between GFR evaluated. *Kidney Int. Rep.,* **4**(6): 854-863.

Wattanavaekin K, Kitporntheranunt M and Kreepala C (2018). Cystatin C as a novel predictor of preterm labor in severe preeclampsia. *Kidney Res. Clin. Pract.,* **37**(4): 338-346.

Wei L, Ye X, Pei X, Wu J and Zhao W (2015). Diagnostic accuracy of serum cystatin C in chronic kidney disease: A meta-analysis. *Clin. Nephrol.,* **84**(2): 86-94.