The reference intervals for renal function indexes in chinese pregnant women

Li Jia, Jiang Yongmei, Peng Leiwen and Yang Yi
The Clinical Laboratory, West China Second University Hospital, Sichuan University

Abstract: The purpose of the present research was to establish reference intervals for renal function indexes (Cystatin C, Blood Urea Nitrogen Creatinine and Uric Acid) in pregnant women in China. Blood samples were collected from 203 healthy non-pregnant women and 623 healthy pregnant women in different trimesters. Samples from 23 healthy consecutive pregnant women were collected to validate. Serum Cystatin C was measured by latex particle enhanced immune turbidity assay (Dade Behring, Germany) using Centaur 2400 (Siemens, New York, USA). Creatinine, Blood Urea Nitrogen and Uric Acid were measured by conventional enzymatic assay using a Centaur 2400 (Siemens, New York, USA). The calculated reference intervals for serum cystatin C were (0.76±0.12) mg/L, (0.70±0.14) mg/L, (0.75±0.16) mg/L, (1.19±0.23) mg/L in control group, 1^{st} trimester, 2^{nd} trimester and 3^{rd} trimester, respectively. Creatinine were (54.21±7.49) µmol/L, (45.90±8.55) µmol/L, (44.56±8.65) µmol/L, (52.78±11.76) µmol/L in each group. Blood Urea Nitrogen were (4.74±1.24) mmol/L, (3.52±1.44) mmol/L, (3.81±2.32) mmol/L, (3.95±1.54) mmol/L. And Uric Acid were (281.35±63.61) µmol/L, (226.93±25.20) µmol/L, (252.39±62.32) µmol/L, (350.86±93.31) µmol/L. In addition, all the data of consecutive pr egnant women were in the central 95% intervals. The results show the necessity to establish the special reference intervals for pregnancy, even each trimester. Different areas show the different intervals, indicating we need own ones in our region, and we provided. And Cystatin C is the most stable between these indexes. But we also should cautiously treat it in the 3st trimester.

Keyword: Renal function, pregnancy, reference intervals, Cr, BUN, UA, Cys C.

INTRODUCTION

Pregnancy, a complex process, will lead to a change in the function of the body, such as the renal function, due to the changing of blood volume, hormone and BMI. Measures of renal function include Glomerular filtration rate (GFR), Creatinine (Cr), Uric Acid (UA), Blood Urea Nitrogen (BUN), and Cystatin C (Cys C). Cr is observed as the routine biomarker of GFR, which is thought to completely across the glomerulus. However, Cr generation is affected by dietary intake and muscle mass, which caused the prediction less. All nucleated cells could produce and excrete Cys C (Grubb, 1992). It is only existed in the extracellular volume, and all cleared by glomerular filtration without tubular secretion (Siew and Ware, 2009) with short time (Koyner *et al*., 2008) and protect as a lysosomal proteinase inhibitor out of the cells, besides as a cysteine protease inhibitor in ones (Zappitelli *et al*., 2007). Also, Cys C concentrations in plasma increase after acute change in the GFR are faster and reach steady state sooner than Cr (Grubb, 1992), so it is more sensitive than Cr to reflect GFR (Xu *et al*., 2006). Some scholars suggest that Cys C could be better than others (Yashiro *et al*., 2009). BUN as the end-product of protein metabolism is excreted through kidney (Willems *et al*., 2009). UA is the end-product of purine metabolism and 2/3 excreted through renal, and 1/3 through intestinal tract (Filler *et al*., 2005). It is also used to observe renal function in clinical practice (Katharine, 2013). Above all, these indexes are well applied in clinical diagnosis (Filler *et al*., 2005). But how, and when or which index happen detail in pregnancy (Uhlmann *et al*., 2001)? And there is little information about the reference intervals for these biomarkers in a large healthy pregnancy in our area (Finney *et al*., 2000). Therefore, we study the reference ranges with health pregnancies, especially, in our region, Chengdu, Sichuan of China, and would like to be applied in other hospitals and diagnose more exactly (Erlandsen *et al*., 1998).

**Corresponding author:* e-mail: huntercet@qq.com

MATERIALS AND METHODS

Samples were collected from 623 pregnant women who visited prenatal clinic at West China Second University Hospital of Sichuan University, China, between March 2010 and October 2013. The inclusion and exclusion criteria were as follow.

The inclusion criteria of pregency

Prim gravid, singleton, and without the diseases as diabetes mellitus, renal disease, hypertension, and liver disease. Then we set up 4 groups: 1. Gestation age between the 10th and 14th week were divided into 1st trimester; 2. 20th -24th into the 2nd trimester; 3. 30th - 34th into the 3rd trimester; and 4. Post pregnant. Foliate, vitamin B12 and iron supplements were taken in all these women during pregnancy

The exclusion criteria of pregency

Systolic pressure>140 mmHg or diastolic pressure >90 mmHg, multiple pregnancy, hypertensive disorders of pregnancy, proteinuria, intrapartum haemorrhage, and pre-term delivery, post-term pregnancy. The other exclusion criteria were an abnormal birth weight (52,500 g or 44,000g), or fetal growth disorders, as Apgar score <8.

The criteria of non-pregnancy: 203 healthy non-pregnant women were selected as controls according the following criteria: no history of pregnancy and chronic diseases, for example, diabetes, anemia, liver disease, hypertension, and renal disease. Therefore, they were selected as controls. Since our study is a retrospective one, no ethical issues that need to be approved by the ethics committee of the hospital are involved.

Validation population

21 consecutive pregnant women from our hospital and all the inclusion, exclusion and other criteria were absolutely as the same to the study population. The women were evaluated during each period as we mentioned above.

Sample collected

All women who were involved in the study should have 30-minute rest in a sitting position before blood samples were obtained [including each group, and the non-pregnant controls (the 20th - 26th day of their menstrual cycle)]. And then, Cys C, BUN, Cr, UA were evaluated from a blood sample, which kept in a Vacutainer tube containing coagulator (Becton-Dickinson, Franklin Lakes, NJ USA). And the centrifugation is 10 minutes at 2500 ×g. Then, take 1 mL of plasma to store at -80°C until examination for the concentration.

The quality control laboratory

The laboratory undertakes all texts, which at the Department of Laboratory Medicine, West China Second University Hospital, Sichuan University, Chengdu, China. Both the National System of External Assessment of the Quality of Results and the College of American Pathology accepts this laboratory.

Test

Cr, BUN and UA were measured by conventional enzymatic assay, using a Centaur 2400 (Siemens, New York, USA) with strict quality control processes, the fitting calibrator and corollary regents.

CysC was measured by latex particle enhanced immune turbidity assay, using Centaur 2400 (Siemens, New York, USA), with strict quality control processes, the fitting calibrator and corollary regents.

STATISTICAL ANALYSIS

Q-Q diagram was used to check the normality. The Dunnets (T3) test was used to compare values between the controls and others. One-Way ANOVA was used to compare the values for the intra group between pregnancies from each time in our study. P<0.05 was considered significant. All data were analyzed by the SPSS, version 19.0 (SPSS, Chicago, IL, USA).

RESULTS

Study population

We present the basic statistical data of the 623 pregnancies (experimental subjects) and that of the 203 non-pregnancies (controls) in table 1. All subjects were healthy and had normal basic indexes. As for age distributions, the mean and range of ages were as follows. Control group, 29.38 years (21-44); 1st trimester, 28.27 years (20-44); 2nd trimester, 28.38 years (21-44); and 3rd trimester, 29.91 years (20-42). It is shown that most of the childbearing ages were covered with no significant difference in each group (P>0.05), which implies that the subjects are representative. The renal function indexes we studied do not depend on body mass index (BMI)[12], although pregnant women's BMI were higher than non-pregnancies (P<0.01). Other physiologic indexes such as blood pressure and fast plasma glucose (FPG) were all within the intervals we expected. To guarantee the validity of our experimental data, we specially eliminate factors, which may disturb the renal function indexes, such as gestational hypertensive disorders and diabetes mellitus. More detailed figures are provided in table 1, showing that the groups match with each other in terms of age, BMI, fasting blood glucose and blood pressure.

The change of renal function indexes

Comparing with the control (non-pregnancy) group, we examined how the indexes of pregnant subjects in the experimental groups changed in different period of pregnancy. The concentrations of Cys C, BUN, Cr and UA decreased in the early periods of pregnancy (1 or/and 2 trimester), and then increased till delivering (fig. 1). Specifically, Cys C decreased in the 1st trimesters with statistical significance (P<0.01) but showed no statistical difference in the 2nd trimester (P>0.05); then it increased in the 3rd trimester with statistical significance (P<0.01). Cr levels in the 1st and 2nd trimesters were less than the control group (P<0.01), but no statistical difference was found in the 3rd trimester (P>0.05). BUN decreased in the first trimester (P<0.01) and increased in the following periods, but it was always lower than that in control group (P<0.01). UA levels in all trimesters were different from that in the control group (P<0.01).

Abbreviation: The goup labels "1" to "4" in the x-axis refers respectively to the control group, 1st trimester, 2nd trimester and 3rd trimester. Abnormal values are marked with five-pointed asterisks "*" and scattered points are marked with circles "○". A double six-pointed asterisk "**" means statistical difference. Graph A shows the

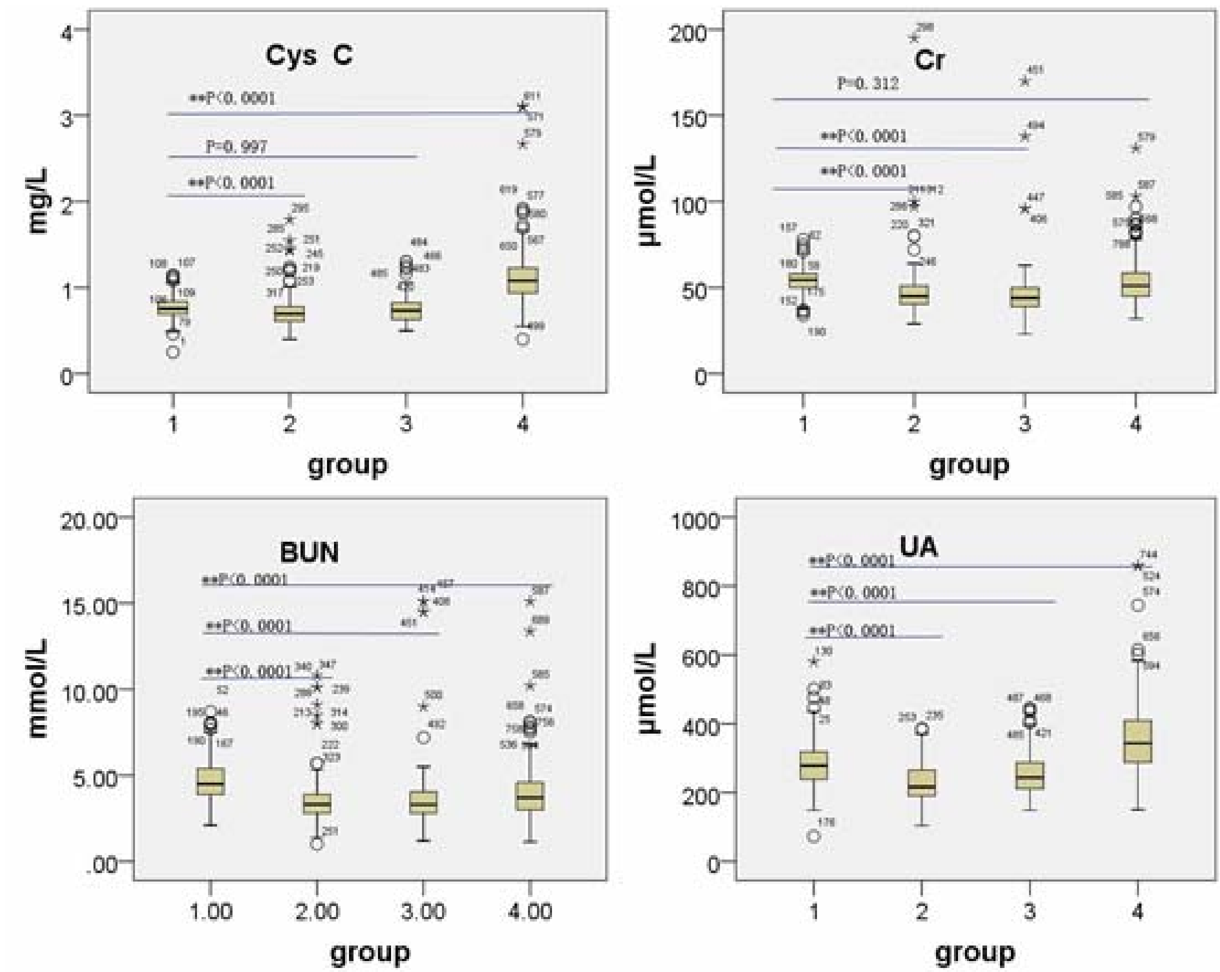

Fig. 1: Distributions and changes of Cys C, Cr, BUN and UA.

Table 1: The basic indexes of pregnancies and non-pregnancy

Index	10-14weeks (n=178)	20-24weeks (n=137)	35-40 weeks (n=308)	Control (n=203)	P value
Age, y	28.27±4.71	28.54±5.65	29.33±6.035	29.38±4.657	0.099
BMI	20.42±2.87	20.50±2.71	22.31±3.82	20.58±3.41	0.000
Blood pressure:	mmHg				
Systolic	110.92±5.88	111.17±5.114	110.58±7.25	110.33±7.13	0.989
Diastolic	71.58±8.28	73.42±7.91	773.08±7.28	72.83±7.49	0.943
FPG	4.26±0.853	4.37±0.99	4.47±0.95	4.52±0.94	0.962

Abbreviation: BMI, body mass index, calculated as weight in kilograms divided by height in meters squared; FPG, fasting plasma glucose. All values are means ±SD unless otherwise indicated.

concentations of Cys C in each group, with the 1st and 3rd trimester groups having significant differences (p<0.0001) compared with the control group. Graph B illustrates the concentrations of Cr and shows significant difference in the 1st and 2nd trimester groups compared with the control group (p<0.0001). Graph C presents the concentrations of BUN, from which significant differences are observed between the control group and each experimental group. Graph D provides the concentrations of UA in each groups, showing significant difference in each experimental groups in comparison with the control group.

Reference values

The indexes all were subjected to Gaussian distribution. The reference values according to each group are showed in table 2, which were calculated after eliminating the abnormal data.

The validation

The indexes for 21 consecutive pregnant women in each trimester compared with the central 95% interval of our results, showing our ranges were available because the validation were all in the reference intervals our established.

DISCUSSION

As we known, kidney structure is mostly affected during gestation, so that renal function will change more or less in this period (Galteau MM, Guyon M, Gueguen R and Siest G, 2001). To the best of our knowledge, only a few studies show the difference about the renal function indexes between healthy pregnant and non-pregnant women, especially, less to establish the reference ranges of Cys C, BUN, Cr, and UA in pregnancy in our area. In our study, we accomplished them.

Our study included 203 healthy women as control, and 623 pregnant women (respectively, 1st trimester-178s, 2nd trimester-137s and 3rd trimester-308s). Table 1 shows age, blood pressure, and serum glucose are no difference between control and pregnancy, suggesting the experience matching age, fasting blood glucose and BP, and also indicating we excluded the most related interference factors. Our values were in expected intervals for healthy pregnant women or non-pregnant women. And the renal function indexes, which we studied, do not depend on body mass index (Larsson *et al*., 2008), even though pregnant women's BMI were higher than non-pregnancies (P<0.01) (Obrenovic *et al*., 2011).

As expected, our present results of Cys C, BUN, Cr, UA levels all have difference between pregnant and non-pregnant women. All of them decrease at the 1st trimester and then increase as follow, although those in 2nd trimester are still lower than control. The reasons are quite complicated. The kidney structure changing, blood volume increasing and special hormone releasing during gestation could cause the GFR increase and Cys C, Cr, BUN, and UA dilution (Hans *et al*., 2008). Lately, these metabolites increase as pregnant women metabolic growing. However, the differences are ignored in clinical in our country, where the reference intervals for pregnant women are still the general ones, including both pregnancy and non-pregnancy. Our results showed the concentrations of these indexes in pregnancy were changed against non-pregnancy. If the reference intervals in non-pregnant women are still used in pregnancy, many misdiagnoses, missed diagnosis, or error therapy will come. Moreover, it is essential to establish its own reference intervals for each laboratory according the IFCC and the National Committee for Clinical Laboratory Standards (now the CLSI) (Mussap *et al*., 2002). But all the processing, including the clinical characterization of proper populations and the selection for the establishment of reference values, is time-consuming, and will take more money (Gabriel *et al*., 2012). It is difficult to accomplish in the primary hospitals in China. We have the similar demographic and geographic with them, which could transfer the results from ours after easy validation.

Our present results show Cys C, BUN, Cr, UA levels all have difference between pregnant and non-pregnant women. Therefore, we analysis in detail as follow:

Cys C level in our control group (0.76±0.12)mg/L is higher than Uhlmann *et al*., 2001 reported their Cys C in non-pregnant women the average of 0.65±0.1 mg/L, and lower than Erlandsen *et al*., (1998) reported the median of 0.84 mg/l. And we compare with Larsson *et al*., (2008) who found the increase of Cys C levels during pregnancy (0.5-0.82mg/L in 28-31 weeks, 0.5-0.98mg/L in 31-34 weeks, 0.58-1.30mg/L in 34-38 weeks), and Obrenovic *et al*., (2011) who showed 0.69±0.16 mg/l vs. 0.78±0.26 mg/l vs. 1.21 ± 0.30 mg/l. Comparing the Cr levels with ours in non-pregnant women, Hans *et al*., 2008) shows the similar average of 53.38μmol/L (the reference interval, 36.60-70.92μmol/L). And our Cr level in pregnant women are lower than Obrenovic *et al*., (2011) (59.11±6.71 μmol/l, 66.03±12.32 μmol/l, 65.69±9.54 μmol/l). BUN is more instable than Cr and Cys C in gravidas. Our concentration in non-pregnancy is lower than Oliveira *et al*. (5.55±1.24 mg/L). Oliveira *et al*. researched healthy volunteers included both male and female (mean age ±standard deviation: 29±4 yr, the similar years) in Italy. We suggest that their research contains both male and female, which gender and age can influence the level of BUN, and male's is significant higher than female's (P<0.01). UA is volatile due to being influenced by diets as we all known. Our result of UA shows large and high ranges than non-pregnancies because of pregnant women change of structure of diet and more caloric taking in so that secreting more, especially in 3rd trimester. The levels of the renal function indexes are much different. Gender, age, diet, weight, height and current cigarette smoking could influence the concentrations of the renal function indexes, and even the different local iodine status, equipment and reagents may cause the difference. And one of the reasons may our control group included non-pregnant women who are more restricted than others (who included all healthy general population), and the other reason maybe the different race and region. Even though these levels are different from ours, the general trends are similar to us. So all indicate that different region need the own reference intervals for Cys C, especially in pregnancy. Our research also has the disadvantages which is a cross-sectional study.

The study population is inconsecutive because it is so difficult to collect. However, we considered 21 consecutive pregnant women to validate the results, which are all in the reference intervals of ours, indicating our intervals are available according the NCCLS global consensus guideline. And the population included has its limitations that almost are from Chengdu or peripheral regions in Sichuan, China. So our results may available to the hospitals in Sichuan of China, and hospitals in other region should transfer and validate carefully.

Table 2: the reference values in each group

		study groups		
	Control (n=203)	1st trimester (n=178)	2nd trimester (n=137)	3rd trimester (n=308)
Cys C (mg/L)	0.76[a]	0.70	0.75	1.19
	0.76±0.12[b]	0.70±0.14	0.75±0.16	1.19±0.23
Cr	54.21	45.90	44.56	52.78
(μmol/L)	54.21±7.49	45.90±8.55	44.56±8.65	52.78±11.76
BUN (mmol/L)	4.74	3.52	3.81	3.95
	4.74±1.24	3.52±1.44	3.81±2.32	3.95±1.54
UA	281.35	226.93	252.39	353.86
(μmol/L)	281.35±63.615	226.93±25.202	252.39±62.323	350.86±93.311

Abbreviations: Cys C means cystatin C; BUN: blood urea nitrogen; Cr: creatinine; UA: Uric acid. A showed average of the value; B showed means ±SD; C showed the range of the data (minimum, maximum). All results were calculated after eliminating abnormal data.

Table 3: the validation values in consecutive pregnant women

	consecutive pregnant women (n=21)		
	1st trimester	2nd trimester	3rd trimester
Cys C(mg/L)	(0.56, 0.97)[a]	(0.56, 1.15)	(0.79, 1.35)
	(0.42, 0.97)[b]	(0.52, 1.25)	(0.74, 1.64)
Cr(μmol/L)	(30, 62)	(23, 59)	(37, 76)
	(29.17, 63.55)	(28.12, 61.52)	(28.10, 78.23)
BUN(mmol/L)	(2.10, 5.70)	(2.41, 5.40)	(1.80, 6.10)
	(0.70, 6.34)	(1.31, 8.35)	(0.93, 6.97)
UA(μmol/L)	(174, 340)	(176, 379)	(181, 502)
	(112.93, 341.43)	(128.32, 392.40)	(161.53, 544.98)

Abbreviations: the indexes of renal function for 21 consecutive pregnant women in each trimester. 'a' showed the whole range of the 21 consecutive pregnant women's indexes; 'b' showed the central 95% intervals of our results.

CONCLUSION

It is essential to establish the special reference intervals for renal function in pregnancy. And our results are suitable to apply for Chinese pregnant women in our region of China.

REFERENCES

El-Shafey EM, El-Nagar GF, Selim MF, El-Sorogy HA, Sabry AA (2009). Is serum cystatin C an accurate endogenous marker of glomerular filtration rate for detection of early renal impairment in patients with type 2 diabetes mellitus? *Ren Fail.*, **31**: 355-359.

Erlandsen EJ, Randers E and Kristensen JH (1998). Reference intervals for serum cystatin C and serum creatinine in adults. *Clin. Chem. Lab. Med.*, **36**: 393-397.

Erik J. Uhlmann, Karl G. Hock, Charla Issitt, M. Rhonda Sneeringer, Denise R. Cervelli and Rober T (2001). Gorman, and Mitchell G. Scott, Reference Intervals for Plasma Cystatin C in Healthy Volunteers and Renal Patients, as Measured by the Dade Behring BN II System, and Correlation with Creatinine. *Clinical Chemistry*, **47**: 2031-2033.

Filler G, Bokenkamp A, Hofmann W, Le Bricon T, Martinez-Bru C and Grubb A (2005). Cystatin C as a marker of GFR-history, indications and future research. *Clin. Biochem.*, **38**: 1-8.

Finney H, Newman DJ and Price CP (2000). Adult reference ranges for serum cystatin C, creatinine and predicted creatinine clearance. *Ann. Clin. Biochem.*, **37**: 49-59.

Gabriel Lima-Oliveira, M.S., Gian Luca Salvagno, Giuseppe Lippi, Matteo Gelati, Martina Montagnana, Elisa Danese, Geraldo Picheth, and Gian Cesare Guidi (2012). Influence of a Regular, Standardized Meal on Clinical Chemistry Analyses. *Ann. Lab. Med.*, **32**: 250-256.

Galteau MM, Guyon M, Gueguen R and Siest G (2001). Determination of serum cystatin C: Biological variation and reference values. *Clin. Chem. Lab Med.*, **39**: 850-857.

Grubb A (1992). Diagnostic value of analysis of cystatin C and protein HC in biological fluids. *Clin. Nephrol.*, **38**: S20-S27.

Hans Pottel, Nicolas Vrydags, Boris Mahieu, Emmanuel Vandewynckele, Kathleen Croes, Frank Martens

(2008). Establishing age/sex related serum creatinine reference intervals from hospital laboratory data based on different statistical methods, *Clinica. Chimica. Acta.,* **396**: 49-55.

Katharine LC and Richard AL (2013). Renal Physiology of Pregnancy. *Advances in Chronic Kidney Disease***,** **20**(3): 209-214.

Koyner JL, Bennett MR and Worcester EM *et al* (2008). Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery. *Kidney Int.*, **74**: 1059-1069.

Larsson A, Palm M, Hansson L and Axelsson O (2008). Reference values for clinical chemistry tests during normal pregnancy. *BJOG.*, **115**: 874-881.

Mussap M, Fanos V, Pizzini C, Marcolongo A, Chiaffoni G and Plebani M (2002). Predictive value of amniotic fluid cystaitn C levels for the early identification of fetuses with obstructive uropathies. *Int. I. Obstet. Gynaecol.,* **109**: 778-783.

Nejat M, Pickering JW and Walker RJ *et al* (2010). Urinary cystatin C is diagnostic of acute kidney injury and sepsis, and predicts mortality in the intensive care unit. *Crit. Care,* **14**: R85.

Obrenovic R, Petrovic D, Majkic-Singh N, Trbojevic-Stankovic J and Stojimirovic B (2011). Serum cystatin c levels in normal pregnancy. *Clinical Nephrology,* **76**: 174-179.

Siew ED and Ware LB and Gebretsadik T *et al*. (2009). Urine neutrophil gelatinase associated lipocalin moderately predicts acute kidney injury in critically ill adults. *J. Am. Soc. Nephrol.*, **20**: 1823-1832

Uhlmann EJ, Hock KG, Issitt C, Sneeringer MR, Cervelli DR, Gorman RT and Scott MG (2001). Reference intervals for plasma cystatin C in healthy volunteers and renal patients, as measured by the Dade Behring BN II system, and correlation with creatinine. *Clin. Chem.,* **47**: 2031-2033.

Willems D, Wolff F, Mekhali F and Gillet C (2009). Cystatin C for early detection of renal impairment in diabetes. *Clin. Biochem.,* **42**: 108-110.

Xu H, Lu Y, Teng D, Wang J, Wang L and Li Y (2006). Assessment of glomerular filtration rate in renal transplant patients using serum cystatin C. Transplant Proc., **38**: 2006-2008.

Yashiro M, Kamata T, Segawa H, Kadoya Y, Murakami T and Muso E (2009). Comparisons of cystatin C with creatinine for evaluation of renal function in chronic kidney disease. *Clin. Exp. Nephrol.,* **13**: 598-604.

Zappitelli M, Washburn KK and Arikan AA *et al* (2007). Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: A prospective cohort study. *Crit Care,* **11**: R84.