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CREATININE CLEARANCE AS A MARKER FOR DIAGNOSING THE SEVERITY OF PREECLEMPSIA

Satya Prakash*[1] and Neha Sharma [2]

Department of Biochemistry, Rajendra Institute of Medical Sciences (RIMS), Ranchi, Jharkhand, India
Department of Biochemistry, Shri Ram Murti Smarak Institute of Medical Sciences (SRMSIMS), Bareilly, Uttar Pradesh, India

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Correspondence to Author:

Neha Sharma

Department of Biochemistry, Shri Ram Murti Smarak Institute of Medical Sciences (SRMSIMS), Bareilly, Uttar Pradesh, India

E-mail: neha16.sharma@gmail.com

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ABSTRACT:

Background: For many years, creatinine clearance has become increasingly popular because of several advantages. The major error arises in clearance test when the timing of urine collection is inaccurate and the bladder is incompletely empty.

Aim: In view of the aforementioned controversial literature, it was decided to evaluate the relation-ship between the renal changes (creatinine clearance) in pre-eclamptic, eclamptic, preeclamptic toxeaemic, non-pregnant and normal pregnant women.

Setting and Design: The present investigations were undertaken to study creatinine clearance in normal non-pregnant women, pregnant women in different trimesters, Preeclamptic toxaemic patients and eclamptic patients, between the age group 25-32 years admitted in Rajendra Institute of Medical Sciences, Ranchi during the period from June 2008 to September 2009.

Methods and Material: Control group consisted of 15 normal non-pregnant women, 10 cases in first trimester, 30 cases in 2nd trimester, 20 cases in 3rd trimester, 60 cases of Preeclamptic toxaemia and 20 cases of eclampsia. Creatinine clearance measurements were done by enzymatic method and modified Jaffe's kinetic method.

Statical Analysis: Statistical analysis was performed using GraphPad Prism version 5.00 for Windows, GraphPad software, San Diego, CAUSA, www.graphpad.com.

Results: Creatinine clearance, in normal non-pregnant cases, the mean was 102.63 ml/min. In eclamptic cases, the mean found was around 71.98 ml/min. The difference between group is highly significant except when control and mild cases were compared ($p>0.005$).

Conclusions: Clearance fall gradually in cases from mild to severe. However, it is a good index to gauge the renal status in these patients.

INTRODUCTION: Hypertension in pregnancy is a major cause of maternal death and also a major source of maternal and perinatal morbidity and perinatal mortality [1]. Preeclampsia is a pregnancy specific syndrome of reduced organ perfusion secondary to vasospasm and endothelial activation. Proteinuria is an important sign of preeclampsia and rightfully concluded that the diagnosis is questionable in its absence. It can be defined as a multisystem disorder of unknown etiology characterized by development of hypertension to the extent of 140/90 mmHg or more with proteinuria

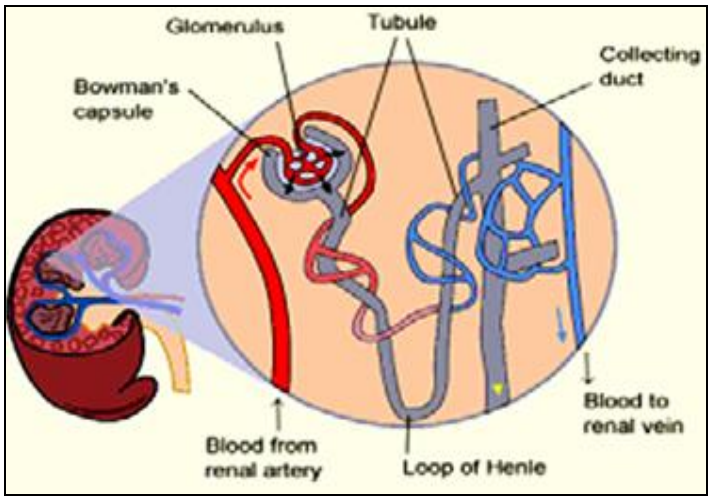
induced by pregnancy after the 20th week [2] Numerous studies had reported that renal function was reduced in preeclamptic pregnancies. Creatine is a naturally occuring nitrogenous organic acid that helps to supply energy to muscle cells. In humans, half of stored creatine originates from food (meat and fish), while the other half is synthesized mainly in the liver, pancrease and kidneys from three amino acids: arginine, glycine and methionine. After synthesis, 95% of creatine is stored as phosphocreatine (creatine phosphate) in skeletal muscles, while the rest is stored in the brain and heart.

Creatinine is the metabolic waste product resulting from the breakdown of creatine (which remains as creatine phosphate in muscle), and is usually produced at a fairly constant rate by the body (depending on muscle mass).Chemically, creatinine is a spontaneously formed cyclic derivative of creatine.[3.] Creatine phosphate is a high energy phosphate bond compound that supplies energy for muscle contraction.[4]

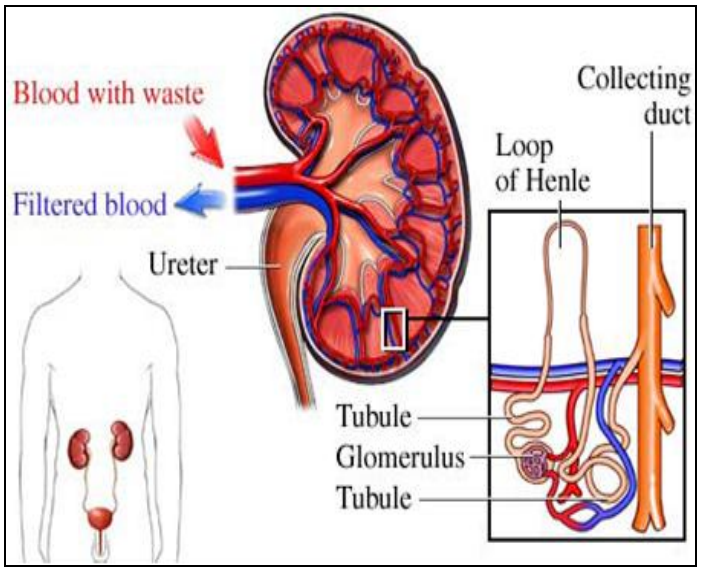
Creatinine is eliminated by glomerular filtration through the kidneys and excreted in urine without tubular reabsorption. In renal dysfunction, the ability of kidneys to filter creatinine is diminished leading to a rise in serum creatinine. Therefore, serum creatinine level is used as an indicator of renal function [5]. One of the most widely used endogenous substance that avoids pricks and hypersensitivity reactions is creatinine.In dogs, rabbits and humans, clearance of creatinine can also be used to determaine the GFR. In man, some creatinine is secreted by the tubules and some may be reabsorbed [6]. The values agree quite well with the GFR values measured with inulin because, although the value for Uc, V is high as a result of tubular secretion, the value of P is also high as a result of non specific chromogens and the errors tend to cancel [7].

Serum creatinine though is a simple test and is the most commonly used indicator of renal function,a rise in blood creatinine levels is observed only with marked damage to functioning nephrons. Therefore this test is not suitable for detecting early stage kidney malfunctioning. A better estimation of kidney function is given by the creatinine clearance test. Endogenous creatinine clearance is easy to measure and is a worthwhile index of renal function [5].

Creatinine has constant values in the serum and urine for every individual; it is higher in males than females.Creatinine has constant values in the serum and urine for every individual; it is higher in males than females. Significant differences in ranges between males and females can be eliminated by correcting for lean body weight [8].



Creatinine clearance is defined as the volume of plasma (ml) that is cleared of creatinine per minute. It is a useful measure to estimate the glomerular filtration rate (GFR) of the kidneys. GFR is estimated by measuring a substance having a steady concentrationin the blood, freely filtered by the kidneys and excreted at a constant rate in urine, neither reabsorbed nor actively secreted. Since creatinine fulfills all these requirements, its determination is a sensitive method to test kidney function [3].



N.B. GFR can be also determined using an exogenous substance (inulin) by constant infusion till a steady state concentration of inulin is maintained in blood then measuring inulin clearance. However, creatinine clearance is an easier, less time-consuming and more practical method since creatinine is endogenously produced in the body.

The increased afferent arteriolar tone in preeclampsia may protect the glomerulus from damage due to high systemic arterial pressures. preeclampsia is associated with impaired renal function, meanwhile there is considerable evidence suggesting that reactive oxygen species were implicated in the pathogenesis of immunologically mediated renal injury [9]. Accordingly this proved that total anti-oxidant status in preeclampsia is correlated to creatinine clearance. Status of glomerules or renal parechyma can be estimated by measuring glomerular Filtration Rate(GFR).

It can be measured in humans by measuring the excretion and plasma level of a substance that is freely filtered through the glomeruli and neither secreted nor absorbed by the tubules.The amount of such a substance in the urine per unit of time must have been provided by filtering exactly the number of millilitre of plasma that contained this amount. Therefore, if the substance is designated by the letter X, the GFR is equal to the concentration of X in urine (Ux) times urine flow per unit of time (V) divided by the arterial plasma level of X (Px) i.e. UxV/Px. This value is called the CLEARANCE of X.Px is, ofcourse, the same in all parts of arterial circulation and if X is not metabolized to any extent in the tissues, the level of X in peripheral venous plasma can be substituted for the arterial for the arterial plasma level [3, 5, 10].

MATERIALS AND METHODS: The present study was conducted on normal non-pregnant women, Pregnant women in different trimesters, Preeclamptic toxaemic patients and eclamptic patients admitted in Rajendra Institute of Medical Sciences, Ranchi during the period from June 2008 to September 2009. The study was approved by the ethical committee of the institution and a written informed consent was taken from all the patients and controls in accordance with the protocol. Here, 2 hour urine clearance test has been done. The 2 hour sample of urine meticulously collected in outdoor, indoor and labour room.

In order to assure an adequate urine flow and thereby minimize the error resulting from incomplete bladder emptying, especially when short collection periods (like in this study of 2 hr.) are employed, the patient must be adequately hydrated prior to the test. This is achieved by telling patient to drink about 500ml of water over a 10 – 15 minutes time immediately prior to the test. For diagnosing preeclampsia following criteria is very helpful:

Minimum criteria:

BP ≥ 140/90 mmHg after 20 weeks gestation, Proteinuria ≥ 300mg/24hours or ≥ 1+ dipstick Increased certainty of preeclampsia:- BP ≥ 160/110mmHg, Protinuria ≥ 300mg/24 hours or ≥ 2+ dipstick, Serum creatinine > 1.2mg/dL unless known to be previously elevated, Platelets < 100,000/mm^3, Microangiopathic hemolysis (increased LDH), Elevated ALT or AST, Persistent headache or other cerebral or visual disturbances,Persistent epigastric pain [11].

At the start of the test, the patient was advised to voids as completely as possible and that urine was discarded. Thereafter, all the urine was collected through the conclusion of the timed period (i.e. 2 hr.) when the patient again voids as completely as possible. the major problem in measuring the 24 hours clearance of creatininehas been in getting complete collection of urine.A 6 hour sample was also recommended during the time of a single shift of nurses on duty. Significant volume of urine fill the increased dead space of the urinary tract during pregnancy, which was the source of error in mesuring any clearance.

If the rate of urine flow was low at the begining of the test when bladder was empty and the urine discarded, the dead space was contain creatinine already excreted but which was included in the test sample.Then, the so obtained urine sample was appropriately preserved by refrigeration [12]. A suitable blood sample was obtained at any convenient time immediately before or after the test or while it is in progress:

Creatinine is stable in serum for 1 day at 2-8^oC. Urine is diluted 1:50 times with distilled water before assay [13]. Creatinine kit is based upon Modified Jaffe's Kinetic method [14].

Principle: Picric acid in an alkaline medium reacts with creatinine to form a red – orange coloured complex with the alkaline picrate. Intensity of the colour formed during the fixed time is directly proportional to the amount of creatinine present in the sample.

Creatinine + Alkaline Picrate ⟶ Orange coloured complex.

Contents:

- L 1: Picric acid reagent
- L 2: Buffer reagent
- S : Creatinine Standard (2mg/dl)

All reagents are stable at room temperature; however, the working solution is stable for 15 days at +2 to +8^{o}C and 5 days at +15 to +25^{o}C. Working reagent are prepared by mixing equal volumes of picric acid reagent and buffer reage.

Creatinine Clearance: Endogenous creatinine clearance test was first introduced by Miller and Winkler in 1938.It has many practical advantages. Creatinine is a normal end product of metabolism with a relatively constant plasma concentration and daily urinary excretion that are not greatly influenced by diet, urine flow rate or exercise. Creatinine is reported to reach a maximum concentration in the urine at flow rates below about 0.35 to 0.5 ml/min so that the endogenous plasma creatinine clearance then assumes a linear dependence upon the urine flow rate [15].

Creatinine clearance = $U_{creat} \times V / P_{creat}$

Where, U_{creat}= Urine creatinine concentration(mg/ml)

P_{creat} = Plasma creatinine concentration (mg/ml)

V = Urine flow rate (ml/min)

The calculated clearance is usually corrected to standard body surface of 1.73 m^2 by multiplying by 1.73/S, where S is the body surface area in m^2. The creatinine clearance may be performed over any accurately timed period like 1, 2, 4, 12, or 24 hour intervals (Tobias GJ, McLaughlin RF Jr, Hopper J; 1962). Another parameter like Detailed menstrual history, Clinical examination, Cycle Flow (Any related complaint such as pain, Last menstrual period (LMP) Expected date of delivery), Obstetrical history (Gravida parity, history of previous pregnancy, detail about previous babies), Medical and Surgical history (Hepatic disorder, Renal disorder, Cardiovascular-hypertension, Heart disease, Respiratory disorder, Metabolic diseases such as Diabetes Mellitus, Epilepsy, Malaria), family history (Multiple pregnancy, Diabetes mellitus, Hyper-tension, Congenital heart disease), physical Examination, General examination (Body built Height, Pallor, Blood pressure Pulse Oedema), Systemic examination (chest, CVS), Abdominal examination, (Inspection, Palpation, Fetal heart rate) were recorded during the study period, when patients had continuous periodical referral to the Dept. of Obstetrics and Gynecology, RIMS.

Statical Analysis: All results were expressed as mean ± SD. Differences between means were calculated by independent samples Student‟s 't' test assuming a 95% confidence interval. Correlation was evaluated separately for each age group by Pearson's method. A $p<0.05$ was considered significant. Statistical analysis was performed using GraphPad Prism version 5.00 for Windows, GraphPad software, San Diego, CAUSA, www.graphpad.com.

RESULTS: The present study was undertaken to analyse the relationship between creatinine clearance and pregnant women. This association between creatinine clearance and pregnancy could be established after exclusing all variable causing risk of assay interfarance like Ketosis, hyperbilirubeni-nemia, cephalosporin. Normal value of creatinine clearance for males 90-139 ml/min, for females 80-125 ml/min. Although the cause of preeclampsia remains unknown, evidence for it begins to manifest early in pregnancy with covert pathophysiological changesthat gain momentum across gestation.

Unless delivery supervenes. These changes ultimtely result in multi-organ involvment with a clinical spectrum ranging from barely noticeable to life threatining for both mother and fetus. These adverse maternal and fetal effects develop simultaneously and presumably are a consequences of vasospasm, endothelial dysfunction and ischemia.

The myriad of maternal consequences of the preeclampsia syndrome are described in terms of organ systems,but they frequently overlap.It is enigmatic that there are such wide variations of involvement of these systems in individual pregnancies.

In preeclampsia, the clearance often is depressed. There may be case to case variation. To see the actual status of renal function, creatinine clearance test was done, the result of which are shown in **Table 1**. In normal non – pregnant group, the mean of creatinine clearance was 102.53 ± 6.475 mg/ml. In the 1st st trimester of normal pregnant group, the mean was 142.1 ± 5.824, and in 3rd trimester, the mean was 128.7 ± 6.480. When each group was compared, the difference i.e. the rise fall in creatinine clearance was statistically significant. **Table 2** compares creatinine clearance values expressed in mg / ml, in normal pregnant cases, in mild, moderate and severe pre-eclamptic cases and in eclamptic cases. The mean value obtained for normal pregnant (control) group was 132.7±7.02, in mild pre-eclamptic group was 127.81±6.09, in moderate pre-eclamptic group was 103.81 ± 5012, in severe cases was 77.68 ± 4.02 and in eclamptics, the mean was 71.98±3.20 mg/ml. The fall in creatinine clearance values, when the two groups are compared was found statistically significant. The aim of this study was decided to evaluate the relation-ship between the renal changes (creatinine clearance) in pre-eclamptic, eclamptic, preeclamptic toxeaemic ,non pregnant and normal pregnant women.

TABLE 1: CREATININE CLEARANCE ACCORDING TO GESTATIONAL AGE IN NORMAL PREGNANCY

Group	Normal size	Mean	S.D.	S.E.	t-value	P-value	Significant
Non pregnant	15	102.53	±6.475	12.271	4.72	<.05	Significant
1st trimester normal pregnancy	10	127.3	±**5.729**	13.280	5.23	<.01	
2nd trimester normal pregnancy	30	142.1	±5.824	10.325			Significant
3rd trimester normal pregnancy	20	128.7	±6.480	7.328	3.92	<.01	Significant

Results are expressed as Mean ± S.D., S.E., t-value & p – value

TABLE 2: CREATININE CLEARANCE IN mg/ml ACCORDING TO GESTATIONAL AGE IN NORMAL AND TOXAEMIA OF PREGNANCY

Group	Type of cases	Mean	Range S.D.	S.E.	Variance	t-value	p- value	Significant
Normal preg. Cases	50	132.7	110-156	7.79	280.95	3.52	<.01	Significant
Mild cases	22	127.81	100-142	14.52	211.08	1.2192	<.05	Significant
Moderate	17	103.81	72-120	3.382	194.4	4.59	<.01	Significant
Severe	22	77.68	70-102	4.17	383.95	3.52	<.01	Significant
Eclamplics	20	71.98	60-82.5	3.18	285.72			Significant

Results are expressed as mean ± S.D., Variance, S.E., t-value, p-value

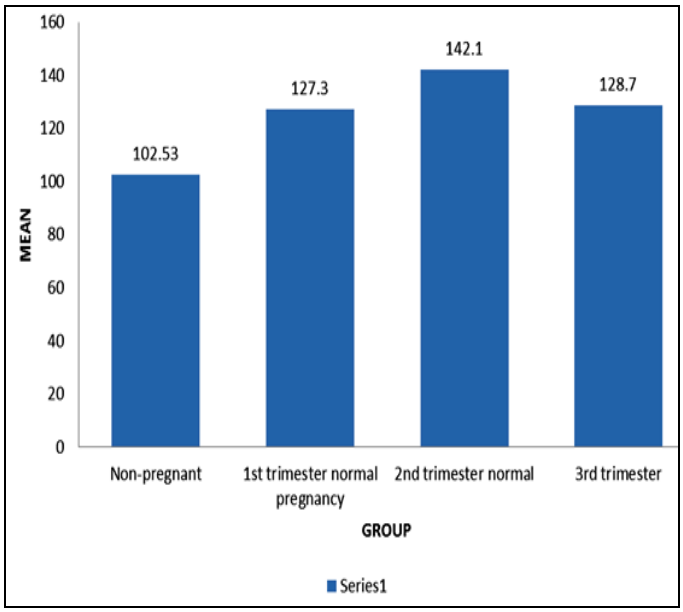


FIGURE 1: CREATININE CLEARANCE ACCORDING TO GESTATIONAL AGE IN NORMAL PREGNANCY

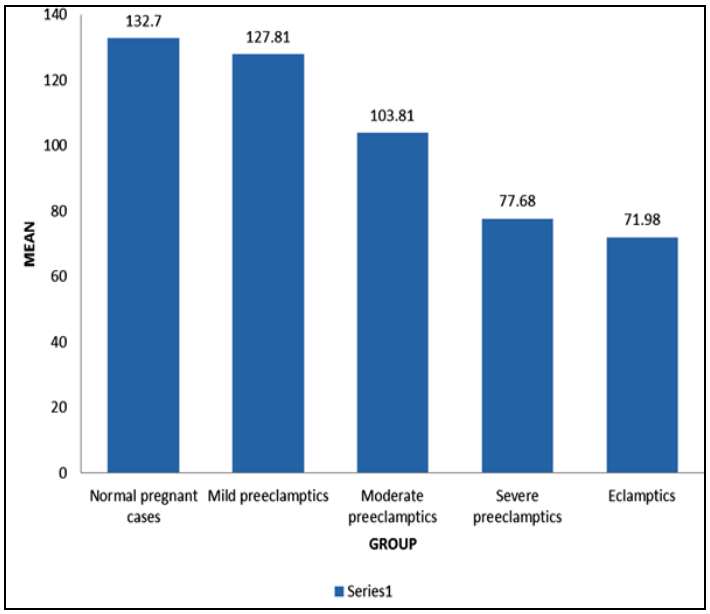


FIGURE 2: CREATININE CLEARANCE ACCORDING TO GESTATIONAL AGE IN TOXAEMIA OF PREGNANCY

Discussion: Preeclampsia is a major complication of human pregnancies, affecting 5-7% of pregnant women. Preeclampsia is characterised by an increase in vascular tone (vasospasm) that is frequently associated with enhanced platelet aggregation and therefore, reduced utero-placental flow. In addition, coupling of these factors with disturbed renal function worsens peri-natal outcome.

Preeclampsia usually is well established before the patient develops symptoms. She may be aware of some puffiness of the fingers and face, which is common in normal pregnancy, but hypertension, proteinuria and increase in uric acid level in blood and decrease in clearance tests may be present for days or weeks before she has any subjective complaints. Hence, the importance of peri-natal care, a primary objective of which is the early detection of the signs and laboratory parameters in preeclampsia.

Creatinine clearance is diminished in very young infants but increases to reach adult levels, when corrected for body surface area, by age. A marked increase in creatinine clearance which occurs in pregnancy parallels the increase in glomerular filtration rate [16]**.** A recent review of investigating renal hemodynamic changes in pre-eclamptic and normal pregnancies showed that, on average, there is a reduction in GFR and a 24% reduction in effective renal plasma flow (ERPF) in pre-eclampsia compared to normal pregnancy values [17].

The average clearance of exogenous creatinine was found to be 139 ml/min.,in normal non-pregnant women. It was increased to an average of 186ml/min. During most of the pregnancy and fell some what to 153ml/min during the last two months. The ratio of exogenous creatinine clearance to inulin clearance varies from 0.75 to 1.90 and averaged 1.09:1 until the last two months when the mean was 1.02:1 [18, 19].

Simultaneous clearance of endogenous creatinine and inulin in normal pregnant women and found the clearance ratio to vary from 0.5 to 1.42.The mean creatinine clearance in their normal nonpregnant female was 105 ml/min. It increased to 170 ml/min. In mid pregnancy and decreased progressively throughout third trimester [20].

In any degree of renal impairement, the urea clearance is less than the inulin clearance and creatinine clearance is greater. When inulin cleared is less than about 20ml/min., it is closed to the average of the simultaneous clearance of urea and creatinine [5]. Significant decrease in creatinine clearance in preeclamptic patients and this decrease was more pronounced in severe cases. There is considerable evidence suggesting that reactive O_2 species are implicated in the pathogenesis of ischaemiotoxic and immunologically mediated renal injury.

In experimental immuno glomerulonephritis, reactive O_2 species are generated by both polymorphs and monocytes and resident glomerular cells (mainly mesangial cells). Their formation results in morphologic lesions and in modification of glomerular permeability to protein through activation of proteases and reduction of proteoglycan synthesis [9].

Our study has some limitations, that it does not include subjects below 25 years and above 32 years, which would have given a better picture of the effect of, gestational age in normal pregnancy and toxemia on the creatinine clearance across ages. Dietary factors, which also influence serum creatinine and urine creatinine levels, were not taken into consideration in our study. Finally, a larger sample size would no doubt have given more dependable results.

CONCLUSION: Thus the present study has, documanted that Creatinine clearance fall gradually in cases from mild to severe Preeclamptic cases. But the difference in fall of the clearance values were not highly significant in mild and moderate cases when compared to normal 3rd and 2nd trimester values. However, it is a good index to gauge the renal status in these patients. Its position as a prognostic marker and diagnostic parameter comes only next to serum uric acid levels.

Finally this research work revealed that when the parameter – creatinine clearance values can predict the course of the disease in these pregnant patients and can be easily given the status of very early markers coming just behind angiotensin sensitivity test in suspected cases.

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