Salivary Creatinine Estimation as an Alternative to Serum Creatinine in Patients with Obstetric Related Acute Kidney Disease

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Abstract: *Introduction: Obstetric related acute kidney disease (ORAKD) contributes significantly to maternal & foetal morbidity & mortality. Its incidence is 3-7% of overall cases of acute kidney injury (AKI) in India. It is most commonly observed in the post partum period & followed by cases seen in third trimester. Sepsis, preeclampsia & eclampsia are the leading causes of ORAKD in developing countries, while in developed countries, chronic hypertension & renal pathologies are the main causes. This clinical entity requires frequent serum analysis for diagnosis & monitoring of clinical outcomes & for evaluation of prognosis. Serum creatinine is considered as a gold standard renal function test in ORAKD. Blood sampling for estimation of S. creatinine is an invasive procedure, in contrast to its estimation from salivary assays which is a non invasive, simple, & cost effective procedure. Salivary creatinine evaluation is very helpful in patients suffering from clotting disorders like haemophilia & when venous access is compromised. The values of salivary creatinine show a positive correlation with values of serum creatinine. Aim: 1. To establish a correlation between serum & salivary creatinine levels. 2. To evaluate the role of salivary creatinine as an alternative modality of diagnosis & management in ORAKD. Methodology: A prospective observational study was conducted on 200 women in the age group of 20-40 years, in the third trimester of pregnancy, in the Department of Obstetrics & Gynaecology, MGM MCH. The cases were divided into two groups. Group I consisted of 100 cases of ORAKD as a result of various etiologies such as PIH, preeclampsia, eclampsia & sepsis. Group II consisted of 100 cases which served as healthy control group. The study was carried out for a period of one year from May 2017 & the subjects were followed until delivery & up to 6 weeks post partum. Cases & controls were compared on various parameters & the results statistically analysed. Results: Serum & salivary creatinine showed a positive correlation in cases of ORAKD (group I) r=0.832. In the control group II, a negative correlation was found in serum & salivary creatinine levels r= -0.321. Conclusion: Salivary creatinine estimation can be employed as an alternative diagnostic modality to serum creatinine estimation in ORAKD cases.*

Keywords: Obstetric related acute kidney disease, Serum creatinine, Preeclampsia, Eclampsia, Sepsis, Renal Function Test

1. Introduction

Obstetric related acute kidney disease (ORAKD) is an abrupt deterioration in renal function. The incidence of ORAKD is increasing worldwide, in developing countries due to increased prevalence of hypertensive disorders in pregnancy. In developed nations due to a rising trend in diabetes mellitus & hypertension. The essence of its management lies in the identification & treatment of the underlying cause along with serial monitoring of therapeutic outcomes. Creatinine is a waste by product of muscle metabolism that is primarily excreted by the kidneys & hence is a gold standard marker of renal function. Monitoring of renal function by serum creatinine estimation is an invasive procedure, also causing additional blood loss due to frequent sampling. On the other hand, salivary estimation is non invasive, simple, & cost effective alternative. Creatinine values obtained from saliva is a reasonably accurate reflection of the same in serum.

Physiological Properties of Saliva

Saliva is a clear bio-fluid composed of 99% water, 0.3% protein & remaining as inorganic components. It is an ultra-filterate of blood & is composed of secretions from the major & the minor salivary glands, mucosal transudations & exfoliated epithelial cells. It contains hormones, proteins, enzymes, antibodies, antimicrobial components & cytokines. It has several biomarkers found in normal & pathological conditions. There is a free exchange of blood based molecules in the salivary fluid by mechanism of active & passive transport, diffusion, ultrafilteration & through gap junctions. Various studies have identified different microbial, immunologic & molecular markers, the expression of which helps in the evaluation of pathological states. Hence saliva is a potential diagnostic medium for oral as well as systemic conditions.

Salivary Creatinine & Renal Pathology

Various studies have been conducted to evaluate the significance of salivary creatinine as a biomarker in renal pathology. Jonathon Lloyd et al in 1996 showed that salivary creatinine was about 10-15% of the serum levels, due to ultrafilteration of creatinine in saliva.

Xia et al in 2012 demonstrated the levels of urea, creatinine& ureic acid in chronic kidney disease (CKD). Levels of salivary creatinine in CKD patients were significantly higher as compared to healthy subjects, also its concentration in stage IV & stage V CKD patients were higher as compared to early stage CKD patients (p<0.05).

Reda et al showed a significant positive correlation (p<0.05) between serum & salivary creatinine in healthy

individuals, CKD patients & End stage renal disease (ESRD) patients (r= 0.83, 0.58 & 0.59 respectively).

Venkathapathy (2014) et al also showed a positive correlation between the two in the CKD group (r=0.731) at p value <0.05. However, the study showed a negative correlation between serum & salivary creatinine in the control group (r= -0.326) at p value <0.05.

2. Aims and Objectives

1. To establish a correlation between serum & salivary creatinine levels
2. To evaluate the role of salivary creatinine as an alternative modality of diagnosis & management in ORAKD.

3. Materials and Methods

Cohorts of 200 pregnant women, in the age group of 20-40 years, in the third trimester of pregnancy were admitted in the Department of Obstetrics & Gynaecology, MGM MCH, Jamshedpur, East Singhbhum, Jharkhand.

A prospective observational study was carried out over a period of one year from May 2017. The study participants were divided into two groups consisting of 100 subjects each. Group I consisted of 100 cases of ORAKD as a result of various etiologies such as PIH, preeclampsia, eclampsia & sepsis. Group II consisted of 100 cases which served as healthy control group. Both the groups were comparable in demographic features & followed up until delivery &upto 6 weeks post partum.

Criteria of diagnosis:

Acute kidney injury (AKI) is defined on the basis of Risk, Injury, Failure, Loss of function & End-stage renal disease (RIFLE) criteria& The Acute Kidney Injury Network (AKIN) classification. ORAKD is defined as AKI diagnosed anytime during pregnancy or upto 6 weeks post partum.

Preeclampsia- It is diagnosed when BP $\geq$ 140/90 mmHg for the first time after 20 weeks of gestation & proteinuria $\geq$ 300 mg/24 hrs or 1+ proteinuria on dipstick.

Eclampsia- Preeclampsia with grand mal tonic clonic convulsions without any other attributable cause.

Sepsis is defined according to the criteria laid down by the American College of Chest Physicians. It is the clinical syndrome that results from a dysregulated inflammatory response to an infection that is non resolving& deleterious, often leading to organ dysfunction. It is defined as the presence (probable or documented) of infection together with systemic manifestations of infection.

Rifle Criteria for Acute Kidney Injury:

STAGE 1 (RISK): Increased S creatinine x 1.5, or GFR decrease >25%, Urine Output< 0.5ml/kg/hr x6 hr

STAGE 2 (INJURY): Increased S creatinine x2, or GFR decrease >50%, Urine Output < 0.5ml/kg/hr x12 hr
STAGE 3 (FAILURE): Increased S creatinine x3, or GFR decrease >75%, or S creatinine$\geq$4 mg/dl, Acute rise $\geq$0.5 mg/dl, Urine output <0.3 ml/kg/hr x24 hr, or anuria x12 hr
STAGE 4 (LOSS): Persistent ARF = Complete loss of renal function >4 weeks
STAGE 5 (END STAGE RENAL DISEASE): ESRD >3 months
AKIN CLASSIFICATION OF ACUTE KIDNEY INJURY:
STAGE 1: Increased S creatinine x1.5 or $\geq$ 0.3mg/dl or UO < 0.5 ml/kg/hr x 6hr
STAGE 2: Increased S creatinine x 2 or UO< 0.5ml/kg/hr x 12hr
STAGE 3: Increased S creatinine x 3 or S creatinine$\geq$4 mg/dl (with acute rise of $\geq$0.5 mg/dl), UO< 0.3ml/kg/hr x 24 hr, anuria x 12hr

Criteria of inclusion: Patients diagnosed as cases of ORAKD, after giving written informed consent.

Criteria of exclusion:

1. Women with known chronic kidney disease or chronic hypertension, pre-existing diabetes mellitus & renal transplant recipients.
2. Women with oral pathology, pre malignant or malignant lesions.
3. Women unwilling to participate.

Study Design: Prospective study

Methodology: 200 cases, who fulfilled the inclusion criteria were selected for the study. GFR estimation was done using Cockcroft Gault formula & the cases of ORAKD were found to be in stage II & stage III of RIFLE criteria corresponding to stage 2 & stage 3 of AKIN classification.

Sample Collection: The samples were obtained between 8:00 AM & 11:00 AM to minimize the effect of diurnal variation. 2mL of blood was drawn from ante cubital vein under aseptic condition. 2mL of saliva was obtained in a sterile graduated container under resting conditions. The subjects were asked to thoroughly rinse mouth with distilled water & refrain from eating & drinking 60 minutes prior to sample collection. The samples were centrifuged at 3000 rpm for 10 minutes. Salivary supernatant & serum were separated & the samples were assayed in automatic analyser. Creatinine estimation was done using creatinine estimation kit by Jaffe Kinetic reaction. All the serum & salivary assays were performed in the Department of Clinical Pathology, MGM MCH. The results were analysed using standard statistical software.
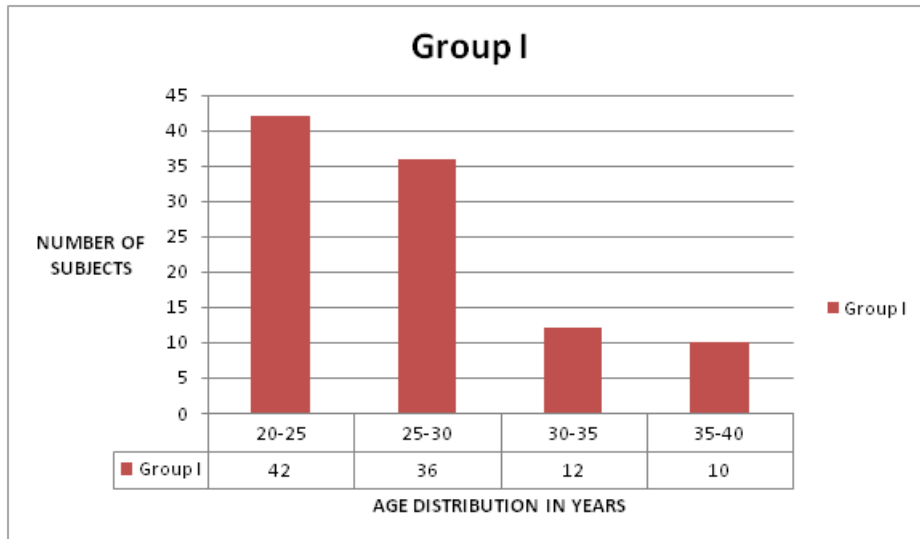
4. Results and Distribution

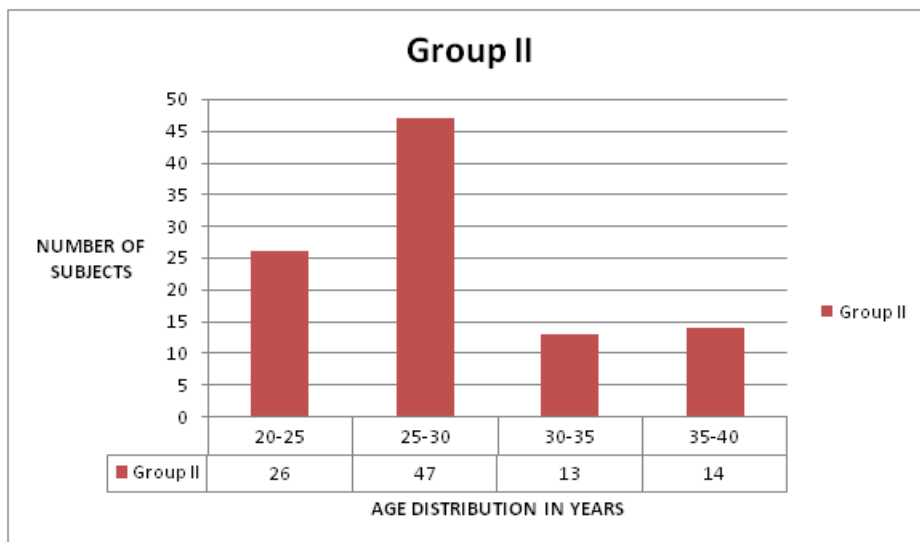**Figure 1(a):** Age distribution of subjects in group I

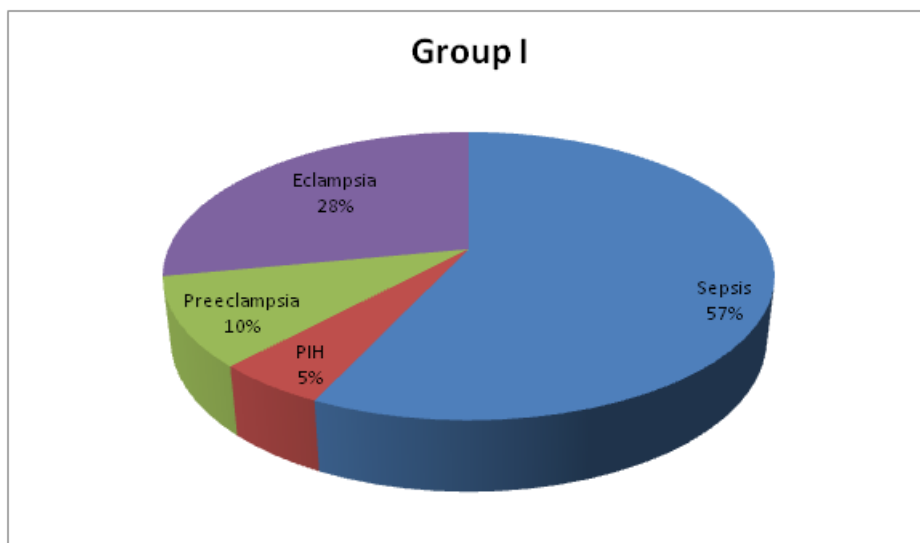**Figure 1(b):** Age distribution of subjects in group II

Figure 2: Distribution of subjects in group I on the basis of etiology
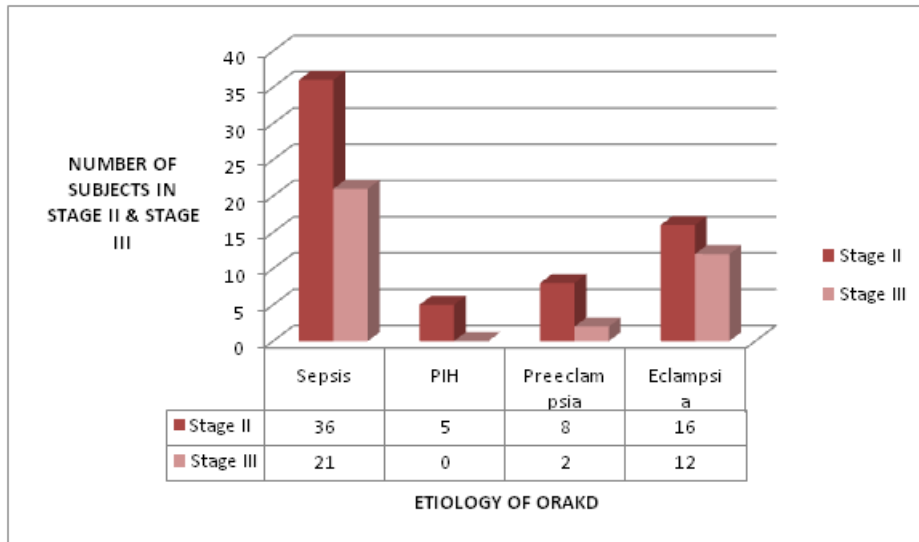
Figure 3: Distribution of cases in group I on the basis of staging of ORAKD

Table 1: Comparison of serum & salivary creatinine levels in group I & group II

Group I	Range	Mean	Standard Deviation	Standard Error	P value
Serum creatinine	2.8-15.7mg/dl	5.92	3.062	0.296	<0.001
Salivary creatinine	0.2-2.5mg/dl	0.68	0.476	0.045	<0.001

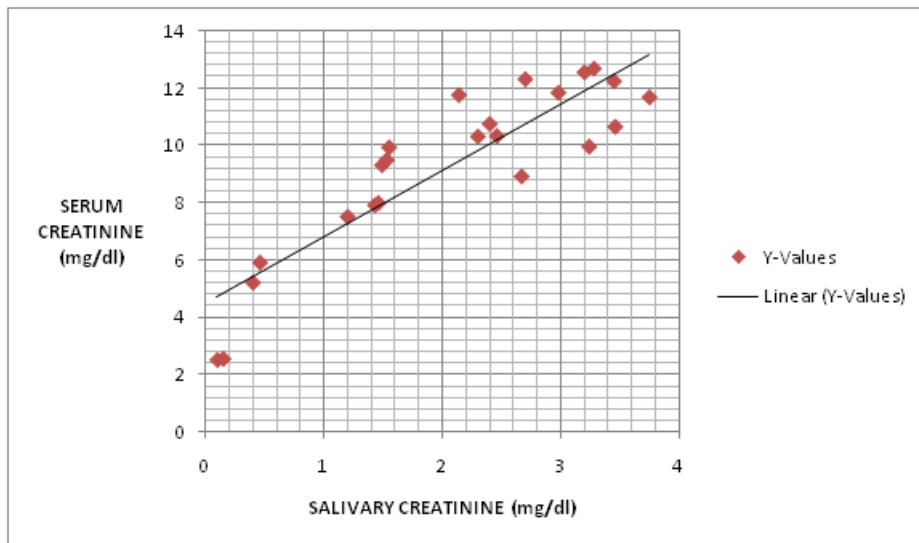| Group II	Range	Mean	Standard Deviation	Standard Error	P value
Serum creatinine	0.5-1.4 mg/dl	0.82	0.169	0.027	<0.001
Salivary creatinine	0.1-0.4mg/dl	0.16	0.03	0.010	<0.001

Figure 4: Scatter plot showing linear correlation between salivary & serum creatinine in group I

Linear regression coefficient r= 0.832
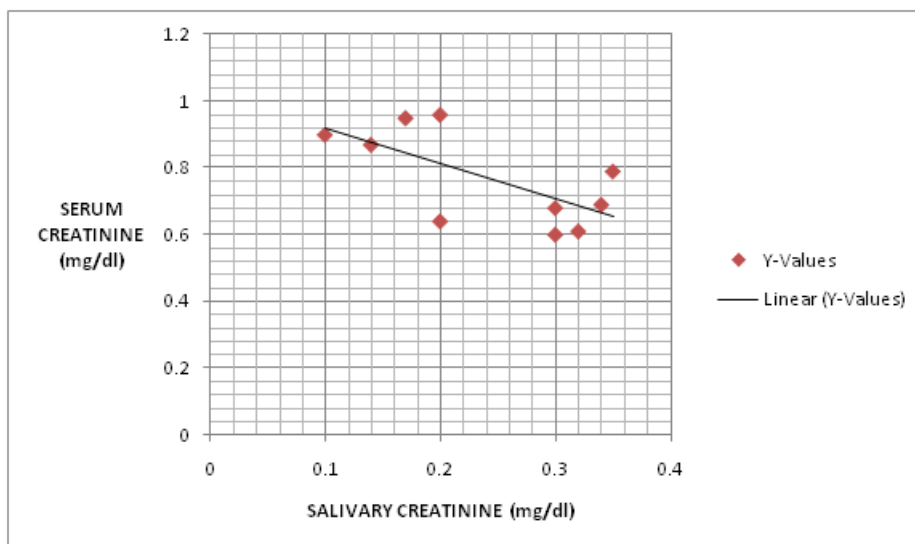
R^2 linear= $(0.832)^2$ =0.692

Figure 5: Scatter plot showing linear correlation between salivary & serum creatinine in group II

Linear regression coefficient r = -0.321

R^2 linear = $(-0.321)^2$ = 0.103

5. Discussion and Observation

Age wise distribution of subjects in group I & group II is done. Mean age of subjects in group I is 27 years. Mean age of subjects in group II is 28.25 years (Figure 1(a), 1(b))

On the basis of etiology of ORAKD, 57% cases in group I are due to sepsis, 28% due to eclampsia, 10% due to preeclamptic & 5% due to pregnancy induced hypertension (Figure 2).

The cases of ORAKD in group I were found to be in stage II & stage III according to RIFLE criteria of acute kidney injury & AKIN classification. Out of 57 cases of acute kidney injury due to sepsis, 63.15% cases belonged to stage II & 36.84% cases belonged to stage III. In the PIH group, 100% cases had ORAKD stage II. 80% of preeclamptic cases were in stage II, while 20% were in stage III. Out of 28 cases of eclampsia, 57.14% cases belonged to stage II & 42.85% cases belonged to stage III of acute kidney injury (Figure3).

In group I, serum creatinine values ranged between 2.8-15.7 mg/dl, with a mean of 5.92 (SD=3.062) & the salivary values ranged between 0.2-2.5 mg/dl, with a mean of 0.68 (SD= 0.476), at p value< 0.001, which is found to be statistically significant.

In group II, serum creatinine values ranged between 0.5-1.4 mg/dl, with a mean of 0.82 (SD=0.169), while salivary values ranged between 0.1-0.4 mg/dl, with a mean of 0.16 (SD=0.03), at p value <0.001, which is statistically significant (Table 1)

To find a correlation between serum & salivary creatinine, & if changes in serum creatinine were accompanied by changes in salivary creatinine, a correlation analysis of both the groups was done using a scatter plot. The correlation between the two was found to be positive in group I (r= 0.832), & negative in the control group II (r= -0.321). (Figure 4, 5)

Creatinine is a waste by product of metabolism with a molecular weight of 113 Da & molecular weight of 3.2 A units. All the creatinine that is excreted at the glomerulus is excreted by the renal tubules without reabsorption, hence, its blood value is used as an index of renal function. The normal range of serum creatinine is 0.6 -1.5 mg/dl & salivary creatinine is 0.05- 0.2 mg/dl. The normal values found in the control group II falls within this range.

A significantly high creatinine level is found in both serum & saliva in cases of ORAKD in group I. This is because the kidneys are unable to excrete creatinine in cases of kidney injury & renal failure. The reason for increased salivary concentration of creatinine is due to a positive concentration gradient, where creatinine diffuses from serum to saliva. It may also be possible that the body utilises saliva as an alternate pathway of excretion of creatinine when renal route is compromised. Hence a positive correlation was found between salivary & serum creatinine values in patients in group I

Creatinine has a high molecular weight with a low lipid solubility, thus in healthy controls (group II), it is unable to diffuse easily across the cells & tight intercellular junction. Therefore, a negative correlation value is obtained between salivary & serum creatinine in group II.

Thus the results of this study suggest that salivary estimation of creatinine is an advantageous alternative to serum creatinine estimation in ORAKD patients.

6. Conclusion

A rising trend is seen in cases of ORAKD due to different etiologies, necessitating frequent estimation of creatinine to diagnose as well as to monitor the response to treatment. For this purpose taking samples of saliva instead of blood is suitable & convenient for the patient, also reduces the

risk of occupational hazard to laboratory personnel. The results obtained through this study satisfactorily suggest that saliva can be used as a non invasive diagnostic modality for creatinine estimation in cases of acute kidney injury.

Conflicts of interest: None declared

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