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Estimated Glomerular Filtration rate and Random Urine Protein: Creatinine ratio in Normal Pregnancies in Makurdi, NigeriaAgbecha, A.*¹, and Iduh, M.U.²

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Abstract

Renal impairment may occur before or during pregnancy with an overall detrimental impact on both the mother and foetus, necessitating the close monitoring of renal function, in a bid to minimize maternal and foetal complications. The study aims at determining the estimated glomerular filtration rate (eGFR), random urine Protein: Creatinine ratio (UP:Cr) in normal pregnancies with the view of providing reference values for monitoring renal function during pregnancy. The case-control study compared urine protein, urine creatinine, serum creatinine, eGFR, UP: Cr of normal pregnant with age matched non- pregnant women. A significant ($p < 0.001$) change was observed in the levels of urine protein, urine creatinine, UP:Cr in the control, second and third-trimester groups when compared. Urine protein, urine creatinine and UP:Cr were significantly ($p < 0.01$) higher in second trimester pregnancies than controls. A significant ($p < 0.000$) increase in urine protein and UP:Cr was observed in third trimester pregnancies compared to non- pregnant controls. Comparing second and third trimester groups showed that urine protein and UP:Cr were significantly ($p < 0.05$) higher in the third trimester group. A significant positive correlation was observed between gestation age and urine protein ($p < 0.05$, $r = 0.217$), gestation age and UP:Cr ($p < 0.01$, $r = 0.369$) in the pregnant population studied. However, an inverse correlation was observed between urine creatinine and gestation age ($p < 0.01$, $r = -0.251$). A strong significant inverse correlation was observed between serum creatinine and eGFR ($p < 0.01$, $r = -0.938$) in the pregnant group. Our

study shows that normal pregnancy impacts on renal function as reflected by changes in renal function indices. Routine biochemical assessment of renal function is recommended during antenatal care, aimed at identifying abnormal changes that may impact on maternal and foetal health.

Keywords: *Estimated glomerular filtration rate, urine Protein: Creatinine ratio, pregnancy, serum creatinine*

Introduction

Renal function is altered as a result of hormonal and haemodynamic changes occurring during pregnancy. Physiologic adaption is impaired in women with risk factors for inappropriate haemodynamic responses, which increases the risk of adverse pregnancy outcomes (Piccoli *et al.*, 2015; Piccoli *et al.*, 2010). Midterm estimated glomerular filtration rate (eGFR) values below and above the reference range of 120–150 ml/min per 1.73 m² is shown to be associated with adverse pregnancy outcomes: premature birth, low birth weight, and preeclampsia (Park *et al.*, 2018). Elevated serum creatinine levels have been reported in 3rd term normotensive pregnant patients who delivered small-for-gestational-age (SGA) fetuses (Akahori *et al.*, 2012). Pathophysiologic mechanisms have shown that renal function and filtration are involved in preeclampsia (Maynard and Thadhani, 2009). The Torino-Cagliari observational study demonstrated that the risk of adverse pregnancy outcomes is linked to chronic kidney disease (CKD), including stage 1CKD (Piccoli *et al.*, 2015).

Glomerular filtration rate (GFR) is generally accepted as the best overall index of kidney function, and a decrease in GFR has diagnostic, prognostic, and patient treatment implications (Kidney Disease Improving Global Outcomes, 2013; Niewczas *et al.*, 2017; Stevens *et al.*, 2006). Glomerular filtration rate can be directly measured (mGFR) based on the clearance of an exogenous filtration marker, but this method is impractical for routine practice. To overcome this limitation, clinical practice guidelines recommend the estimation of glomerular filtration rate using validated equations based on serum concentration of endogenous filtration markers (creatinine, cystatin C) and demographic variables [age, gender, and race] (Kidney Disease Improving Global Outcomes, 2013; Levey *et al.*, 2014). The use of eGFR has not been well studied in the pregnant population. However, few studies that examined the suitability of the formulas in the pregnant population reported a fair performance, relative to the mGFR involving the 24-hour inulin and creatinine clearance test (Alper *et al.*, 2007; Smith *et al.*, 2008). Gestational eGFR levels has been determined and observed to be significantly associated with adverse pregnancy outcomes in a large observational study involving singleton pregnancies (Park *et al.*, 2018).

The quantification of proteinuria in pregnancy is indicated in the monitoring of preexisting kidney disease and for the diagnosis of preeclampsia. Twenty-four-hour urine collection, although the gold standard for proteinuria quantification, has several limitations. It is cumbersome for the patient, it is often inaccurate because of under collection, and result availability is delayed for at least 24 h while the collection is being completed (Christopher-Stine *et al.*, 2004; Levey *et al.*, 1999). Random urinary protein:creatinine ratio is a reliable indicator of significant proteinuria (>300 mg/day) in non- ambulatory pregnant women, irrespective of sampling time during the day and may reasonably be used as an alternative to the 24-h urine collection method (Leanõs-Miranda *et al.*, 2007). The aim of this present study is to determine eGFR, random urine Protein: Creatinine ratio in normal pregnancies compared to non pregnant females with the view of providing reference values to enable

clinicians comprehensively monitor renal changes during pregnancy

Materials and Methods

Study Area

This case control study was conducted amongst antenatal clinic attendees and general outpatients of Federal Medical Centre Makurdi, after obtaining institutional ethical clearance.

Selection of Participants and Study Design

Individuals were eligible to participate in the study if they: (a) were within the reproductive age of 18 to 35 years; (b) had no history of hypertension and were not using antihypertensive medications; (c) were free of any other major systemic illnesses (e.g. renal disease, gout, liver disease, cancer, diabetes mellitus); (d) were free from pregnancy complications. Individuals who refused to take part in the study were excluded. After an informed consent was sought and the inclusion criteria fulfilled, the hospital-based study recruited a total of 120 women. This comprised of 40 mid-term and 40 third term pregnant women selected from a population of normal pregnant women attending the antenatal clinic. Also 40 apparently healthy aged- matched females visiting the general out patients department for their general health checkup were recruited into the study as controls. The study compared the renal indices of the pregnant groups and non pregnant controls.

Collection of Blood and Urine Samples

Participant's blood samples were collected into plain vacutainer tubes, centrifuged at 3000 rpm for 10 minutes within 1 hour of blood collection to obtain serum. Random urine samples were collected into clean universal containers. The determination of serum and urine creatinine levels was done using Randox reagent kits (Randox Laboratories Ltd., County Antrim, UK) on a spectrophotometer (Optima SP-300 Spectrophotometer; Optima INC. Tokyo, Japan). Urine protein was determined spectrophotometrically adopting the turbidimetric method. Estimated glomerular filtration rate was calculated from serum creatinine values and demographic variables according to the new equation of Levey *et al.*

(2009). Urine protein:creatinine ratio was calculated and expressed in mg/mg.

Statistical Analysis

Data were presented as means and standard deviations for continuous variables. Analysis of variance was used for between-group assessments followed by least significant difference *post hoc* honestly significant difference test. All statistical analyses were performed using the IBM Armonk, NY, USA, SPSS version 21. A two-sided $p < 0.05$ was considered statistically significant.

Results

Table 1 shows serum creatinine, urine protein, urine creatinine, UP:Cr, eGFR in pregnant and non-pregnant controls. A significant ($p < 0.001$) change was observed in the levels of urine protein, urine creatinine and UP:Cr in the control, second and third-trimester groups compared. No significant ($p > 0.05$) change in eGFR, serum creatinine and maternal age was observed among the three groups compared. A breakdown of the results as revealed by a *post hoc* analysis is presented in table 2. Urine protein, urine creatinine and UP:Cr were significantly ($p < 0.01$) higher in second trimester

pregnancies than controls, where as no significant ($p > 0.05$) change was observed in serum creatinine and eGFR. A significant ($p < 0.000$) increase in urine protein and UP:Cr was observed in third trimester pregnancies compared to non-pregnant controls. However, a non significant difference was observed in serum creatinine, urine creatinine and eGFR when the pregnant and non-pregnant groups were compared. Comparing second and third trimester groups shows that urine protein and UP:Cr were significantly ($p < 0.05$) higher in the third trimester group, where as a non significant ($p > 0.05$) change in serum creatinine, urine creatinine and eGFR was observed between the two pregnant groups. Table 3 presents data on the correlations between serum creatinine, urine protein, urine creatinine, UP:Cr, eGFR and pregnancy stages. A significant positive correlation was observed between gestation age and urine protein ($p < 0.05$, $r = 0.217$), gestation age and UP:Cr ($p < 0.01$, $r = 0.369$) in the pregnant population studied. However, an inverse correlation was observed between urine creatinine and gestation age ($p < 0.01$, $r = -0.251$). A strong significant inverse correlation was observed between serum creatinine and eGFR ($p < 0.01$, $r = -0.938$) in the pregnant group.

Table 1: Renal indices in age matched pregnant and non-pregnant females

| Parameter | Control n=40 | 2 nd Trimester n=40 | 3 rd Trimester n=73 | F- value | p- value |
|---------------------------------------|-----------------|--------------------------------------|--------------------------------------|-------------|-------------|
| Age years | 28.83±5.17 | 27.30±4.61 | 28.38±4.49 | 1.14 | 0.323 |
| S. Creat (µmol/l) | 58.83±32.06 | 65.68±28.50 | 65.57±27.57 | 0.81 | 0.448 |
| U. Protein (mg/dl) | 5.66±2.74 | 11.13±5.58 | 13.64±7.38 | 22.74 | 0.000* |
| U. Creat (mg/dl) | 62.63±15.36 | 82.06±26.29 | 72.39±28.98 | 5.81 | 0.004* |
| U. P: Cr (mg/mg) | 0.09±0.04 | 0.14±0.08 | 0.20±0.09 | 26.30 | 0.000* |
| eGFR (ml/min/1.73 m ²) | 134.83±47.71 | 126.50±38.41 | 129.57±33.75 | 1.38 | 0.254 |

*Significant, S. Creat- serum creatinine, U. protein –urine protein, U. creat-urine creatinine, U.P:Cr-urine protein creatinine ratio, eGFR- estimated glomerular filtration rate.

Table 2: Post hoc test comparing renal indices among the pregnancy terms with controls

| Parameter | Control n=40 | 2 nd Trimester n=40 | P- value | Control n=40 | 3 rd Trimester n=73 | P- value | 2 nd Trimester n=40 | 3 rd Trimester n=73 | p- value |
|------------|-----------------|--------------------------------------|-------------|-----------------|--------------------------------------|-------------|--------------------------------------|--------------------------------------|-------------|
| Age | 28.83±5.17 | 27.30±4.61 | 0.164 | 28.83±5.17 | 28.38±4.49 | 0.634 | 27.30±4.61 | 28.38±4.49 | 0.243 |
| S. Creat | 58.83±32.06 | 65.68±28.50 | 0.309 | 58.83±32.06 | 65.57±27.57 | 0.239 | 65.68±28.50 | 65.57±27.57 | 0.985 |
| U. Protein | 5.66±2.74 | 11.13±5.58 | 0.000* | 5.66±2.74 | 13.64±7.38 | 0.000* | 11.13±5.58 | 13.64±7.38 | 0.036* |
| U. Creat | 62.63±15.36 | 82.06±26.29 | 0.001* | 62.63±15.36 | 72.39±28.98 | 0.053 | 82.06±26.29 | 72.39±28.98 | 0.056 |
| U. P:Cr | 0.09±0.04 | 0.14±0.08 | 0.002* | 0.09±0.04 | 0.20±0.09 | 0.000* | 0.14±0.08 | 0.20±0.09 | 0.000* |
| eGFR | 134.83±47.71 | 126.50±38.41 | 0.363 | 134.83±47.71 | 129.57±33.75 | 0.141 | 126.50±38.41 | 129.57±33.75 | 0.850 |

*Significant, S. Creat- serum creatinine, U. protein –urine protein, U. creat-urine creatinine, U.P:Cr-urine protein creatinine ratio, eGFR- estimated glomerular filtration rate

Table 3: correlations between renal indices and pregnancy stages

| Parameters | Age | Gest. Age | U. Protein | U. Creat. | UP:Cr | S. Creat. |
|------------|---------|-----------|---------------|--------------|--------|-----------|
| Gest. Age | 0.094 | | | | | |
| U. Protein | -0.157 | 0.217* | | | | |
| U. Creat. | -0.083 | -0.251** | 0.373** | | | |
| UP:Cr | -0.087 | 0.369** | 0.667** | -0.352** | | |
| S. Creat. | 0.049 | 0.048 | -0.085 | -0.068 | -0.038 | |
| Egfr | -0.189* | -0.022 | 0.076 | 0.069 | 0.025 | -0.938** |

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

Discussion

The assessment of renal function in pregnancy other than the standard serum creatinine measurement may involve the determination of eGFR and urine protein:creatinine ratio. However, normal gestational ranges have not been established. The aim of this study is to define the difference in serum creatinine level, eGFR, urine protein:creatinine ratio in healthy pregnancy population compared with non-pregnant females to enhance the assessment of adverse pregnancy outcomes associated with maternal kidney function.

Results of the present study shows that urine protein creatinine ratio in pregnant women is higher in pregnant women than non-pregnant females. This finding is in line with that of Saxena *et al.* (2013). Random urine protein:creatinine ratio of non-pregnant females (0.08) as well as mid-trimester pregnancy (0.12) reported by Saxena *et al.* (2013) is similar

respectively with the 0.09 and 0.14 reported in this study. However, pre-elamptic protein creatinine ratio values reported by Shahbazian and Hosseini-Asl (2008) [0.2], Al *et al.* (2004) [0.19], and Dwyer *et al.* (2008) [0.19] are similar with normal third trimester pregnancy values [0.2] observed by our study.

The present study observed an eGFR of 126.5 ml/min/1.73 m² in second and 129.57 ml/min/1.73 m² in third trimester pregnancies. This finding is similar to the observations of Park *et al.* (2017) who found an eGFR within the range of 120–150 ml/min/1.73 m² in mid-term pregnancies. Similar with the present study Bae *et al.* (2017) found an eGFR of 128ml/min/1.73m² in normal third trimester pregnancies.

The present study observed serum creatinine values of non-pregnant controls, 2nd and 3rd trimester pregnancy as 58.83, 65.68 and 65.57

$\mu\text{mol/l}$ respectively. This in consonance with serum creatinine values of non-pregnant controls reported by Afolabi et al. (2016) [58.00 $\mu\text{mol/l}$], Babay et al. (2005) [58.00 $\mu\text{mol/l}$], and Schoenmakers et al. (2013) [59.00 $\mu\text{mol/l}$]. Serum creatinine levels of 64.00, 64.00, 66.00 $\mu\text{mol/l}$ has been respectively reported in 2nd trimester pregnancies by Al-Kuran et al. (2012), Pahl et al. (2001) and Järnfelt-Samsioe et al. (1985). In third trimester pregnancies 66.00, 64.00, 65.00 $\mu\text{mol/l}$ has been previously reported by Heguilén et al. (2007), Lohsiriwat et al. (2008) and Miri-Dashe et al. (2014) respectively. Equivalent serum creatinine values observed in 2nd and 3rd trimester normal pregnancies by this present study is in consonance with the observations of Iqbal et al. (2003), and Larsson et al. (2008).

The pattern of renal indices observed in this present study may be linked with haemodynamic changes that occur during normal pregnancy. Significant alterations in haemodynamics in normal pregnancy has been previously reported by Piccoli et al. (2010), these include; increases in cardiac output, sodium, and water retention, leading to blood volume expansion and reduction in systemic vascular resistance. The increase in renal blood flow and the involvement of several pathways, including the renin-aldosterone-angiotensin system or relaxin pathway, has been postulated as potential mechanisms of midterm renal hyperfiltration (Cadnapaphornchai et al., 2001; Conrad 2004; Davison et al., 2004). Decrease in concentration of serum creatinine has been linked with the physiologic increase in GFR during pregnancy. Thus, normal serum creatinine levels in the non-pregnant could depict renal impairment in a pregnant woman (Wiles et al., 2018).

Our study observed renal changes during normal pregnancy and these changes if exaggerated, may have impact on maternal and foetal health. We recommend routine biochemical assessment of renal function during antenatal care for timely identification and interventions aimed at achieving good maternal and foetal outcomes.

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