Journal of Indian Academy of Obstetrics and Gynaecology July 2021

Vol. 3, Issue 1

Original Article

MATERNAL PROTEINURIA IN TWIN COMPARED WITH SINGLETON PREGNANCIES

Mallick A[1]✉, Shirsath S[1], Jana D[2]

ABSTRACT

BACKGROUND: The exact amount of albumin filtered each day by kidneys is controversial. Normal rate of albumin excretion is less than 20 mg/day. The upper limit of the urinary protein excretion is 150 mg/d in normal non–pregnant women. Total protein excretion, however, increases to 150-250 mg daily in normal pregnancy due to increase in blood volume and, therefore, the glomerular filtration rate. This study was conducted to compare 24-hour urinary protein excretion in twin and singleton pregnancies, not complicated by hypertension.

MATERIALS AND METHODS: This is a prospective study done in the department of Obstetrics and Gynecology in R.G. Kar Medical College and Hospital, Kolkata from June, 2015 to May, 2016. A total of 86 women (43 twin and 43 singleton pregnancies) participated in this study. Six collections were inadequate based on creatinine excretion and were excluded. So, 80 women (40 twin and 40 singleton pregnancies) comprised the total cohort.

RESULT: In our study four twin pregnancies (10%) were found to have proteinuria ≥ 300 mg/day at the time of the specimen collection but no singleton pregnancy had this level of proteinuria. Of this4 twin pregnancies, 3 twin pregnancies were normotensive, yet they showed proteinuria ≥ 300 mg/day. Only one of this 4 twin pregnancies (who had proteinuria ≥ 300 mg/day) subsequently developed hypertensive disorder in pregnancy though statistically not significant (p 0.1238).

CONCLUSION: Twin pregnancy has more proteinuria as measured by 24-hour urine protein, than singleton pregnancy. And they are more likely to have proteinuria without hypertension and this value can exceed 300 mg/day.

KEY WORDS: Proteinuria, Singleton pregnancy, Albumin, Twin.

INTRODUCTION

The concept of maternal proteinuria and its diagnosis in pregnancy is utmost important. The exact amount of albumin filtered each day by kidneys is controversial. Normal rate of albumin excretion is less than 20 mg/day. The upper limit of the urinary protein excretion is 150 mg/d in normal non–pregnant women1. Total protein excretion, however, increases to 150-250 mg daily in normal pregnancy due to increase in blood volume and glomerular filtration rate too. The cut-off value for pathologic proteinuria in pregnancy (accepted as 300 mg of total protein

per 24 hours) was established using samples from pregnancies without preexisting medical conditions and prior to the onset of labor2. Proteinuria is an important criterion in diagnosing pre-eclampsia in pregnancy3. Pre-eclampsia is best described as a pregnancy-specific syndrome that can affect virtually every organ system. And, although pre-eclampsia is much more than simply gestational hypertension with proteinuria, appearance of proteinuria remains an important diagnostic criterion. Thus, proteinuria is an objective marker and reflects the systemwide endothelial leak, which characterizes the pre-eclampsia syndrome.

Presence of 30mg of protein in 100 ml of urine results in a positive reaction (1+) on a urinary dipstick. This is not very accurate since the severity of proteinuria is a function of quantity of protein as well as urine volume. Quantitative method for urine protein estimation are 24 hour urine protein estimation and Urine protein/creatinine ratio. Despite recent updates, urinary protein excretion remains an important parameter in evaluation of pre-eclampsia and differentiate preeclampsia from gestational hypertension. Twin pregnancy experience a 2-3 times increase risk of pre-eclampsia4 and likely to be affected more by all hypertensive disorders5 . Hypertensive disorders due to pregnancy are more likely to develop with multiple fetuses. The exact incidence attributable to twin gestation is difficult to determine because twin pregnancies are more likely to deliver preterm before pre-eclampsia can develop and because women with twin pregnancies are often older and multiparous. The incidence of pregnancy-related hypertension in women with twins is 20 percent at Parkland Hospital. Case-control analyses suggest that pre pregnancy body mass index (BMI) ≥ 30 kg/m2 and egg donation are additional independent risk factors for pre-eclampsia. The risk for pregnancy associated hypertension was significantly increased for triplets and quadruplets (11 and 12 percent, respectively) compared with that for twins (8%)6 . These data suggest that fetal number and placental mass are involved in pre-eclampsia pathogenesis. Although one study suggests that urinary protein excretion, as measured by urinary protein –to-creatinine ratio, is higher in twin pregnancy during third trimester7 , the same cut off value for proteinuria for diagnosis of pre-eclampsia is used for both twin and singleton pregnancy. No clear cut off value of proteinuria for diagnosis of pre-eclampsia in twin is mentioned in the literature. In a recent study done by Somerson et al 8 shows that mean 24-hour urinary protein excretion in twin pregnancies is greater than in singletons. These data suggest a reevaluation of the diagnostic criteria for pre-eclampsia in twin pregnancies. Hence more study is needed to address this issue. The objective of this study will be to determine 24 hour urinary protein excretion and the prevalence of proteinuria in twin and singleton pregnancies, which is not complicated by hypertension and this will give a clue whether urinary protein excretion value(in 24 hours) for the diagnosis of preeclampsia to be re-evaluated differently or not, in singleton and twin pregnancy.

MATERIALS AND METHODS

This is a prospective study done in the department of Obstetrics and Gynecology in R.G. Kar Medical College and Hospital, Kolkata from June, 2015 to May, 2016 with the aim of comparing 24-hour urinary protein excretion in twin and singleton pregnancies not complicated by hypertension. Pregnant women in between 18–45 years attending antenatal clinic in R.G. Kar Medical College and Hospital and carrying either twin or singleton pregnancies of 24-36 weeks gestation were included in the study. Women with urinary tract infections, any hypertension at initial checkup, presentational diabetes, autoimmune disorders, known renal disease, vaginal bleeding, and higher-order multiple gestations were excluded. Following ethical clearance and informed consent from the participants, the study began. Blood pressure was measured and normotensive participants between 24 weeks to 36 weeks were asked to submit 24 hour urine. Blood samples were drawn at the time of urine collection, for serum creatinine estimation. Participants with blood pressure ≥140/90 mm of Hg or urine suggestive of infection were discarded. To ensure adequate follow up to

observe for the development of hypertension, participants who delivered within 2 weeks of submitting the 24-hour urine, were excluded. Participants were advised to discard the first morning urine sample and collect all urine in dark container for 24 hour period ending with the next morning's void. They were instructed to avoid strenuous exercise and intercourse during the time of urine collection. Adequacy of collection were assessed using creatinine excretion as described by Clark et al15 with a range of 11-25 mg/kg was considered adequate at the time of specimen collection. Women admitted in antenatal ward were eligible only during the first 3 days of admission. Total urinary protein excretion was measured by the Biochemistry department of R.G. Kar Medical College and Hospital. All urine specimens were processed within 1 hour of arrival to the laboratory. To determine the total protein concentrations in the 24-hour urine specimens, the total urine volume (dL) was multiplied by the total urine protein concentration (mg/dL). 24-hour urine protein estimation was done by Eshbach's illuminometer. Demographic and clinical characteristics were noted. Participants were followed for up to 6 weeks postpartum to monitor for the development of hypertension, which was defined as new-onset blood pressure ≥140 mmHg systolic or ≥90 mmHg diastolic. The primary outcome was to calculate mean 24-hour urinary protein excretion. Secondary outcomes were proteinuria ≥300 mg in 24 hours and the incidence of hypertensive disorder during pregnancy.

In a study by Osmund son et al 8 proteinuria (300mg /day protein excretion or greater) occurred in 38% of twin and 8.2% of singleton pregnancies. Keeping in alpha error of 0.05 and 80% power, and attrition of 50% total sample size was calculated, which came to be 86 with 43 in each arm. So ultimately a total of 86 women (43 twin and 43 singleton pregnancies) were included in this study.

Results: Out of the 86 participants, 6 participants collections were inadequate based on creatinine excretion and were excluded. So, 80 women (40 twin and 40 singleton pregnancies) comprised the final cohort. The two groups were similar in most baseline demographic and clinical characteristics. Twin pregnancies delivered at an earlier gestational age compared to singleton pregnancies. Mean 24-hour urinary protein excretion was higher in twin than singleton pregnancies (196.30 mg compared with 145.45 mg, P <0.0001). The upper limit of the 95% confidence interval (CI) for the mean urinary protein excretion was 216. 2 mg in twin and 157. 14 mg in singleton pregnancies. Four twin pregnancies (ten percent) were found to have proteinuria ≥ 300 mg/day at the time of the specimen collection but no singleton pregnancy had this level of proteinuria. Ten percent of singleton (four participants) and fifteen percent of twin pregnancy (six participants) subsequently developed hypertensive disorder in pregnancy (total 10 participants). When the data were reanalyzed excluding these 10 participants (n = 70), the findings were consistent with the overall analysis i.e. mean 24-hour urinary protein excretion was higher in twin than singleton pregnancies (193.11765 mg compared with 141.33333 mg, P <0.0001) and 75% of twin who had 24 hour proteinuria ≥ 300 mg were normotensive. This study demonstrates that women with twin pregnancies excrete more protein as measured by a 24 hour urine collection.

Characteristic	Singleton Pregnancies	Twin Pregnancies	P value
AGE	24.45 ± 3.741	24.85 ± 3.325	0.6147
RELIGION – HINDU	16 (40)	12 (30)	0.4819
RELIGION – MUSLIM	24 (60)	28 (70)	0.4819
NULLIPARITY	20 (50)	22 (55)	0.8228
MULTIPARITY	20 (50)	18 (45)	0.8228
GESTATIONAL AGE AT DATA COLLECTION	28.98 ± 2.516	28.48 ± 2.230	0.3498
BMI	23.5625± 1.5561	23.7850± 1.1153	0.4689
SBP	119.15 ± 10.953	120.75 ± 10.961	0.5156
DBP	75.95 ± 6.118	77.40 ± 5.995	0.2876
URINE	17.25 ±	18 ±	0.232

CREATININE	2.90667	2.6602	3
SERUM CREATININE	0.72 ± 0.156	0.7 ± 0.165	0.5791
24 HOUR URINE PROTEIN	145.45 ± 38.276	196.30 ± 62.223	<0.0001
TOTAL PROTEIN ≥300mg	00	4(10)	0.1238
GESTATIONAL AGE AT DELIVERY	38.1 ± 2.318	35.43 ± 1.752	<0.0001
HYPERTENSIVE DISORDER IN PREGNANCY	4(10)	6(15)	0.7353
24 HOUR URINE PROTEIN EXCLUDING THOSE WHO DEVELOP HYPERTENSION .LATER	141.33333± 37.47761	193.11765 ±59.76356	<0.0001

Table: Difference of mean of various Characteristics vs Pregnancies

Discussion

This study supports the hypothesis that baseline urinary protein excretion is greater in twin pregnancies. In this study mean 24-hour urinary protein excretion was higher in twin than singleton pregnancies (196.30 mg compared with 145.45mg P<0.0001).The upper limit of the 95% confidence interval (CI) for the mean urinary protein excretion was 216.2 mg in twin and 157.69 mg in singleton pregnancies. And even after excluding the subjects who subsequently develop hypertension mean 24- hour urinary protein excretion was still higher in twin than singleton pregnancies (193.12mg compared with 141.33 mg, P <0.0001). Previously Osmund son et al[8] compared 24-hour urinary protein excretion in twin and singleton pregnancies not complicated by hypertension. They evaluated mean 24-hour urinary protein excretion in twin and singleton pregnancies between 24 weeks and 36 weeks of gestation. Mean urinary protein excretion was higher in twin compared with singleton pregnancies (269.36124.1 mg compared with 204.3692.5 mg, P 0.004). Published studies comparing urinary protein excretion in twin and singleton pregnancies are limited. Smith et al compared urine protein-to creatinine ratios in 51 twin and 51 singleton pregnancies at three time points across gestation. They found that the urine protein-to-creatinine ratio increased significantly over gestation in all pregnancies. Additionally, they found that the odds of an elevated urine protein-to-creatinine ratio—defined as greater than 0.19—was significantly higher in twin compared with singleton pregnancies but only in the late third trimester (34–38 weeks of gestation). Despite the lack of published studies comparing twin and singleton renal physiology, it is biologically plausible that urine protein excretion is higher in twin pregnancies. Pregnancy increases filtration of urinary proteins resulting in increased urine protein excretion compared with the nonpregnant state [9]. This is thought to occur as a result of progesterone-induced permeability of the glomerular basement membrane and a 50% increase in the glomerular filtration rate established as early as the first trimester[10] . In twin pregnancies, cardiac output increases by an additional 20% and blood volume increases by an additional 10% compared with singleton pregnancies [11]. Theoretically, increased cardiac output could lead to an increased glomerular filtration rate resulting in more filtration of protein and more protein excretion. Alternatively, greater proteinuria in twin pregnancy might represent slightly greater accumulation of placental derived vasoactive factors such as sFlt-1, which has been associated with albuminuria in normal pregnancies[12]. So our study strengthen the hypothesis that baseline urinary protein excretion is greater in twin pregnancies. Although 300 mg/day of urinary protein excretion is considered as abnormal in pregnancy, it is not clear how this threshold originated[10] . In a study by Higbee K[13] on

normal values of urinary albumin and total protein excretion during pregnancy, 270 healthy pregnant women ≤ 35 years without a history of diabetes, hypertension, pyelonephritis, preeclampsia, or renal or connective tissue disease were evaluated. They found a mean urinary protein excretion of 117 mg and an upper 95% CI limit of 260 mg. Kuo and colleagues[14] reported an upper limit of the 95% CI of less than 150 mg among their population of 205 women with singleton pregnancies. But mean 24-hour urine protein excretion was not reported in this study. So our study also adds to the current literature regarding normal values for urinary protein excretion in pregnancy.

In our study four twin pregnancies (10%) were found to have proteinuria ≥ 300 mg/day at the time of the specimen collection but no singleton pregnancy had this level of proteinuria. And only one of these twin pregnancies (who had proteinuria ≥ 300 mg/day) subsequently developed hypertensive disorder in pregnancy. Rest three twin pregnancies were normotensive, yet they showed proteinuria ≥ 300 mg/day. Though statistical analysis of 24-hour urine protein ≥ 300 mg in singleton and twin pregnancies was not significant (P 0.1238) in our study, in the study by Osmund son et al[8] proteinuria ≥ 300 mg/day occurred in 38.0% of twin and 8.2% of singleton pregnancies and statistical analysis of their study showed significant proteinuria (≥ 300mg /day) in twin pregnancies compared with singleton pregnancies (P<0.001). Proteinuria ≥ 300mg /day is the cut off value for the diagnosis of hypertensive disorder in pregnancy and currently this value stands for all pregnancies ─ singleton/ twin/ higher order multiple gestations. Our study showed three normotensive twin pregnancies had proteinuria ≥ 300 mg/day. And similar finding was noted in the study by Osmundson et al[8]. Hypertensive disorder in pregnancy is an important cause of maternal morbidity and mortality in pregnancy. So, an appropriate criterion for its diagnosis is utmost important. These data suggest a re-evaluation of the diagnostic criteria for pre-eclampsia in twin pregnancies. Hence more study is needed to address this issue.

In our study four singleton and six twin pregnancies developed hypertensive disorder in pregnancy later in study period. So a total of 10 pregnancies out of 80 participants developed hypertensive disorder in pregnancy. Incidence of hypertensive disorder in pregnancy in our study is 8% which correlated with the reported incidence of pre-eclampsia (2 - 8% of pregnancies worldwide).

So in conclusion, twin pregnancy had significantly more proteinuria as measured by 24 hour urine protein, than singleton pregnancy. And they are more likely to have proteinuria without hypertension and this value can exceed 300 mg/day. So, a reevaluation of the diagnostic criteria for preeclampsia in twin pregnancies is needed.

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Received: 11.07.2021
Accepted: 29.07.2021
Published online: 30.07.2021
Citation: Mallick A, Shirsath S, Jana D.Maternal proteinuria in twin compared with singleton pregnancies. J Indian Acad Obstet Gynecol. 2021;3(1):10-15

1. Department of Obstetrics and Gynaecology, College of Medicine and JNM Hospital, WBUHS, Kalyani, Nadia, West Bengal
2. Department of Gynecology and Obstetrics Institute of Post-graduate Medical Education and Research, A.J.C. Bose Road, Kolkata-700020, West Bengal, India.
✉ Email: arumallick88@gmail.com