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Original Article

Obstetrics and Gynaecology Section

Detection of Creatinine in Vaginal Secretions of Women with Premature Rupture of Membranes in Third Trimester from Semiurban Population: A Cross-sectional Study

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ABSTRACT

Introduction: Spontaneous rupture of the membranes any time beyond the 28th week of pregnancy but before the onset of labour is called Premature Rupture of Membranes (PROM). There are many techniques for diagnosing PROM that is based on both clinical assessments and biological studies. Each of these procedures has benefits and drawbacks. Since, foetal urine is the major source of amniotic fluid, the presence of vagina and a high creatinine level may aid in the diagnosis of PROM.

Aim: To determine the cut-off levels of serum creatinine in predicting the PROM.

Materials and Methods: A cross-sectional study was conducted at Dr. D.Y. Patil Medical College, Hospital and Research Centre in Pune, Maharashtra, India from June 1 2020 to June 5 2021. Two equal groups of 140 each pregnant women with gestational ages ranging from 28-42 weeks were created: one with a history of vaginal leaking (the study group P), and the other with gestationally matched no leakage (the control group C). Patient's details such as age, gestational age, and obstetric history were noted. Each patient was examined and cervicovaginal secretions were collected. A 5 mL of saline was flushed in the posterior fornix of vagina. This fluid with vaginal wash was aspirated and creatinine concentration from vaginal fluid was determined by Jaffee synthetic chemical colorimetric method. The Student's t-test and the Chi-square test were used to analyse the data.

Results: The study group's vaginal fluid creatinine level was substantially higher (p-value <0.001). The cut-off value of 0.6 mg/dL had highest sensitivity 96.1% and specificity 100%. The area under the Receiver Operating Characteristics (ROC) curve was 0.917. The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of serum creatinine at cut-off 0.6 mg/dL was 100% and 96.3%, respectively.

Conclusion: Vaginal fluid creatinine is simple and easily available, which makes it acceptable to the candidate for a diagnosis of PROM.

Keywords: Amniotic membrane, Fetoprotein, Fibronectin, Gonadotropin, Prelabour, Prolactin

INTRODUCTION

Rupture of the amniotic sac before the onset of labour is referred to as PROM. Risk factors include: low socio-economic status, underweight women, preterm labour history, urine infection, vaginal bleeding, cerclage, and amniocentesis [1]. It complicates about 8-10% of term pregnancies and approximately 1% of preterm pregnancies [2]. Gestational age and co-morbidities determine the course of treatment [3]. One of the most frequent side-effects of preterm PROM is early delivery. The length of the latent period, which is the interval between membrane rupture and delivery, is inversely related to the gestational age at which PROM takes place [4]. An analysis of a study evaluating patients with preterm PROM between 16 and 26 weeks' gestation found that 57% of patients delivered within one week, and 22% had a latent period of four weeks [5]. For instance, a large study of patients at term found that 95% of patients delivered within roughly one day of PROM [4]. PROM is diagnosed by amniotic fluid at posterior fornix with a speculum examination. Nitrazine test observing a basic pH change on indicator paper. A fern test of dried amniotic fluid under the microscope [6]. An ultrasound, but rarely done as one cannot differentiate PROM from other causes of oligohydramnios [7]. Detection of alpha fetoprotein, insulin growth factor binding protein-1, foetal fibronectin, human chorionic gonadotropin, prolactin, urea, creatinine [8-12].

For gestational age-specific obstetric measures to improve perinatal outcome and reduce significant consequences, like cord prolapse and infectious morbidity, a primary and accurate diagnosis of preterm PROM would be necessary (chorioamnionitis, neonatal sepsis). On the other hand, a false-positive preterm PROM diagnosis may result in needless obstetric treatments, such as hospitalisation, the administration of antibiotics and corticosteroids, or even labour induction. Therefore, an early and precise PROM diagnosis is essential for maximising pregnancy outcomes; in fact, it is so important that an amnio-dye test (also known as a tampon test) may be advised if, other tests for preterm PROM are in conclusive and if, the pregnancy is far from the term. Amniocentesis is used during this test, and the amniotic cavity is injected with dye [13].

The PROM diagnosis is challenging and unreliable with conventional techniques. The present study sought to establish the cut-off value and assess the validity of vaginal washing fluid creatinine for PROM diagnosis.

MATERIALS AND METHODS

A cross-sectional study was conducted at Dr. D.Y. Patil Medical College, Hospital and Research Centre in Pune, Maharashtra, India, from June 1 2020 to June 5 2021. Institutional Ethics Committee (IEC) approval was obtained (IESC/PGS/2019/137). Prior to the study, the patients provided written informed consent.

Inclusion criteria:

- Age group >18 years
- Confirmed PROM cases by positive nitrazine test and positive fern test
- Gestational age 24 to 36 weeks

- Cervical dilatation < 3 cm
- High-risk pregnancies include those in which the mother experienced preterm PROM during a previous pregnancy, preterm labour in the past, direct abdominal trauma, polyhydramnios, or numerous pregnancies.

Exclusion criteria:

- Active vaginal bleeding
- Preeclampsia
- Liver or kidney disease
- Vaginal bleeding or vaginal infection
- Foetal congenital anomalies or intrauterine foetal death
- Any circumstances that might affect the creatinine amounts in vaginal fluid.

Sample size calculation: The sample size included 280 women who complained of PROM, fitting the inclusion criteria. The prevalence of PROM was reported to be 10% [2], precision was set at 5%=0.05, Z value at 95% confidence=1.96. Therefore, a total of 140 patients with confirmed PROM and 140 patients without PROM were enrolled in the study.

Study Procedure

Patient's details such as age, gestational age, obstetric history were noted. General and systemic examination was performed. Each patient was examined and cervicovaginal secretions were collected. These secretions were subjected to:

- **Fern test:** A small drop of sample was spread evenly on a glass slide and air dried. It was observed under the light microscope (10x magnification) for an arborisation pattern. A single observer performed the test in all patients.
- **Nitrazine test:** In the deep vagina, a cotton tip applicator was used to deposit the substance onto nitrazine paper. Change in colour was suggested, if pH <6.5 and test was considered positive [14].
- **Measurement of creatinine levels:** The posterior fornix of the vagina was flushed with 5 mL of saline. The same syringe was used to aspirate both fluids, which were then submitted right away to the central laboratory for creatinine measurement. Creatinine concentration from vaginal fluid was determined by Jaffee synthetic chemical colorimetric method [15].

STATISTICAL ANALYSIS

Mean and Standard Deviation (SD) were used to present quantitative data. According to the findings of the normalcy test, a comparison between the research groups was carried out using an unpaired t-test. The Fisher's-exact test, Student's t-test, and Chi-square test were used to see, whether there was any association between the research groups. A p-value <0.05 was considered significant. Analysis of Variance (ANOVA) test was used to find whether there was a significant association between serum creatinine levels with the gestational age of patients. For statistical analysis, appropriate statistical software was utilised, such as MS Excel and Statistical Package for the Social Sciences (SPSS) version 20.0.

RESULTS

The majority of the patients in group P were in the age range of 21 to 30. The average age for group P was 26.49±5.03 years. Most of the patients in group C were between the ages of 21 and 30. In group C, the average age was 27.11±4.80 years [Table/Fig-1].

It was observed that 48.6% and 51.4% patients in group P were primigravida and multigravida, respectively, while 42.1% and 57.9% patients in group C were primigravida and multigravida, respectively [Table/Fig-2].

The mean gestational age of patients in group P and group C was 31.09±3.35 weeks and 31.53±3.37 weeks, respectively [Table/Fig-3].

Age (years)	Group P		Group C		p-value
	N	%	N	%	
18-20	20	14.3	13	9.3	0.411
21-30	89	63.6	92	65.7	
>30	31	22.1	35	25	
Total	140	100	140	100	
Mean±SD (years)	26.49±5.03		27.11±4.80		

[Table/Fig-1]: Distribution of patients according to age.
Test used: Student's t-test

Parity	Group P		Group C		p-value
	N	%	N	%	
Primigravida	68	48.6	59	42.1	0.337
Multigravida	72	51.4	81	57.9	
Total	140	100	140	100	

[Table/Fig-2]: Distribution of patients according to parity.
Test used: Chi-square test

Gestational age (weeks)	Group P		Group C		p-value
	N	%	N	%	
24-28	31	22.1	27	19.3	0.274
29-32	53	37.8	49	35	
33-36	56	40.1	64	45.7	
Total	140	100	140	100	
Mean±SD (weeks)	31.09±3.35		31.53±3.37		

[Table/Fig-3]: Distribution of patients according to gestational age.
Test used: Student's t-test

In group P, the cervix was 1 cm dilated in 47.9% patients, while it was 2 cm and 3 cm dilated in 30% and 22.1% patients, respectively. In group C, the cervix was 1 cm dilated in 49.3% patients, while it was 2 cms and 3 cms dilated in 31.4% and 19.3% patients, respectively [Table/Fig-4].

Cervical dilatation (cm)	Group P		Group C		p-value
	N	%	N	%	
1	67	47.9	69	49.3	0.839
2	42	30	44	31.4	
3	31	22.1	27	19.3	
Total	140	100	140	100	

[Table/Fig-4]: Distribution of patients according to cervical dilatation.
Test used: Chi-square test

The mean vaginal fluid creatinine was considerably higher in group P than group C [Table/Fig-5].

There was no significant association of serum creatinine levels with gestational age of patients as per ANOVA test (p>0.05) in both the groups [Table/Fig-6]. A cut-off value of 0.6 mg/dL for serum creatinine had the best sensitivity (96.1%) and specificity (100%). Serum creatinine's positive and negative predictive values are 100% and 96.3%, respectively. The ROC curve's area under it, was 0.917 [Table/Fig-7,8].

Vaginal fluid creatinine (mg/dL)	Group P	Group C	p-value
	Mean±SD	Mean±SD	
	1.78±0.52	0.36±0.14	0.001

[Table/Fig-5]: Patient distribution according to vaginal fluid creatinine (mg/dL).
Test used: Student's t-test

DISCUSSION

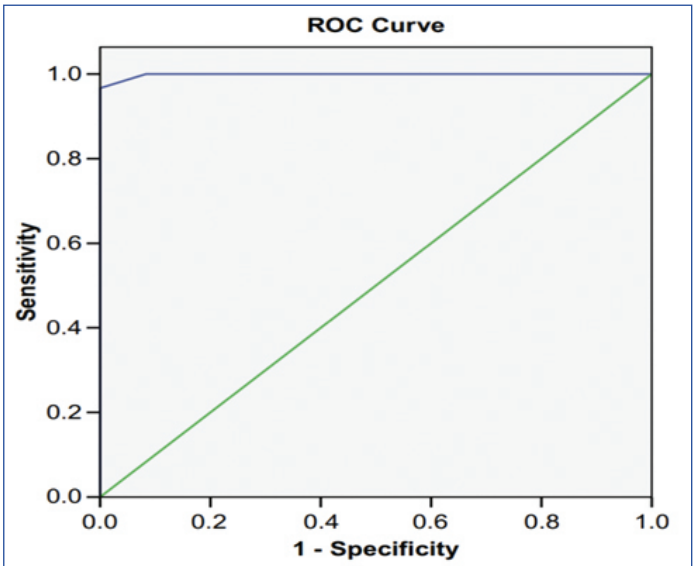
There is no non invasive method that can be used as the gold standard. Other non invasive diagnostic methods, which include pooling assessment, microscopic fern test, and pH examination

Gestational age (weeks)	Serum creatinine (mg/dL)			
	Group P		Group C	
	Mean	SD	Mean	SD
24-28	1.85	0.52	0.32	0.12
29-32	1.76	0.52	0.34	0.15
33-36	1.75	0.53	0.40	0.14
p-value	0.669		0.8	

[Table/Fig-6]: Association of serum creatinine levels with gestational age of patients. Test used: ANOVA test

Serum creatinine				
Cut-off	1	0.8	0.6	0.4
Sensitivity	78.7%	85.3%	96.1%	86.1%
Specitivity	100%	100%	100%	100%
Positive predictive value	100%	100%	100%	100%
Nagative predictive value	81.7%	86.8%	96.3%	88.1%

[Table/Fig-7]: Serum creatinine cut-off value.



[Table/Fig-8]: ROC curve analysis for determination of optimal cut-off value of serum creatinine in predicting the PROM.

of cervicovaginal discharge (nitrazine test) are not cost-effective, and diagnostic accuracy is not high, if time has passed, since membranes ruptured. The gold standard test currently used for diagnosis of PROM, the amnio-dye test, is invasive and there are risks of infection, abruption, and abortion [16]. Recently, the focus of studies has been to detect various biochemical markers in cervicovaginal discharge, when ROM occurs. These markers include alpha fetoprotein, foetal fibronectin, insulin growth factor binding protein-1, prolactin, beta human chorionic gonadotropin, urea, lactate, placental alpha-microglobulin-1 and 2, and creatinine. Authors have speculated that, analysis of vaginal creatinine and urea can be used as a foetal maturation test, in cases of preterm labour, as the creatinine level depends on gestational age [17-19]. In the present study, the majority of the patients (63.6%) in group P were in the age group of 21-30 years. The mean age in group P was 26.49±5.03 years. The mean age in group C was 27.11±4.80 years. There was no significant difference between the groups as per Student's t-test (p-value >0.05). In a study by Sharma A et al., mean ages were similar, in all three groups (23.44, 23.40, and 23.30 in Group 1 (Confirmed PROM), group 2 (Unconfirmed PROM), and control group respectively) [18].

In the present study, 68 (48.6%) and 72 (51.4%) patients in Group P were primigravida and multigravida, respectively, while 59 (42.1%) and 81 (57.9%) patients in group C were primigravida and multigravida respectively. Sharma A et al., study, showed most of the patients were primiparous and were well-matched in all the three groups [18]. The mean gestational age of patients in group P and group C was 31.09±3.35 weeks and 31.53±3.37 weeks respectively. There was no significant difference between the groups. This was similar to the studies of Sharma A et al., and Begum J et al., [18,19]. Sharma A et al., found mean gestational age in weeks were 34.86, 34.98, 35.11 in group 1, group 2 and control group, respectively [18]. Begum J et al., study, evaluating cervicovaginal fluid for urea and creatinine for diagnosis of PROM had no particular difference between both groups with respect to maternal age, obstetric score and gestational age of the participants [19]. A creatinine concentration of 1.75 mg/dL or more correlated significantly with a gestational age of 37 weeks or more [3]. It was observed in present study that, the mean vaginal fluid creatinine was strikingly higher in group P than in group C. The mean vaginal fluid creatinine in groups I, II, and III were 1.74±0.8 mg/dL, 0.45±0.2 mg/dL and 0.25±0.1 mg/dL, respectively. The creatinine level was significantly higher in the confirmed group (group I) than in the other two groups (p-value <0.0010) as reported in Zanjani MS and Haghighi L study [20]. The mean urea levels were 26.35 mg/dL in the study group 1 and 3.12 mg/dL in the control group in Sharma A et al., study [18].

Malchi F et al., reported, the overall mean of creatinine in the case group was significantly higher than the control. It was noted that the sensitivity and specificity of creatinine was higher than urea in the diagnosis of PROM [21]. Sharma A et al., cross-sectional study assessing creatinine levels in vaginal fluid as a marker of PROM found cervicovaginal fluid creatinine levels were more marked in women with confirmed leaking than the women in the control group [18]. The findings of these studies were in agreement with the findings of the index study. In a study done by Zanjani MS and Haghighi L study, evaluating the reliability of vaginal fluid creatinine for the diagnosis of PROM showed mean vaginal fluid creatinine in groups I, II, and III were 1.74±0.8, 0.45±0.2 and 0.25±0.1 mg/dL, respectively. The creatinine level was significantly higher in the confirmed group (group I) than in the other two groups (p<0.0010) [20]. In the present study, for serum creatinine a cut-off 0.6 mg/dL had highest sensitivity 96.1% and specificity of 96.3%. The area under the ROC curve was 0.917. Similar observations were noted in other studies [17-19,22-24]. The sensitivity, specificity, positive predictivity, and negative predictivity were all 100% in detecting PROM by evaluation of vaginal fluid urea and creatinine concentration with a cut-off value of 12 and 0.6 mg/dL, respectively [17].

Limitation(s)

Contamination of sample by blood, urine, or meconium. The estimation of vaginal fluid creatinine level is effective for patients not in labour and it is not accurate for patients with congenital foetal malformations.

CONCLUSION(S)

Correct and prompt diagnosis of the condition is crucial to achieving the best pregnancy result. A proper diagnosis should serve as the foundation for management. This test is appealing in clinical practice due to its accessibility and simplicity. Vaginal fluid creatinine is, therefore, a potential contender to be accepted as a test for PROM diagnosis.

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