Vaginal Fluid Creatinine versus Human Chorionic Gonadotropin for Diagnosis of Premature Rupture of Membranes.

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Abstract: This prospective case-control study was done to compare the reliability of vaginal fluid creatinine and quantitative human chorionic gonadotropin for diagnosis of premature rupture of membranes**. Patients and Methods:** The study included 150 pregnant women between 25-34 weeks of gestation attending Al-Azhar University Hospitals. They were divided into three groups: Group (I) consisted of 50 patients with positive history of vaginal leakage and positive fluid leakage observed using sterile Cusco speculum. Group (II) consisted of 50 patients with positive history of vaginal leakage and negative fluid leakage observed using sterile Cusco speculum. Group (III) consisted of 50 pregnant women without any complaint or complication. All patients underwent full history, general examination, abdominal examination and sterile Cusco speculum examination. The vagina was washed by injection with a syringe filled with 3ml of saline solution, and 3ml the washing fluid was collected from the posterior vaginal fornix. The collected fluid was sent immediately to the laboratory for measuring of vaginal fluid creatinine & quantitative HCG. **Results:** The study showed that there was no significant statistical difference between confirmed, suspected and control groups as regard maternal age, parity and gestational age. There was significant statistical difference between confirmed, suspected and control groups as regard amniotic fluid index. The number of patients with AFI≤ 9 cm was 32 patients in confirmed group, 17 patients in suspected group and 4 patients in the control group. On the other hand the patients with the AFI >9 cm was 18 patients in confirmed group, 33 patients in suspected group and 46 patients in the control group. Analysis of results using Receiver-operator characteristic (ROC) curve showed that the best cutoff point for vaginal fluid creatinine among the studied groups in our study was 0.7 mg/dl with sensitivity, specificity, +ve predictive value, -ve predictive value and accuracy were all 100%. The number of patients who exceeded the cutoff point for vaginal fluid creatinine was 50 patients in confirmed group, 22 patients in suspected group and no patients in the control group. Analysis of results using ROC curve showed that the best cutoff point for vaginal fluid HCG among the studied groups in our study was 47.0 mIU/mL with sensitivity 94%, specificity 86%, +ve predictive value 93.1%, -ve predictive value 87.8% and accuracy 91.3%. The number of patients who exceeded the cutoff point for vaginal fluid HCG was 50 patients in confirmed group, 27 patients in suspected group and 8 patients in the control group. From the results of our study we could show that both vaginal fluid creatinine and HCG concentrations are good predictors of PROM but measurement of vaginal fluid creatinine is more reliable and less expensive than measurement of vaginal fluid HCG in diagnosing PROM.

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1. Introduction:

Premature rupture of membranes (PROM) is a condition which occurs in beyond 37 weeks gestation and has presented by rupture of membranes before the onset of labor. Preterm premature rupture of membranes (PPROM) is ROM prior to 37 weeks' gestation. Spontaneous premature rupture of the membranes (SPROM) is ROM after or with the onset of labor**. (Deering *et al.,* 2007).**

Premature rupture of the membranes (PROM) occurs in 10% of all gestations and about 2–4% of preterm pregnancies, with complications such as infection and preterm birth **(Kafali and Oksuzler, 2007).**

Spontaneous membrane rupture occurs physiologically at term either before or after the onset of symptomatic contractions. This is believed to be related to progressive weakening of the membranes seen with advancing gestation, largely due to collagen remodeling and cellular apoptosis. When PROM occurs before term, the process of membrane weakening may be accelerated by a number of factors such as stretch, infection, inflammation and local hypoxia (**Francois and Goffinet, 2005).**

Numerous risk factors are associated with PROM as smoking, low socioeconomic status, negros, history of sexually transmitted infections, history of previous preterm delivery, uterine over distension (e.g. polyhydramnios and multiple pregnancy) **(Savitz *et al.*, 1991).**

There is evidence demonstrating an association between ascending infection from the lower genital tract and PPROM. In women with PPROM about one third of pregnancies have positive amniotic fluid cultures and studies have shown that bacteria have the ability to cross intact membranes **(Carroll *et al.*, 1996).**

Diagnosis of PROM is confirmed when there is a demonstration of amniotic fluid leakage from the cervix, but more difficult when there is doubt as to whether PROM has occurred or not. Failure to identify patients with membrane rupture can result in failure to implement obstetric measures, while the false diagnosis can lead to inappropriate interventions such as hospitalization or labor induction (**Medina and Hill, 2006).**

The methods used to diagnose PROM are variable and based as much on clinical evaluation as on biological tests, which are useful in the cases of clinically asymptomatic patients and/or in the ones with unclear PROM. These tests include the measurement of vaginal pH, prolactin, α-fetoprotein, di-amine oxidase, insulin-like growth factor binding protein-1 (IGFBP- 1), human chorionic gonadotropin and fetal fibronectin. All these tests have advantages and drawbacks. Up till now there is no gold standard diagnostic test for PROM **(Kafali and Oksuzler, 2006).**

Gurbuz *et al.*, 2003, hypothesized that vaginal fluid creatinine may be helpful in diagnosing PROM because fetal urine is the most important source of amniotic fluid in the second half of pregnancy.

The beta subunit of human chorionic gonadotropin (β-hCG) has been evaluated as a possible predictor of preterm delivery and as a marker for PPROM. Human chorionic gonadotropin is produced by trophoblastic tissue, which is present in varying degrees in serum, urine, and amniotic fluid during pregnancy. Previous investigators have established quantitative ranges and thresholds of HCG concentrations in pregnant women with and without ruptured membranes during each trimester **(Kim *et al.*, 2005).**

Aim of the study:

Comparing the reliability of vaginal fluid creatinine and quantitative human chorionic gonadotropin for diagnosis of premature rupture of membranes. The achieved goal of this study is to compare the reliability of vaginal fluid creatinine and vaginal fluid HCG. The false diagnosis of rupture of membranes can lead to inappropriate intervention such as hospitalization or induction of labor. Therefore, it is highly desirable to establish a definite diagnosis of rupture of membranes in uncertain cases without delay, however, traditional diagnostic methods and tests has some limitation and cannot be applied to all patients with 100% accuracy.

2.Patients & Methods

This is a prospective case control study was done at Al-Azhar University Hospitals for the period of 2 years. The study was conducted on 150 pregnant women with the following inclusion and exclusion criteria:

Inclusion criteria:

1- Gestational age between 25- 34 weeks

2- Single intrauterine pregnancy

3- No fetal congenital malformation

4- Not suffering from any medical problems as diabetes mellitus or heart disease with pregnancy

Exclusion criteria:

1- Gestational age below 25 week

2- Multiple intrauterine pregnancies

3-Fetal distress

4-Vaginal bleeding

5- Any pregnancy with any medical problems as diabetes mellitus or heart disease. They are divided into three groups: Group(I) (Confirmed PROM group), Group (II) (Suspected but unconfirmed PROM group), Group (III) (Control group).

- **Group I (Confirmed PROM group)**

This group included 50 pregnant women with positive history of vaginal leakage and positive fluid leakage from the cervix observed using sterile Cusco speculum examination.

- **Group II (Suspected but unconfirmed PROM group)**

This group included 50 pregnant women with positive history of vaginal leakage and negative fluid leakage from the cervix observed using sterile Cusco speculum examination. Or vaginal pooling with negative nitrazine paper test

- **Group III (Control group):** This group included 50 normal pregnant women who attended the outpatient clinic for routine antenatal care with no signs or symptoms suggestive of PROM.

All patients included in the study were subjected to:

1- All pregnant women included, signed an informed consent explaining the aim of study.

2-Full history taking**:**

Including, age, parity, last menstrual period, amniotic fluid leakage (onset, amount, duration, color of the fluid, etc…).

General examination:

Including, blood pressure, pulse, temperature, etc…

4-Abdominal examination:

Including, fundal level, uterine contraction, fetal heart rate.

5-Local examination by sterile Cusco speculum:

To detect amniotic fluid leakage coming from the cervix and for sample collection.

6-Transabdominal U/S for:

Gestational age, amniotic fluid index calculated by 4 quadrants method, fetal life, placental site and congenital fetal malformation.

Sample collection:

- Patients lied in lithotomy position.
- Sterile vaginal examination using a sterile Cusco speculum under complete aseptic conditions was done then vaginal fluid sampling was taken as follows: 3 ml of sterile saline solution was injected into the posterior vaginal fornix and 3 ml was aspirated by the same syringe and sent to the laboratory for measuring of vaginal fluid creatinine & quantitative HCG.
- Any sample contaminated with blood was excluded.

Method of measuring creatinine & HCG:

- The sample was placed in a plastic tube then we put the tube in the centrifuge for 10 min.
- We aspirate 0.5 ml of the centrifuged sample then we put it in Hitachi Roche 902 Automatic Analyzer for 15 min.
- Lastly we record the level of creatinine & HCG.

Statistical Methodology:

Analysis of data was done by IBM computer using SPSS (statistical program for social science) version 19 as follows:

- **Description** of quantitative variables as mean, SD and range.
- **Description** of qualitative variables as number and percentage.
- **ANOVA test (Analysis of Variance)** was used to compare quantitative variables between groups.
- **Chi-square test** was used to compare qualitative variables between groups.
- **ROC(Receiver operator characteristic curve)** was used to find out the overall predictivity of parameter in and to find out the best **Cutoff** point with detection of **Sensitivity, specificity, +ve predictive value (+PV), -ve predictive value (-PV) and accuracy** at this **Cutoff** point.
- **Diagnostic validity test:** It includes:

a. The diagnostic sensitivity: It is the percentage of diseased cases truly diagnosed (TP) among total diseased cases (TP+FN):

$$\textbf{Sn\%} = \frac{\textbf{True Positive}}{\textbf{True positive + false negative}}$$

b. **The diagnostic specificity**: It is the percentage of non-diseased truly excluded by the test (TN) among total non-diseased cases (TN+FP):

$$\textbf{=Sp\%} = \frac{\textbf{True Negative}}{\textbf{True negative + false positive}}$$

c. **The predictive value for a +ve test:** It is the percentage of cases truly diagnosed among total positive cases:

$$\textbf{P+\%} = \frac{\textbf{True Positive}}{\textbf{True positive + false positive}}$$

b. **The predictive value for a -ve test:** It is the percentage of cases truly negative among total negative cases:

$$\textbf{P-\%} = \frac{\textbf{True Negative}}{\textbf{True negative + false negative}}$$

d. **The efficacy or the diagnostic accuracy of the test:** It is the percentage of cases truly diseased plus truly non-diseased among total cases:

$$\text{Eff. \%} = \frac{\text{True Positive + True Negative}}{\text{True positive + false positive + true negative + false negative}}$$

Level of significance:

- <0.05 significant
- <0.001 highly significant
- >0.05 not significant

3. Results:

The study included 150 pregnant women between 25-34 weeks of gestation divided into three groups (confirmed, suspected and control groups).

Table (1): Comparison between confirmed, suspected and control groups as regard age of the patients.

Group	Age (years)					Analysis of variance	
	Range			Mean	± SD	F	*P*-value
Confirmed PROM	18.00	-	40.00	26.18	± 5.50	0.166	0.847
Suspected PROM	18.00	-	40.00	26.82	± 5.89		
Control	18.00	-	40.00	26.34	± 5.95		

Table (1) shows that there was no statistical significant difference between confirmed, suspected and control groups as regard age of the patients (P>0.05).

Table (2): Comparison between confirmed, suspected and control groups as regard gestational age of the patients.

Group	Gestational Age (weeks)					Analysis of variance	
	Range			Mean	± SD	F	*P*-value
Confirmed PROM	26.00	-	34.00	30.20	± 2.25	1.24	0.29
Suspected PROM	26.00	-	34.00	30.06	± 1.87		
Control	25.00	-	33.00	30.76	± 2.81		

Table (2) shows that there was no statistical significant difference between confirmed, suspected and control groups as regard gestational age of the patients (*P*>0.05).

Table (3): Comparison between confirmed, suspected and control groups as regard parity of all patients.

Parity		Group			Total
		Confirmed PROM	Suspected PROM	Control	
PG	Number	14	9	10	33
	%	28.0%	18.0%	20.0%	22.0%
P1	Number	14	14	11	39
	%	28.0%	28.0%	22.0%	26.0%
P2	Number	11	15	12	38
	%	22.0%	30.0%	24.0%	25.3%
P3	Number	5	8	9	22
	%	10.0%	16.0%	18.0%	14.7%
P4-7	Number	6	4	8	18
	%	12.0%	8.0%	16.0%	12.0%
Total	Number	50	50	50	150
	%	100.0%	100.0%	100.0%	100.0%
Analysis using **Chi-Square Tests**		X^2		4.934	
		P-value		0.765	

Table (3) shows that there was no statistical significant difference between confirmed, suspected and control groups as regard parity of all patients (P>0.05).

Table (4): Comparison between confirmed, suspected and control groups as regard amniotic fluid index (Normal = 9:15 cm).

Group		AFI <=9 cm		AFI >9 cm		Total	
		N	%	N	%	N	%.
Confirmed PROM		32	64.00	18	36.00	50	100.00
Suspected PROM		17	34.00	33	66.00	50	100.00
Control		4	8.00	46	92.00	50	100.00
Total		53	35.33	97	64.66	150	100.00
Chi-square	**X2**	21.726					
	***P*-value**	<0.001					

Table (4) shows that the number of patients with AFI< 9 cm was 32 patients in confirmed group, 17 patients in suspected group and 4 patients in the control group. On the other hand the patients with the AFI > 9 cm was 18 patients in confirmed group, 33 patients in suspected group and 46 patients in the control group so there was significant statistical difference between confirmed, suspected and control groups as regard amniotic fluid index.

Table (5): Creatinine level in the vaginal fluid among the three groups.

Group	Creatinine level (mg/dl)					Analysis of variance	
	Range			Mean	± SD	F	*P*-value
Confirmed ROM	0.75	-	2.04	1.20	± 0.33	213.204	<0.001
Suspected PROM	0.00	-	1.88	0.35	± 0.26		
Control	0.00	-	0.27	0.07	± 0.08		

Table (5) shows that there was very high significant difference between confirmed, suspected and control groups as regard creatinine level in vaginal fluid (*P*<0.001).

Table (6): HCG level in the vaginal fluid among the three groups.

Group	HCG level (mIU/mL)					Analysis of Variance	
	Range			Mean	±SD	F	*P*-value
Confirmed PROM	165.2	-	1002.6	448.5	±254.8	90.744	<0.001
Suspected PROM	0.00	-	846.16	125.9	±105.3		
Control	0.00	-	58.96	32.91	±15.50		

Table (6) shows that there was very high significant difference between confirmed, suspected and control groups as regard HCG level in vaginal fluid (*P*-value <0.001).

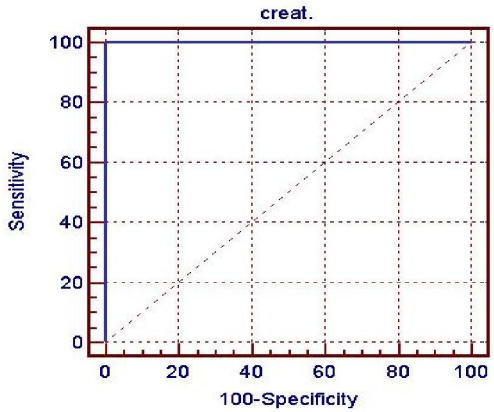

Figure (1): Receiver-operator characteristic curve of creatinine level in diagnosis of PROM among confirmed, suspected, control groups.

Analysis of results using Receiver-operator characteristic curve showed that the best cutoff point for vaginal fluid creatinine among the studied groups in our study was 0.7 mg/dl with Sensitivity, specificity, +ve predictive value, -ve predictive value and accuracy were all 100%.

The number of patients who exceeded the cutoff point for vaginal fluid creatinine was 50 patients in confirmed group, 22 patients in suspected group and no patients in the control group.

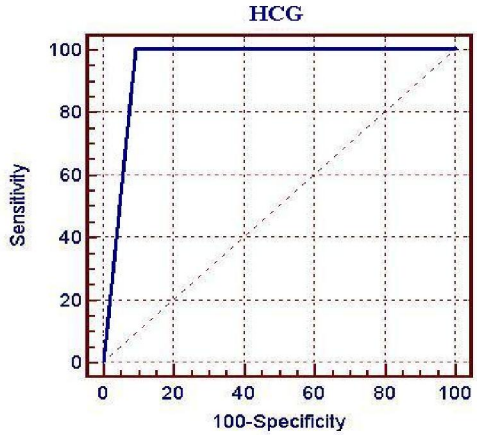

Figure (2): Receiver-operator characteristic curve of HCG level in diagnosis of PROM among confirmed, suspected, control groups.

Analysis of results using Receiver-operator characteristic curve showed that the best cutoff point for vaginal fluid HCG among the studied groups in our study was 47 mIU/mL with Sensitivity 94%, specificity 86%, +ve predictive value 93.1%, -ve predictive value 87.8% and accuracy 91.3%.

The number of patients who exceeded the cutoff point for vaginal fluid HCG was 50 patients in confirmed group, 27 patients in suspected group and 8 patients in the control group.

4. Discussion:

Premature rupture of membranes (PROM) is a condition which occurs in pregnancy when the amniotic sac ruptures before the onset of labor. Preterm prelabor rupture of membranes (PPROM) is a condition which occurs in pregnancy when the amniotic sac ruptures before 37 weeks of gestation **(Deering *et al.*, 2007).**

Preterm PROM complicates 2% to 20% of all deliveries. The accurate diagnosis of Preterm PROM is important, because it is associated with infectious morbidity of mother and fetus, cord prolapse, and preterm labor **(Caughey *et al.*, 2008).**

Diagnosis of PROM is easy when there is a demonstration of amniotic fluid leakage from the cervix, but become difficult when there is doubt as to whether PROM has occurred or not. Failure to identify patients with membrane rupture can result in failure to implement obstetric measures, while the false diagnosis can lead to inappropriate interventions such as hospitalization or labor induction **(Kim *et al.*, 2005).**

Traditionally, the diagnosis of PROM has relied on a combination of factors, including the patient's history, identification of gross pooling of amniotic fluid in vagina, the ferning test, and the nitrazine test. However, in equivocal cases of PROM, the traditional method has been associated with both false-positive and false-negative results. The ferning test should be performed on a sample collected from the posterior fornix or lateral vaginal sidewall to avoid cervical mucus, which may yield a false positive result. The Nitrazine test can be "falsely positive" if the vaginal pH is increased by blood or semen contamination or alkaline antiseptics, or if bacterial vaginosis is present.

Prolonged leakage with minimal residual fluid can lead to a false negative Nitrazine or ferning test. Alternative biochemical markers for diagnosing PROM have been investigated. Markers such as diamino-oxydase, prolactin, alpha-fetoprotein, fetal fibronectin, and IGFBP-1 have advantages and disadvantages.However, despite the improved diagnostic value of these markers; they have not become popular because of their complexity and cost **(Esim *et al.*, 2003).**

The absence of a non-invasive gold standard method for the diagnosis of PROM has led to the search for the alternative biochemical markers which have high amniotic concentration **(Kim *et al.*, 2005).**

Gurbuz *et al.* (2003) hypothesized that vaginal fluid creatinine may be helpful in diagnosing PROM because fetal urine is the most important source of amniotic fluid in the second half of pregnancy. They evaluated the value of vaginal fluid creatinine for diagnosis of PROM, they included only two groups, one confirmed and the other control group. The study did not compare the value of creatinine with any other method for diagnosis of PROM. In this study the cutoff point for vaginal fluid creatinine among the studied groups was 0.12 mg/dL with sensitivity, specificity, +ve predictive value, -ve predictive value and accuracy were all 100%. The study concluded that creatinine assay is cheaper and faster than other methods, and has higher sensitivity and specificity to establish accurate diagnosis.

In our study we added a third group of suspected PROM rather than the confirmed and the control group. The suspected group is the group which would actually get the benefit from our work. As we included this suspected group, we had to use AFI as an indicator which may refer to the possibility of membrane rupture. The amniotic fluid index was low in 17 patients of the suspected group, in these patients vaginal fluid creatinine was more than 0.7 mg/dL. In our study the cutoff point for vaginal fluid creatinine among the studied groups was 0.7 mg/dL with sensitivity, specificity, +ve predictive value, -ve predictive value and accuracy were all 100%.

Kafali and Oksuzler (2007) evaluated the value of vaginal fluid urea and creatinine for diagnosis of PROM. They included three groups as we did but they compared urea with creatinine for diagnosis of PROM. In this study the cutoff point for vaginal fluid creatinine among the studied groups was 0.6 mg/dl with sensitivity, specificity, +ve predictive value, -ve predictive value and accuracy were all 100%. The study concluded that vaginal fluid urea and creatinine determination for the diagnosis of PROM is a reliable, simple and rapid test.

Esim *et al.* (2003) hypothesized that vaginal fluid HCG may be helpful in diagnosing PROM because HCG is a glycoprotein produced exclusively by syncitiotrophoblasts in the placenta and present at a certain level in the vaginal fluid. They evaluated the value of vaginal fluid HCG for diagnosis of PROM, they included three groups as we did but they did not compare the value of HCG with any other method for diagnosis of PROM. In this study the cutoff point for vaginal fluid HCG among the studied groups was 65.0 m IU/mL with sensitivity 68%, specificity 95%, positive predictive value 82%, negative predictive value 90% and accuracy 87%. The study concluded that vaginal fluid HCG determination for the diagnosis of PROM is reliable, simple and rapid test.

In our study the cutoff point for vaginal fluid HCG among the studied groups was 47.0 mIU/mL with sensitivity 94%, specificity 86%, +ve predictive value 93.1%, -ve predictive value 87.8% and accuracy 91.3%.

Kim *et al.* (2005) evaluated the value of vaginal fluid HCG for diagnosis of PROM, they included four groups. The study did not compare the value of HCG with any other method for diagnosis of PROM. In this study the best cutoff point for vaginal fluid HCG among the studied groups was 39.8mIU/mL with sensitivity 95.5%, specificity 94.7%, positive predictive value 91.3% and negative predictive value 97.3%. The study concluded that measurement of vaginal fluid β-hCG may be reliable, simple, and rapid test in diagnosing PROM.

Conclusion:

Our study demonstrated that measuring both vaginal fluid creatinine and HCG concentrations are good predictors of PROM but measurement of vaginal fluid creatinine is more reliable and less expensive than measurement of vaginal fluid HCG in diagnosing PROM. Further studies are needed to assess the use of those cut-off values of the vaginal fluid creatinine and HCG to establish the diagnosis of PROM.

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