Maternal Serum Amyloid A level in Pregnancies Complicated with Preterm Premature Rupture of Membranes

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ABSTRACT

Background: serum amyloid A (SAA) is a cytokine-inducible acute-phase reactant whose plasma concentrations can exceed 1 mg/mL during an acute-phase response (500 to 1000 fold of plasma levels greater than in the non inflammatory state) thus representing an ideal marker for clinical use. Preterm premature rupture of membranes (PPROM) complicates only 2% of pregnancies but is associated with 40% of preterm deliveries and can result in significant neonatal morbidity (Prematurity, sepsis and pulmonary hypoplasia) and mortality. **Aim of the work:** this study aimed to find out association between maternal serum amyloid A level and preterm premature rupture of membranes. Also to compare SAA, CRP levels, N/L ratio in the study group. **Patients and methods:** this study is a cross sectional study conducted in Ain Shams University Maternity Hospital from December 2015 – December 2016 on 58 pregnant women. Women have been allocated in this study, represented in two groups:

1- Study group: including 29 women complaining of preterm premature rupture of membranes. 2- Control group: including 29 women as control group with no complain. Venous blood sample was taken from each participant (study group within 1hour from onset of PPROM, control group during their follow up visit to the clinics). Serum amyloid A, Micro C reactive pretein, total WBCs and neutrophil/lymphocyte ratio (NLR) were calculated. **Results:** the results point out that PPROM cases had significantly lower GA and APGAR scores at 1 min and more prone to neonatal sepsis which may lead to death. PPROM women have significantly higher total WBC, N/L ratio CRP and serum amyloid A. There were significant positive correlations between amyloid-A, N/L ratio& CRP in both groups. Serum amyloid A level above 2 ng/ml is a risk factor for PPROM and low Apgar score at 1 min. but has low predictive value. CRP with cut off value 5.0 mg/dl has better predictive value in discrimination between PPROM group and control group.**Conclusion:** Results assessed possible association between maternal SAA, maternal and fetal parameters in pregnancies complicated with PPROM.

Keywords: preterm premature rupture of membranes, serum amyloid A, C reactive protein, Neutrophil/Lymphocyte ratio.

INTRODUCTION

Preterm premature rupture of membranes (PPROM) is defined as spontaneous membranes rupture that occurs before the onset of labor and 37 weeks gestation [(1)] .

PPROM complicates only 2% of pregnancies but is associated with 40% of preterm deliveries and can result in significant neonatal morbidity and mortality. The three causes of neonatal death associated with PPROM are prematurity, sepsis and pulmonary hypoplasia. Women with intrauterine infection deliver earlier than non-infected women and infants born with sepsis have mortality four times higher than those without sepsis. In addition, there are maternal risks associated with chorioamnionitis [(2)].

PPROM is largely a clinical diagnosis. It is typically suggested by a history of watery vaginal discharge and confirmed on sterile speculum examination. The traditional minimally invasive gold standard for the diagnosis of ROM relies on clinician ability to document 3 clinical signs on sterile speculum examination: (1) visual pooling of clear fluid in the posterior fornix of the vagina or leakage of fluid from the cervical os; (2) an alkaline pH of the cervicovaginal discharge, which is typically demonstrated by seeing whether the discharge turns yellow nitrazine paper to blue (nitrazine test); and/or (3) microscopic ferning of the cervicovaginal discharge on drying. Evidence of diminished amniotic fluid volume (by Leopold's examination or ultrasound) alone cannot confirmthe diagnosis, but may help to suggest it in the appropriate clinical setting [(3)].PPROM is multifactorial in nature. Infection and inflammation appears to play an important role in its aetiology [(4)].Serum amyloid A (SAA), the most prominent representative amongst acute-phase proteins can reach plasma levels 500 to

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1000 fold greater than in the non inflammatory state, thus representing an ideal marker for clinical use. Measurements of acute-phase proteins in serum are of value in the assessment of the activity and response to therapy of inflammation in several diseases. In most studies a parallel increase of SAA and C-reactive protein (CRP) has been observed, although some studies have delineated SAA as the more sensitive inflammatory parameter [(5)].

SAA is a member of apolipoproteins associated with high density lipoproteins in plasma. It is also associated with inflammatory response highly similar to erythrocyte sedimentation rate and CRP [(6)].

Stimulation of hepatic production of acute-phase proteins is by proinflammatory cytokines. The functions of positive acute-phase proteins (APP) are regarded as important in optimization and trapping of microorganism and their products, in activating the complement system, in binding cellular remnants like nuclear fractions, in neutralizing enzymes, scavenging free hemoglobin and radicals, and in modulating the host's immune response [(7)].

Studies have examined direct effects of SAA on a number of immune cells. For instance, SAA has been reported to cause chemotaxis of several cell types. The receptor that causes the chemotactic activity was identified as formyl peptide receptor1 (FPRL1), which is a low-affinity receptor for N-Formyl-methionyl-leucyl-phenylalanine (FMLP) and binds to lipoxin A4.6. This receptor has also been identified as being responsible for other activities such as neutrophil IL-8 release or intracellular calcium mobilization [(8)]. In a previous study, serum proadrenomedullin and SAA levels were reported to be elevated in patients with PPROM and PPROM having subclinical chorioamnionitis [(9)].

AIM OF THE WORK

To compare serum amyloid A level in women with PPROM and controls. Also to determine CRP, total WBCs and N/L ratio in the study and control group.

PATIENTS AND METHODS

This study was conducted in Ain Shams University Maternity Hospital from December 2015 – December 2016.

The study included 29 women in each group.

- Study group: including 29 women complaining of preterm premature rupture of membranes (Gestational age between 24 and less than 37 weeks).
- Control group: with no complain.

Cases with intrauterine infection (maternal fever, significant maternal tachycardia, uterine tenderness, cervical motion tenderness, purulent vaginal discharge) fetal anomalies, women with antepartum bleeding were excluded from the study.

All patients were subjected to all of the following:

- Complete history taking emphasizing on:
- Gestational age of present pregnancy: determination according to last menstrual period (if patient sure about the date and had 3 regular cycles before the pregnancy or has ultrasound report done in first trimester) and confirmation by ultrasound assessment.
- Complaint and present history:
  - Onset of the vaginal leakage.
  - Nature of the discharge: watery or mucoid.
  - Colour of the discharge: clear, yellow or green.
  - Odour (Odorless or offensive).
  - Associated symptoms: bleeding, decreased fetal kicks and regular uterine contraction.
- Medical history: to exclude any maternal disease like preeclampsia, systemic lupus erythematosis, rheumatoid arthritis, amyloidosis, atherosclerosis, antiphospholipid syndrome, coronary artery disease and systemic sclerosis.
- Surgical history:
  - Any cervical surgeries.
  - Previous cesarean section
- General examination stressing on vital signs to assess hemodynamic stability of the patient.
- Abdominal examination and auscultation of fetal heart sounds by electronic heart rate recording (Sonicaid) and CTG .
- Pelvic examination under complete aseptic condition to diagnose PPROM by a sterile speculum examination demonstrating pooling of fluid in the posterior vaginal fornix and asses cervical dilatation.
- Venous blood sample was taken from each participant (study group within 1-9 hours from onset of PPROM, control group during their follow up visit to the clinics) samples were collected, centrifuged and stored.
- Serum amyloid A, Micro CRP, total WBCs and neutrophil/lymphocyte ratio (NLR) were calculated.

The study was done after approval of ethical board of Ain-Shams university and an informed written consent was taken from each participant in the study.

Statistical analysis

Data were analyzed using SPSS© Statistics version 21 (IBM© Corp., Armonk, NY, USA).

Normality of numerical data distribution was examined using the Shapiro-Wilk test. Normally distributed numerical variables were presented as mean ± SD and intergroup differences were compared using the unpaired t tests. Non-normally distributed numerical variables were presented as median and interquartile range and between-group differences were compared using the Mann-Whitney test.

Correlations were tested using the Spearman rank correlation. The correlation coefficient (Spearman rho) is interpreted as follows:

Table (1): Receiver-operating characteristic (ROC) curve analysis:

Correlation coefficient (Spearman rho)	Strength of correlation
<0.2	Very weak
0.2 – 0.39	Weak
0.4 – 0.59	Moderate
0.6 – 0.79	Strong
0.8 – 1	Very strong

Receiver-operating characteristic (ROC) curve analysis was used to examine the diagnostic (or predictive) value of amyloid A, NLR, or CRP. The area under the ROC curve (AUC) is interpreted as follows:

Table (2): Classification of the power of the diagnostic/ predictive value according to area under the curve:

Area under ROC curve (AUC)	Diagnostic / predictive value
0.9 – 1.0	**Excellent**
0.8 – 0.89	**Good**
0.7 –0.79	**Fair**
0.6 – 0.69	**Poor**

Areas under different ROC curves were compared using the DeLong method. Mulltivariable binary logistic regression analysis was used to test the relation between amyloid A and binary outcomes (PROM and low Apgar score) as adjusted for the gestational age.

Table (3): Results of Laboratory work-up (total WBCs, N/L ratio, CRP and SAA) in PPROM and control groups:

Variable	Control (n=29)	PROM (n=29)	t / U	df / Z	p-value
WBC (x103/ml)	**9.5 ± 2.1**	**11.7 ± 4.2**	**-2.539**	**41.289**	**0.015¶**
N/L ratio	**3.2 (2.65 – 4.63)**	**4.4 (3.00 – 6.75)**	**275**	**-2.265**	**0.023§**
CRP (mg/dl)	**2.5 (1.70 – 5.00)**	**16 (9.05 – 25.75)**	**51**	**-5.751**	**<0.001§**
Amyloid A (ng/ml)	**0.00 (0.00 – 1.70)**	**2.5 (0.00 – 5.00)**	**231**	**-3.077**	**0.002§**

Data are mean ± SD or median (interquartile range). t, t statistic; df, degree of freedom; U, U statistic; Z, Z statistic, ¶Unpaired t test, §Mann-Whitney U test, this table shows there is significant statistical difference between PPROM group and control group as regard WBCs (p value 0.015), N/L ratio (p value 0.023), Serum Amyloid A (p value 0.002), While CRP showed highly significant difference (p value<0.001).

Table (4): Correlation between serum amyloid A level and other numerical variables (Age, BMI, GA, WBCs, N/L ratio, CRP, birth weight, Apgar at 1 min, Apgar at 5 min) among the whole study sample:

	Amyloid A	
Variable	**Spearman's rho**	**p-value**
Age	**0.036**	**0.787**
BMI	**0.140**	**0.294**
Gestational age	**-0.368****	**0.005**
WBCs	**0.418****	**0.001**
N/L ratio	**0.632****	**<0.001**
CRP	**0.648****	**<0.001**
Birth weight	**-0.201**	**0.131**
Apgar 1	**-0.268***	**0.042**
Apgar 5	**-0.161**	**0.228**

*. Correlation is significant at the p <0.05 level, **. Correlation is significant at the p <0.01 level.

According to the table above, there is negative correlation between Serum amyloid A and gestational age (p value 0.005) and Apgar score at 1min (p value 0.042).

There were highly significant positive correlations between Serum amyloid A and NL ratio (p value<0.001), CRP (p value <0.001) and significant correlation with Total WBCs (p value 0.001).

Table (5): Comparison of the areas under the ROC curves associated with N/L ratio, CRP and serum amyloid A:

Marker	AUC	SE	95% CI		
N/L ratio	**0.673**	**0.072**	**0.537 to 0.790**		
CRP	**0.939**	**0.028**	**0.844 to 0.985**		
SAA	**0.725**	**0.064**	**0.592 to 0.834**		
Comparison	**Difference between AUCs**	**SE**	**95% CI**	**z statistic**	**p-value¶**
N/L ratio VS. CRP	**0.266**	**0.060**	**0.148 to 0.384**	**4.426**	**<.0001**
N/L ratio VS. AA	**0.052**	**0.061**	**0.067 to 0.171**	**0.862**	**.389**
CRP VS. SAA	**0.214**	**0.054**	**0.108 to 0.320**	**3.947**	**.0001**

¶DeLong method.

Comparison here of AUC of NLR, CRP and demonstrated better predictive value of CRP over NLR (p value <0.0001) and SAA (p value0 .0001), NLR vs SAA wasn't significant (p value 0.389).

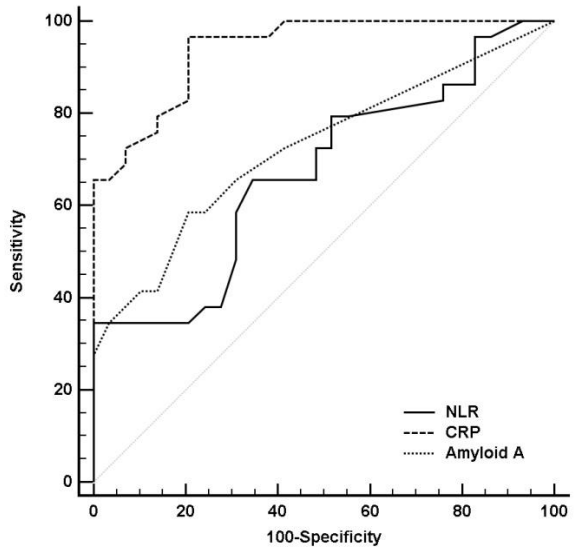

Figure (1): Comparison of the areas under the ROC curves associated with N/L ratio, CRP and serum amyloid A.

Table (6): Multivariable binary logistic regression analysis for the relation between the N/L ratio and occurrence of PPROM as adjusted for the gestational age:

	Regression coefficient	***SE***	***Wald statistic***	***p-value***
Serum amyloid A (ng/ml)	0.944	7995.399	4.243	0.05
CRP level (mg/l)	0.429	975.909	2.96	0.04
NLR	0.910	3095.699	0.000	1.000

after adjustment for the confounding effect of gestational age by multivariable binary logistic regression analysis demonestrated that serum amyloid A and CRP are discriminative factors for preterm premature rupture of membranes.

DISCUSSION

Preterm premature rupture of membranes (PPROM) is associated with severe complications (chorioamnionitis, preterm labour, pulmonary hypoplasia placental abruption, cord prolapse and fetal death) for both the mother and the fetus [10]. SAA is a member of apolipoproteins associated with high density lipoproteins in plasma. It is also associated with inflammatory response highly similar to erythrocyte sedimentation rate and C-reactive protein (CRP) [11].

This subject was chosen to be the target of this research due to its high value on pregnant women and neonatal health and outcome.

This study was designed to compare Serum Amyloid A level in women with PPROM and controls.Also to determine CRP level, total WBCs and N/L ratio in the study and control group. The study included 29 women in each group.

Few studies investigated association between preterm premature rupture of membranes and serum amyloid A and compare it with other inflammatory markers [9].

There was no statistical difference between both groups regarding age and BMI. All Participants have no statistical difference in parity, gravidity and number of abortions.

In the study conducted by **Köseoğlu and colleagues** [6.]; Maternal CRP level (15.88 in PPROM vs 6.99 in the control), N/L ratio (5.79 in PPROM group vs 4.27 in the control) and SAA level (905.16 in PPROM vs 72.71in the control) were higher in PPROM group than control group p value of CRP (0.003), p value of N/L ratio (0.024) and p value of SAA (0.041).

Results of the present study showed significant statistical difference between PPROM group and control group as regard WBCs (p value 0.015), N/L ratio (p value 0.023), Serum Amyloid A (p value 0.002), While CRP showed highly significant difference (p value<0.001).

Another study by **Cekmez and colleagues** [9]. reported that pregnant women with PPROM exhibit increased levels of some inflammatory markers such as total leucocyte count (TLC), CRP, interleukin-6 (IL-6), proadrenomedullin (pro-ADM), and serum amyloid A (SAA). A total of 63 pregnant women, of which 43 with PPROM between 24 and 34 weeks gestation and 20 normal pregnant women without PPROM were included in the study. SAA, had significant correlations with TLC (r: 0.242; $P < 0.05$), CRP (r: 0.488; $P < 0.01$).

These studies results harmonize with the present study analysis of variables and correlations between amyloid A and other findings among PPROM and control groups which showed that SAA levels correlated significantly with CRP levels (r: 0.648; $P<0.001$) and with N/L ratio (r: 0.632; $P<0.001$).

Another study conducted by **Le Ray and colleagues** [12]study which was on 121 women with PPROM between 24 and 34 + 0 weeks of gestation. Association between white blood cells (WBC) count, plasma CRP, interleukin-6 (IL-6), monocyte/macrophage chemo attractant protein-1 (MCP-1) and interferon-gamma-inducible protein-10 (IP-10) levels and histologic chorioamnionitis (HCA) was assessed. Significant increase was observed for CRP, IL6 and total WBCS. In contrast, there were no significant differences,or even trends toward differences, between the two groups for IP-10 and MCP-1 levels.

In previous study, **Caloone and colleagues** [13] in recent and wide study to compare several maternal serum markers after preterm premature rupture of membranes (PPROM). Study design a prospective and multicentric observational study, including six french tertiary referral centres. 295 pregnant women over 18 years, with PPROM between 22 + 0 and 36 + 6 weeks gestation. A blood sample was obtained before delivery and analysed for C-Reactive Protein (CRP), Inter Cellular Adhesion Molecule-1 (ICAM-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Matrix-Metalloproteinase 8 and 9 (MMP-8, MMP-9), Triggering receptor on myeloid cells (TREM-1), and Human Neutrophile Peptides (HNP). The ROC curve with the largest AUC was for CRP (AUC; 0.70; 95% CI; 0.64–0.77) and it was significantly higher than those for MMP-8, MMP-9, or HNP ($P < 0.03$).

This is strongly supported by results of the present study, CRP ROC curve showed excellent diagnostic / predictive value (p value <.0001) at a cut-off value of 5.0 mg/l, the area under the curve was 0.9.

In the current study, ROC curve of N/L ratio has drawn with cut-off value of 5.8, the area under the curve was 0.673, with poor diagnostic / predictive value (p value 0.011).

ROC curve of Amyloid-A with a cut-off value of 2.0 pg/ml showed that area under the curve was 0.725, which represent fair predictive value (p value.0005).

This suggest that their levels represent risk factors for PPROM (p value <0.05).

Köseoğluand colleagues [6.] showed that after analysis of AUC of ROC curves, odds ratio for CRP, N/L ratio and serum amyloid A and according to the logestic regression method are discriminative factors for PPROM.

Similarly, results of the present study after adjustment for the confounding effect of gestational age by multivariable binary logistic regression analysis demonestrated that serum amyloid A and CRP are discriminative factors for preterm premature rupture of membranes.

CONCLUSION

Serum amyloid A correlated positively with CRP and N/L ratio in PPROM cases and represent a risk factor for PPROMCRP has the advantages of being easily available and cheaper than SAA However, the result of this test alone doesn't justify a decision for immediate delivery. So, further studies with larger sample size are needed to define cut off values for inflammatory markers in PPROM cases for clinical decisions of immediate delivery to prevent neonatal complications.

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