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Article

Maternal serum and amniotic fluid cytokines in patients with preterm premature rupture of membranes with and without intrauterine infection

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Abstract

Objectives: To evaluate the role of interleukins (IL-1, IL-6), tumor necrosis factor α (TNFα) and for the first time interferon gamma (IFNγ) and epidermal growth factor (EGF) in the pathogenesis of premature rupture of membranes (PROM) with and without confirmed intrauterine infection. *Methods:* Amniotic fluid was retrieved by transabdominal amniocentesis from 30 patients with PROM and 20 normal pregnant women with intact membranes of matched gestational age. Microbial state of amniotic cavity included culture for aerobic and anaerobic bacteria, mycoplasmas and ureaplasma whether or not clinical signs of chorioamnionitis were present. Maternal serum and amniotic fluid IL-1, IL-6, TNFα and IFNγ concentrations were determined by the corresponding immunoradiometric assay, whereas EGF concentration was determined by a specific radioimmunoassay. *Results:* Nearly all cases of PROM with infection revealed elevated amniotic fluid cytokines (IL-1β, IL-6, TNFα, IFNγ, EGF) whereas half of them revealed elevated serum cytokines. In cases of PROM without confirmed infection, there were no significant changes of maternal serum cytokines, whereas two-thirds of them revealed elevated amniotic fluid cytokines. *Conclusions:* The rise of cytokines in amniotic fluid of cases of PROM with infection may represent: (a) enhanced macrophage activity for immunosurveillance of the fetus; (b) a preparatory step for the initiation of labor; and (c) a valuable tests for diagnosing chorioamnioitis. The mechanism responsible for PROM in the presence or absence of infection is likely to be of different nature.

Keywords: Premature rupture of membranes; Infection; Cytokines

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1. Introduction

Premature rupture of membranes (PROM) is defined as fetal membranes rupture with leakage of amniotic fluid that precedes the onset of uterine contraction by at least 2 h. Although PROM is a common problem, at present, there is little understanding of its basic physiology and little agreement of the most favorable management. PROM complicates 4–7% of all births and is directly associated with short gestational length and increased perinatal and maternal morality [1,2]. There are certain predisposing conditions associated with the occurrence of PROM such as local infections, cervical incompetence and low socioeconomic condition. Even so, the etiology of PROM remains unknown in the majority of cases. A growing body of evidence indicates the contribution of intrauterine infection in the onset of human parturition. The role of intrauterine infections included bacterial toxins [3], bacterial phospholipases [4], and cytokines such as IL-1, IL-6 and TNFα [5–7]. However, our knowledge of the mechanism of the onset of labor in the absence of infection remains incomplete.

Increased biosynthesis of prostaglandins by intrauterine tissues is widely accepted as a key event in the initiation of parturition [8]. Preterm labor after spontaneous rupture of membranes is associated with a significant increase in amniotic fluid concentrations of prostaglandins [9]. Mechanisms responsible for increased bioavailability of prostaglandins in preterm labor associated with infection has not been well defined. The conventional view has been that bacteria and their products are responsible for the increased biosynthesis of prostaglandins that results in the onset of labor [10]. However, it has been recognized that many of the metabolic derangements associated with microbial infection are mediated by endogenous factors. IL-1, IL-6 and TNFα production in decidual tissue in response to intra-amniotic infection and their effects on the production and regulation of prostaglandin production have been reported [11]. Moreover, the role of epidermal growth factor (EGF) in human parturition by stimulating prostaglandin production by amnion cells has been reported by Romero et al. [12].

This study was undertaken to evaluate the role of IL-1, IL-6, TNFα, and for the first time interferon gamma (INFγ) and EGF in the pathogenesis of PROM in cases with confirmed and non-confirmed intrauterine infection.

2. Patients and methods

This study was conducted on 50 selected pregnant women at their third trimester who more attending the antenatal clinic of King Abul-Aziz and Cairo University Hospitals. Thirty cases were presented with prelabor premature rupture of membranes and 20 cases with normal uncomplicated pregnancy (controls). All women were matched for age (21–32 years), gestational age (37–40 weeks), with a completely normal singleton pregnancy. Rupture of membranes was diagnosed by clinical history of passage of watery discharge or sudden gush of fluid and confirmed by sterile speculum examination. All cases were subjected to complete general and local examination, and laboratory investigations which included blood grouping and Rh typing, complete blood picture, fasting and postprandial blood glucose. Abdominal ultrasonographic examination was performed to all cases to confirm the diagnosis of rupture membranes, to determine fetal gestational age and to identify amniotic fluid pool for amniocentesis. No medical complications or systemic diseases, e.g. diabetes or hypertension were observed in these cases. Amniotic fluid samples were collected by transabdominal amniocentesis using aseptic technique and preserved at −70 °C until the time of assay. Blood- and meconium-stained samples were discarded. After amniocentesis ultrasonographic assessment of fetal heart beat was done for every case. Intra-amniotic infection was defined as the presence of a positive amniotic fluid culture whether or not clinical signs of chorioamnionitis were present. Amniotic fluid was transported to the laboratory in a capped plastic syringe immediately after amniocentesis. Amniotic fluid was plated within 30 min of collection. Cultures for aerobic, anaerobic bacteria, *Mycoplasma hominis* and *Ureaplasma urealyticum* were performed. Methods for bacterial culture and identification have been described previously in detail [13]. Mycoplasma

species were cultured with a commercially available system (Mycotrim-GU, Berkeley, CA, USA). At the same time of amniocentesis 10 ml maternal venous blood from the antecubital vein were collected. All maternal blood samples were centrifuged at 2000 rev./min for 10 min, sera were separated and stored at −70 °C until the time of assay. Amniotic fluid and maternal serum specimens were assayed for IL-1β, IL-6, TNFα, IFNγ by the corresponding immunoradiometric assay, using kits purchased from Medgenix Diagnostics, Belgium. EGF concentration was determined by radioimmunoassay using kits purchased from Diagnostic System Laboratories Inc, Webster, TX, USA. The intra-assay coefficient of variation for IL-1β, IL-6, IFNγ and TNFα were 3.2%, 1.9%, 2.8% and 3.7%, respectively. The interassay coefficient of variation for IL1β, IL-6 and IFNγ and TNFα were 6.2%, 5.4%, 7.4% and 8.1%, respectively. The intra and interassay coefficient of variation for EGF were 4.4% and 6.1%, respectively. Comparison between IL-1β, IL-6, IFNγ, TNFα and EGF levels in controls and cases of PROM was performed with the Wilcoxon non-paired rank sum test. A *P*-value of less than 0.05 was considered significant.

3. Results

Positive amniotic fluid culture was encountered in 24 out of 30 cases of PROM. Maternal serum IL-1β, IL-6 and EGF in cases of PROM with infection (24 cases) were significantly higher than the corresponding levels of controls. The prevalence of abnormally elevated serum levels of IL-1β, IL-6, TNFα, IFNγ and EGF in cases of PROM with infection was encountered in 33.3%, 20.8%, 17%, 12.5% and 52% of cases, respectively (Table 1). Amniotic fluid IL-1β, IL-6, TNFα, IFNγ and EGF levels were significantly increased, when compared with the corresponding levels in amniotic fluid of controls. Abnormal elevated values (values exceeding the upper confidence limit, mean ± 2 S.D. of healthy controls) of amniotic fluid IL-1β, IL-6 and IFNγ were encountered in all cases of PROM with infection. Amniotic fluid EGF and TNFγ concentrations in cases of PROM with infection were markedly elevated in 93% and 90% of cases. These results demonstrate that nearly all cases of PROM with positive amniotic fluid culture revealed significantly elevated concentrations of cytokines IL-β, IL-6, TNFα, IFNγ and EGF, indicating local intrauterine infection. Circulating levels of these cytokines in cases of PROM with infection demonstrated that up to 52% of cases might be associated with generalized systemic infection. Table 2 shows maternal serum and amniotic fluid cytokines levels in cases of PROM without positive amniotic fluid culture. In these cases there were no significant changes of maternal serum IL-1β, IL-6, TNFα, IFNγ and EGF levels when compared to the corresponding levels of controls. On the other hand, 50% to 66.6% of cases of PROM without infection revealed abnormally elevated values of amniotic fluid cytokines. The mean values of amniotic fluid IL-1β, IL-6, TNFα, IFNγ and EGF were significantly higher than the corresponding values of controls. The prevalence of abnormal high values of these cytokines were encountered in 66.6%, 66.6%, 50%, 50% and 50% of cases, respectively.

4. Discussion

The function of cytokines in normal pregnancy and delivery is not fully established. The cytokines IL-1, IL-6 and TNFα, as well as EGF have been detected in human amniotic fluid during pregnancy and labor [5–7,12]. The present study revealed also the presence of IFNγ in amniotic fluid of normal pregnancies. We found that amniotic fluid mean values of IL-1β, IL-6, TNFα, IFNγ and EGF were approximately 4, 140, 4 and 3 times those of maternal serum. It seems that the increased production of cytokines in amniotic fluid indicates fetal or placental contribution. This is in agreement with Menon et al. [11] who reported that amniochorionic membranes are a site of inflammatory cytokine production. The rise of cytokines in amniotic fluid may represent enhanced macrophage activity for immunosurveillance of the fetus. In addition, the elevation of amniotic fluid cytokines may be a preparatory step in the initiation of labor in view that cytokines play an important role in the stimulation of prostaglandin synthesis [5,6,12,14]. In the present

Table 1
Maternal serum and amniotic fluid cytokines levels in cases of PROM with infection compared to controls

Parameter	Maternal serum		Amniotic fluid	
	Controls $n=20$	PROM with infection $n=24$	Controls $n=20$	PROM with infection $n=24$
IL-1β (pg/ml)				
Mean±S.E.	19±0.36	23.12±1.74	76±1.00	236.6±9.76
t/d.f.	2.3188/42		16.2761/42	
P-value	<0.01		<0.0005	
Abnormal values[a]	8/24 (33.3%)		24/24 (100%)	
IL-16 (pg/ml)				
Mean±S.E.	30.75±1.02	36.87±2.89	412.8±7.2	10 212±755
t/d.f.	1.9969/42		12.9790/42	
P-value	<0.05		<0.0005	
Abnormal values[a]	5/24 (20.8%)		24/24 (100%)	
TNFα (pg/ml)				
Mean±S.E.	18.67±0.99	20.65±2.17	68.7±1.95	188.62±6.61
t/d.f.	0.8301/42		17.400/42	
P-value	>0.05		<0.0005	
Abnormal values[a]	4/24 (17%)		38/42 (90%)	
IFNγ (pg/ml)				
Mean±S.E.	1.1±0.04	1.09±0.03	3.00±0.02	5.38±0.27
t/d.f.	0.2001/42		8.7920/42	
P-value	>0.05		<0.0005	
Abnormal values[a]	3/24 (12.5%)		24/24 (100%)	
EGF (pg/ml)				
Mean±S.E.	3.46±0.36	5.6±0.45	0.745±0.056	1.656±0.047
t/d.f.	3.7133/42		12.4624	
P-value	<0.005		<0.0005	
Abnormal values[a]	6/24 (25%)		29/42 (93%)	

[a] Abnormal values are values exceeding the upper 95% confidence limit of normal values.

study, determination of amniotic fluid and serum cytokines were carried out in 24 cases of PROM with intrauterine infection and six cases of PROM without apparent infection. Both groups showed significant increase of amniotic fluid cytokines, compared to controls, but the rise of these cytokines was more pronounced in cases of PROM with infection. The mean fold rise of amniotic fluid IL-1β, IL-6, TNFα, IFNγ and EGF in cases of PROM with intrauterine infection was 3.1, 24.7, 2.75, 1.8 and 2.2, respectively. However, the corresponding fold rise of these cytokines in amniotic fluid of cases of PROM without infection was 1.6, 1.2, 1.4, 1.35 and 1.23, respectively. Meanwhile serum IL-1β, IL-6 and EGF levels in cases of PROM with intrauterine infection were significantly higher than the corresponding levels of controls, whereas serum TNFα and IFNγ levels were not significantly different from those of controls. Abnormal high values of serum IL-1β, IL-6, TNFα, IFNγ and EGF were encountered in 33.3%, 20.8%, 17%, 12.5% and 25% of cases of PROM with infection, respectively. These results demonstrate that nearly all cases of PROM with infection

Table 2
Maternal serum and amniotic fluid cytokines levels in cases of PROM without infection compared to controls

Parameter	Maternal serum		Amniotic fluid	
	Controls $n=20$	PROM without infection $n=6$	Controls $n=20$	PROM without infection $n=6$
IL-1β (pg/ml)				
Mean ± S.E.	19 ± 0.36	21.8 ± 2.0	76.8 ± 1.00	121.3 ± 15.02
t/d.f.	1.3891/24		2.9578/24	
P-value	>0.05		<0.01	
Abnormal values[a]	2/6 (33.3%)		4/6 (66.6%)	
IL-16 (pg/ml)				
Mean ± S.E.	30.75 ± 1.02	32.20 ± 1.10	412.8 ± 7.2	480.05 ± 8.5
t/d.f.	0.9666/24		6.0775/24	
P-value	>0.05		<0.0005	
Abnormal values[a]	2/6 (33.3%)		4/6 (66.6%)	
TNFα (pg/ml)				
Mean ± S.E.	18.67 ± 0.99	16.67 ± 0.56	68.7 ± 1.95	94.5 ± 13.88
t/d.f.	1.7462/24		1.8407/24	
P-value	>0.05		<0.05	
Abnormal values[a]	0/6 (0%)		3/6 (50%)	
IFNγ (pg/ml)				
Mean ± S.E.	1.1 ± 0.04	1.11 ± 0.12	3.00 ± 0.02	4.05 ± 0.52
t/d.f.	0.0971/24		1.9404/24	
P-value	>0.05		<0.05	
Abnormal values[a]	0/6 (0%)		3/6 (50%)	
EGF (pg/ml)				
Mean ± S.E.	3.46 ± 0.36	3.5 ± 0.40	0.745 ± 0.056	0.918 ± 0.075
t/d.f.	0.07433/24		1.8483/24	
P-value	>0.05		<0.05	
Abnormal values[a]	0/6 (0%)		3/6 (50%)	

[a] Abnormal values are values exceeding the upper 95% confidence limit of normal values.

had elevated levels of amniotic fluid cytokines and approximately half of them were associated with increased maternal serum cytokines. Murtha et al. [15] reported that maternal serum IL-6 concentrations are elevated in patients with PROM with clinical or histologic chorioamnionitis. It seems that amniochorionic membranes are the site of inflammatory cytokines production and these cytokines may act as an inflammatory mediators, leading to systemic and local changes at the fetomaternal interface and activating fetomaternal immune system against intrauterine infection. Our findings support the hypothesis that cytokines may play a role in the initiation of preterm labor associated with intra-amniotic infection by stimulation of prostaglandin biosynthesis. Moreover, amniotic fluid cytokines are sensitive tests for the prospective diagnosis of acute chorioamnionitis and the identification of neonates at risk for significant morbidity and mortality.

The present data are consistent with the findings of Rizzo et al. [16] who demonstrated that intra-amniotic infection is associated with increased IL-6 concentration in cervical secretion which is

related to amniotic fluid levels. Moreover, Romero et al. [17] proposed that preterm labor in the setting of infection results from the action of proinflammatory cytokines secreted as part of the fetal and/or maternal host response to microbial invasion. They reported that a systemic fetal proinflammatory cytokine response is followed by the onset of spontaneous preterm labor in patients with preterm PROM. Carroll et al. [18] found that intrauterine infection is associated with increased IL-1β concentration in fetal plasma and amniotic fluid. Fukuda et al. [19] reported also increased amniotic fluid and cord blood IL-6 levels in PROM. Degradation of the extracellular matrix in fetal membranes with intra-amniotic infection is caused by matrix metalloproteinases derived from chorionic cells through the inflammatory cytokines (TNFα, IL-1) released in response to bacterial infection, prostaglandin E2 is released causing uterine contraction and these effects combine to induce PROM [20].

To the best of our knowledge no previous data were published concerning maternal serum and amniotic fluid interferon gamma (IFNγ) in cases of PROM. IFNγ is a real lymphokine produced by activated T-cells. Despite its clear antiviral and cellular growth regulating activities, its immunomodulatory properties were believed to be the most important [21]. The absence of significant rise of maternal serum IFNγ of cases of PROM, despite its increase in amniotic fluid exclude maternal contribution and it is likely that the placenta produces IFNγ as a result of activated immunity at the fetomaternal interface.

In the present study, cases of PROM with negative amniotic fluid culture showed significant increase of amniotic fluid IL-1β, IL-6, TNFα, IFNγ and EGF concentrations, when compared to the corresponding values of controls. However, the elevation of these cytokines was less than that encountered in cases of PROM with intrauterine infection. On the other hand, maternal serum cytokines concentrations in these cases did not differ significantly from those of controls. Millar et al. [22] proposed a relaxin-mediated pathway which leads to PROM and is independent of infection. The mechanism responsible for PROM in the presence of infection probably involves the macrophage system and its cytokines, while in the absence of infection, the signals for PROM are likely to be of a different nature. In cases of PROM without confirmed infection using the conventional methods, the possibility of intrauterine viral infection could not be ruled out because of the significant increase of the antiviral cytokine IFNγ in amniotic fluid. Alanen [23] reported that intra-amniotic infection during pregnancy can be caused by bacteria, viruses, protozoa and these infections are connected with PROM. He demonstrated that PCR assays are capable of detecting certain clinically important viruses.

It is proposed that the onset of labor and PROM in the setting of infection has survival values. The host (fetus and/or mother) uses a highly preserved phylogenetic mechanism of defense, the macrophage system and its cytokines to trigger the onset of labor or PROM when the intrauterine environment is hostile.

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May 30, 2003

Mohamed Shaarawy M.D.
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Dear Professor Shaarawy:

On behalf of the editors of the *International Journal of Gynecology and Obstetrics* (IJGO) and the Executive Board of the International Federation of Gynecology and Obstetrics (FIGO) I am pleased to notify you and Dr. Shobokshi that your article, "MATERNAL SERUM AND AMNIOTIC FLUID CYTOKINES IN PATIENTS WITH PREMATURE RUPTURE OF MEMBRANES WITH ANE WITHOUT INTRAUTERINE INFECTION", has received Honorable Mention in the IJGO Prize Paper Award for 2002. Although the Honorable Mention recognition does not include a financial reward, we are enclosing award certificates, which we hope you and Dr. Shobokshi will be proud to display. We ask that you pass the certificate on to Dr. Shobokshi on our behalf.

All clinical research articles from developing countries that were published during 2002 were considered for this prize. Selection was made by the editors, in collaboration with the anonymous donor of the funds, and was endorsed by the editorial board of the *International Journal of Gynecology and Obstetrics*.

This award was established for the purpose of encouraging investigators from developing countries to submit their very best clinical research articles for publication in the *International Journal of Gynecology and Obstetrics*, and we hope that receiving this award will indeed encourage you and your colleagues to continue your fine work.

The editors of the IJGO and the Executive Board of FIGO extend our congratulations to you and Dr. Shobokshi on the receipt of this Honorable Mention recognition.

Sincerely,

J. J. Sciarra

John J. Sciarra, M.D., Ph.D.
Editor

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2002

A. Shobokshi, M. Shaarawy

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Maternal serum and amniotic fluid cytokines in patients with preterm premature rupture of membranes with and without intrauterine infection

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عنوان البحث : Maternal Serum and amniatic fluid cyto-kines in patients with preterm premature rupture of membrance with and without intrauterine infection

المؤلفون حسب ترتيب ظهورهم على البحث : Amal S. Shabokshi, Mahamed Shannawy

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