

## The Role of Tumor Necrosis Factor-Alpha (TNF-A) in The Induction of Preterm Labor

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### Abstract

**Background:** Microbial colonization and inflammation in the maternal genital tract has emerged as one of the major risk factors associated with spontaneous preterm birth.

**Objectives:** this study aimed to demonstrate the role of tumor necrosis alpha (TNF- $\alpha$ ) in the induction of preterm labor.

**Materials and methods:** This study was conducted in Babylon Teaching Hospital of Gynecology and Pediatrics from November 2007 to May 2008. A total of 60 pregnant women with preterm labor admitted to Labor Room and 20 control women (10 of them were pregnant at term with bacterial infection, 5 were pregnant at term without bacterial infection, and 5 were normal females not pregnant and not infected) were included in this study. The ages of patients and controls ranged from (17-40) years. Blood samples were collected from both patients and controls to estimate tumor necrosis factor alpha (TNF- $\alpha$  by EASIA (Enzyme Amplified Sensitivity Immunoassay) method.

**Results:** The results show there is significantly higher ( $p < 0.05$ ) in the level of TNF- $\alpha$  in patients with preterm labor compared to all control groups.

**Conclusion:** The results clearly indicate the possible role of TNF- $\alpha$  in the induction of preterm labor.

**Keywords:** preterm labor, TNF- $\alpha$

### الخلاصة

**خلفية الدراسة:** يعد الاستيطان الميكروبي والتهاب القناة التناسلية الأنتوية من أهم عوامل الخطورة المسببة للولادات المبكرة

**الهدف من الدراسة:** معرفة دور عامل التنخر إلفا في إحداث الولادات المبكرة

**المواد وطرسائق العمل:** أجريت الدراسة في مستشفى بابل للنسائية والأطفال للفترة من تشرين الثاني 2007 ولغاية أيار 2008. تضمنت الدراسة 60 امرأة حامل بعانين من ولادة مبكرة وتم إدخالهن إلى صالة الولادة و20 امرأة أخرى كمجموعة سيطرة (10 منهن كن حوامل في الشهر التاسع و يعانين من التهابات بكتيرية و 5 كن حوامل في الشهر التاسع ولايعانين من التهابات بكتيرية و5 كن نساء طبيعيات غير حوامل وليس لديهن التهابات). كانت أعمار المريضات و مجموعة السيطرة تتراوح بين 17 و 40 سنة. تم جمع عينات الدم من كافة عينات الدراسة وتم قياس عامل التنخر ألفا في الأمصال المفصولة باستخدام تقنية الادمصاص المناعي المرتبط بالإنزيم.

**النتائج:** أظهرت النتائج ارتفاع مستويات عامل التنخر إلفا في الحوامل ذات الولادات المبكرة مقارنة مع المجاميع الأخرى.

**الاستنتاج:** النتائج تدل بوضوح على إمكانية عامل التنخر إلفا بإحداث الولادات المبكرة

## Introduction

Preterm birth is defined as delivery of a baby before completed 37 weeks of pregnancy<sup>(1)</sup>. The major risk factors for preterm birth are previous preterm birth, uterine over-distention and uterine abnormalities<sup>(2)</sup>. The main cause of preterm birth is infection which is possible cause in up to 40% of cases<sup>(3)</sup>.

Infection may promote preterm labor by producing prostaglandins that in turn stimulate labor. Prostaglandins production by human amnion can be stimulated by bacterial endotoxins and that many organisms produce phospholipase and thus may potentially initiate preterm labor<sup>(4)</sup>.

A considerable body of evidence supports a role for inflammatory mediators in the mechanisms of preterm labor. Major attention has been focused on the role of proinflammatory cytokines such as TNF- $\alpha$ <sup>(5)</sup>.

Evidence for the participation of TNF- $\alpha$  in preterm labor includes the following: TNF- $\alpha$  stimulate prostaglandin production by amnion, decidua and myometrium<sup>(6)</sup>; human decidua can produce TNF- $\alpha$  in response to bacterial products<sup>(7-8)</sup>; amniotic fluid TNF- $\alpha$  bioactivity and concentrations are elevated in women with preterm labor and intraamniotic infection<sup>(9-10)</sup>. Antibiotics may be of benefit in the prevention of preterm birth, so is the prophylactic use in women with abnormal genital tract colonization, for the prevention of preterm labor<sup>(11)</sup>.

This work aimed To evaluate TNF- $\alpha$  production in women with preterm labor and compare it with control groups (pregnant at term with bacterial infection, pregnant at term without bacterial infection, and normal females not pregnant and not infected).

## Patients & methods

a-A total of sixty pregnant women with preterm labor whose ages range between (17-40) years have been included in this study. Those patients have been clinically diagnosed by gynecologists as having preterm labor, and were admitted to the labor room at Babylon Hospital of Maternity and Pediatrics, during the period from November/2007 to May/2008.

Twenty control women, whose ages range between (20-40) years, were divided into two groups:

b-Fifteen pregnant at term (37 weeks of gestation or more) without infection.

c- Five normal, non-infected females-non pregnant.

## Specimens Collection

### Amniotic Membrane Piece

When the patient become fully dilated she is placed in lithotomic position. Povidone iodine used to wash the vagina and perineum. After delivery of the baby the placenta and membrane are delivered by Schultz method and placed in a sterilized dish. A piece of membranes is taken from the inner side (maternal side) using a sterilized pens and scissor to avoid contamination. Each piece was placed in a sterile tube containing brain-heart infusion broth and incubated aerobically for 24-48 hours at 37C° and then swab or loopfull was taken from this medium and inoculated on culture media (Blood agar, MacConkey agar and Nutrient agar) and incubated aerobically for 24-48 hours at 37C°<sup>(12)</sup>.

Blood samples were collected from women, sera isolated and TNF- $\alpha$  was measured by immunoenzymometric assay using Biosource TNF- $\alpha$  EASIA kit (Biosource Europe S.A.). TNF- $\alpha$  level was measured in picogram / ml.

Statistical Analysis: For statistical analysis SPSS (version 10)

program was used. The results were represented through frequency, mean and standard deviation. Student t-test used to compare between means and study the significance of the difference. P value <0.05 was considered to be statistically significant.

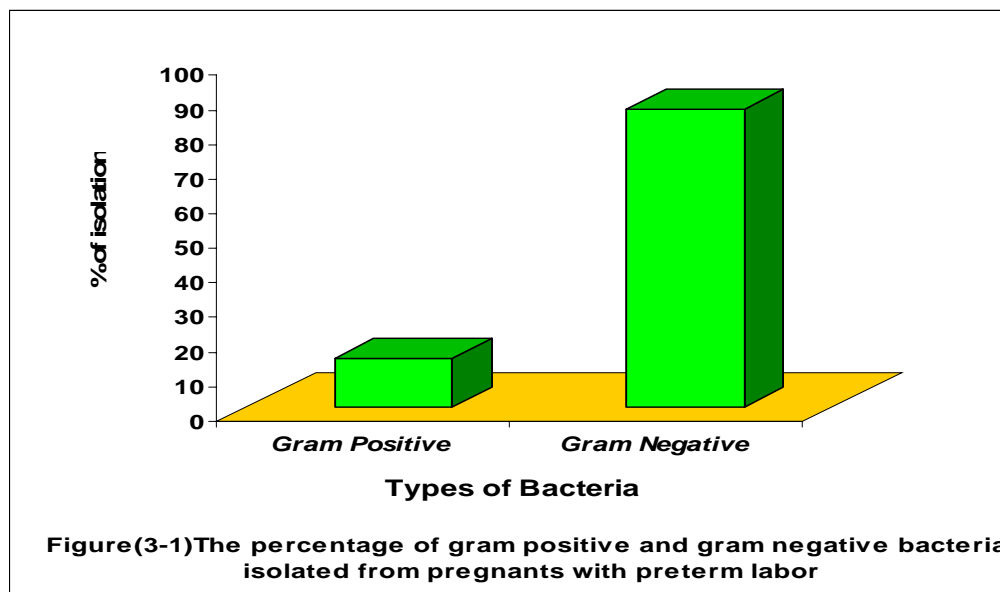
## Results & discussion

### Bacterial isolation

A total of 60 amniotic membrane pieces have been subjected to aerobic culturing on different types of culture media. The results reveal that 57 (95%) samples have positive

bacterial culture, whereas 3 (5%) samples have showed no bacterial growth. This negative growth may be due to the presence of another causative agents for preterm labor such as multiple pregnancy, polyhydramnions, uterine abnormalities, intrauterine death, iatrogenic and cervical incompetence<sup>(13)</sup>.

The results shown that Gram negative bacteria are the predominant bacterial isolates and constitutes about 85.96% (49:57) from the total isolates and compared with Gram positive bacteria which constitutes only 14.04% (8:57) as shown in figure (3-1).



the onset of preterm labor may be due to the presence of endotoxin (lipopolysaccharide) which associates with The high percentage of Gram negative bacteria<sup>(14)</sup>. Deb *et al.*,<sup>(15)</sup> have reported that genitourinary tract or systemic infections of the Gram negative bacteria in pregnant women causes preterm labor. Lipopolysaccharide is the most potent antigenic component of the Gram negative bacterial cell wall, and is known to modulate the expression of various proinflammatory cytokines.

Therefore, women with preterm labor had a high concentration of endotoxin in amniotic fluid than patients who were not in labor<sup>(16)</sup>.

Table (1) show the levels of TNF- $\alpha$  which is considered as an important immunological parameter that enhances the mechanism of preterm labor.

The results show that the mean of TNF- $\alpha$  in preterm labor is 91.287 pg/ml, while control groups which include non infected pregnant at term is 42.239 pg/ml, and normal female

which were used as a standard normal value is 27.883 pg/ml. Thus, there is significant increase in the level of TNF- $\alpha$  in preterm labor when compared to other control groups ( $P < 0.05$ ). This agrees with Htm1 (2007) who has reported that the amniotic fluid proinflammatory mediators (TNF- $\alpha$ ) increase during intra-amniotic infection, preterm labor, or preterm premature rupture of membrane<sup>(17)</sup>.

Spaziani *et al* (1998) suggest that TNF- $\alpha$  may play a role in infection-induced preterm labor by its pleiotropic ability to simultaneously stimulate cyclooxygenase-2 activity, prostaglandin E2 production, and expression of the prostaglandin E2 production receptor sub type Ep1 in human amnion<sup>(18)</sup>. Thus, TNF- $\alpha$  is the major mediators that may be responsible for the induction of preterm labor in this study, and the production of this cytokine is

stimulated by bacterial infection; mainly gram negative bacteria<sup>(19)</sup>. there is a direct association between septic shock and TNF- $\alpha$ . Most cases of septic shock are caused by endotoxin (LPS) – producing Gram negative bacteria. The LPS triggers the monoclear phagocytic cells to produce TNF- $\alpha$  that enhances the local acute inflammatory response characterized by fever, prostaglandins production, hypotension, endothelial injury, and relaxation of smooth muscles. In severe cases, these inflammatory processes result in multi organ failure (MOF)<sup>(20s)</sup>.

Thus in this study, it is clearly to note the role of TNF- $\alpha$ ; which is triggered by LPS of Gram negative bacteria isolated predominantly from the majority of cases (95%), in the induction of preterm labor by mediating the inflammatory process leading to the rupture of amniotic membrane.

Table 1. Tumor necrosis alpha (TNF- $\alpha$ ) for Pregnants with Preterm Labor and Controls

Testing group		TNF- $\alpha$ pg/ml
A-Preterm Pregnant	M*	91.2870
	SD**	33.7728
B-Non infected Pregnant at term	M	42.2398
	SD	12.5475
	Significance between A,C	significant (P < 0.05)
C-Normal Female	M	27.8838
	SD	35.8213
	Significance between A,D	significant (P < 0.05)

\* Mean

\*\* Standard deviation

Thus we conclude that there is an association between elevated pregnant serum TNF- $\alpha$  level and preterm labor, and TNF- $\alpha$  increases significantly in preterm labor when compared to other control groups.

From the results expressed above, we recommended that the use of TNF- $\alpha$  level as a marker to detect women at risk of preterm labor. Studies are needed to evaluate the association of other inflammatory mediators in the

process of preterm labor, and Inhibitory factors for TNF- $\alpha$  may be used as a therapeutic agent in women with preterm labor who have elevated serum TNF- $\alpha$  level.

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