Correlation Between *Chlamydia trachomatis* Infections and Interferon Gamma (IFN-γ) in Women With spontaneous abortion

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الخلاصة:

الهدف من الدراسة هو ايجاد العلاقة ما بين الاصابة بواسطة كلاميديا تراكوماتس وبين الاجهاض وايضا ايجاد الاهمية او عدمها بين الاصابة بالكلاميديا وبين الانترفيرون– كاما في النساء اللواتي عانين من الاجهاض.

تم جمع مصل الدم من النساء اللاتي عانين من الاجهاض وايضا من النساء اللاتي لديهن حمل ناجح وفحصت هذه المصول لاختبار وجود الاجسام المضادة للكلاميديا تراكوماتس "نوع م" وايضا للانترفيرون– كاما بواسطة اختبار الامتزاز المناعي المرتبط بالانزيم ويكون بشكل عدد تجارية.

لقد وجد ان هناك فرقا احصائيا مهما ما بين نسبة المضاد المناعي "نوع م" الخاص بالكلاميديا مع الانترفيرون– كاما في حالة النساء اللاتي عانين من الاجهاض مقارنة باولئك النسوة اللواتي لديهن حمل طبيعي. وايضا وجد ان هناك ارتباطا مناعيا مهما بين نسبة المضاد المناعي "نوع م" الخاص بالكلاميديا مع الانترفيرون كاما في مصول النساء اللواتي عانين من الاجهاض.

بيانات هذه الدراسة رفعت احتمالية ان يكون للمواد المناعية الخلوية نوع "1" دور في امراضية الاجهاض في حالة النساء المصابات بالكلاميديا تراكوماتس.

Abstract:

The objective of this study was to find out the correlation between *Chlamydia trachomatis* infection and production of Interferon gamma (IFN- γ) with respect to abortion in women that had spontaneous abortion.

Serum was collected from the women that had abortion and woman with successful pregnancy, and all samples were analysed for IgM specific antibodies for *Chlamydia trachomatis* and IFN- γ by commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits.

A highly significant difference in the serum level of IgM specific antibodies of *Chlamydia trachomatis* and IFN- γ was found in women that had abortion compared with successful pregnancy (P<0.001), and a highly

significant positive correlation between them (r=0.503, p<0.001) in sera of women that had abortion.

The data of this study strengthen the possibility that IFN-γ may explain the role of type 1 cytokines in the pathogenicity of abortion in the positive group for *Chlamydia trachomatis*.

Introduction:

Chlamydia trachomatis causes genital tract infections are unique due to a lack of clinical indicators of their presence. *Chlamydia trachomatis* is a major cause of infertility[1], ectopic pregnancy[2] and pregnancy loss (3) in women, and probably contributes to infertility in men[3,4].

Evidence exists to support that cytokines play a key role in maintenance of pregnancy via modulation of the immune system and associated cells[5].

During the early stages of pregnancy, the conceptus and placental tissues produce a wide variety of cytokines, including IFNs, ILs, TNF and granulocyte macrophage colony-stimulating factors (GM-CSF)[6].

It is generally accepted that the maintenance of an essentially T_{H2} cytokine environment, namely production of IL-4, IL-5, IL-6, IL-10 and IL-13[7], appear to be essential for successful pregnancy [5,8], Where as T_{H1} cytokines are associated with pregnancy loss [9]. Campbell and Lees[10] demonstrated that, the presence of infectious agents cause a shift of immune response during pregnancy from T_{H2} to T_{H1} which can be observed as an abortion process.

In this study, we attempted to find out the significance of acute infection of *Chlamydia trachomatis* in spontaneous abortion and find out whether or not there is a significant correlation between *Chlamydia trachomatis* infections and IFN-γ in women with spontaneous abortion.

Materials and Methods:
Patients:

This study included eighty (80) women from the Obstetrics and gynecology department of Al- Kadhmiya teaching hospital in Baghdad.

Sixty (60) women were admitted to the hospital for spontaneous abortion for evacuation, and 20 women for successful pregnancy as control group.

Patients ages ranged between ($\leq$ 20 to $\geq$35) years, from the mean of age (23.9 − 28.5) year.

According to the results of Enzyme Linked Immunosorbent Assay (ELISA) for detection of IgM antibodies for *Chlamydia trachomatis*; the patients were divided into three groups:

Group 1:   20 positive for acute *Chlamydia trachomatis* infection.
Group 2:   40 negative for acute *Chlamydia trachomatis* infection.

Group 3:   20 successful pregnancy negative *Chlamydia trachomatis* as a control group.

Sample collection:

For each women included in this study blood samples were collected to obtain the serum.

Enzyme Linked Immunosorbent Assay (ELISA) for the detection of IgM antibodies for *Chlamydia trachomatis*:

Materials provided with kit (NovaTec Immundiagnostica GmbH.Germany). Purified *Chlamydia trachomatis* antigen is coated on the surface of microwells Diluted patient serum is added to the wells, and the *Chlamydia trachomatis* IgM-specific antibody, if present, binds to the antigen. All unbound materials are washed away. Horseradish peroxidase (HRP)-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM-specific antibody in the sample. The results are read by a microwell ELISA reader compared in a parallel manner with calibrator and controls.

ELISA for the detection of IFN- γ in serum:

Materials provided with the kit (Mabtech Australia Pty, Ltd): estimation of IFN-γ (monoclonal antibody 1-D1K and Biotinylated monoclonal antibody 7-B6-1) level in serum or plasma by ELISA method. It was intended for quantification of serum IFN-γ. This ELISA is a two immunological step sandwich type assay. In the first step IFN-γ is captured by a monoclonal antibody bound to the wells of a micro titer plate. In the second step a monoclonal antibody linked to a biotinylated monoclonal antibody is added together with streptavidine-peroxidase conjugate. The solid phase antibody-antigen complex and, in turn, binds the conjugate. After incubation, the wells are washed and the antigen complex bound to the well detected by addition of a chromogenic substrate. The intensity of the coloration is proportional to the IFN-γ concentration in the sample or standard.

Statistical analysis:

ANOVA analysis program was used to calculate the values. The relationship between the indicators (IgM & IFN- γ) was measured qualitatively by using the correlation coefficient (r). Values of $P<0.05$ were considered as statistically significant.

Results:

The sera level of C.trachomatis IgM antibody and IFN-γ was detected by ELISA assay. Table (1) and (2) show the mean value of concentration (pg/ml) of *Chlamydia trachomatis* IgM antibody and IFN-γ in the sera of women which had abortion (positive and negative group of C. trachomatis) compared with that in successful pregnancy (control group).

Table (3) and (4) show a highly significant difference ($p < 0.001$) in the concentrations (pg/ml) of C.trachomatis and IFN-γ among the three groups and within the groups respectively.

Furthermore, the study demonstrated a highly significant correlation between the concentrations (pg/ml) of C.trachomatis IgM antibody and IFN-γ ($r = 0.503$, $p < 0.001$) in abortion, as demonstrated in Figure (1).

Variable	Group	n=80	Mean± SE[a]
C.trachomatis IgM	1	20	6.5 ±0.3
	2	40	3.4 ±0.5
	3	20	3.1 ±0.6

[a]=Standard error.

Table (1): The mean value of concentration (pg/ml) of *Chlamydia trachomatis* IgM antibody among studied groups.

Variable	Group	n=80	Mean± SE[a]
IFN- γ	1	20	950.2±81.3
	2	40	370.2±63.8
	3	20	8.6±0.42

[a]=Standard error

Table (2): The mean value of concentration (pg/ml) of IFN-γ (ELISA assay) in sera of studied groups.

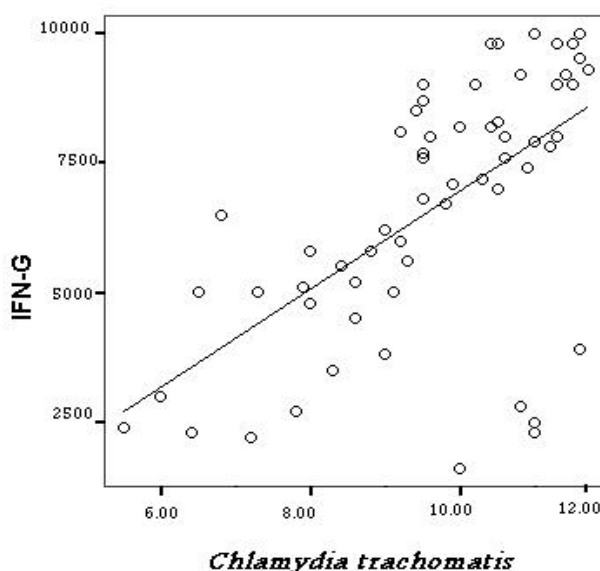| C. trachomatis IgM	P value
Among the groups	p<0.001**
Between group 1 and 2	p<0.001**
Between group 1 and 3	p<0.001**
Between group 2 and 3	p>0.05[n]

**highly significant difference ; [n] = not significant difference.

Table (3): **The significance of difference in the mean value of concentration (pg/ml) of *C.trachomatis* IgM in sera of studied groups.**

IFN—γ	P value
Among the groups	p<0.001**
Between group 1 and 2	p<0.001**
Between group 1 and 3	p<0.001**
Between group 2 and 3	p<0.001**

**highly significant difference

Table (4): **The significance of difference in the mean value of concentration (pg/ml) of IFN-γ (ELISA assay) in sera of studied groups.**

(IgM)

Figure (1): Correlation between *Chlamydia trachomatis* (IgM) and IFN-γ (r=0.503, p<0.001) in abortion.

Discussion:

This study has shown increased level of (IgM) anti-*Chlamydia trachomatis* antibody in women with abortion compared with that of women with successful pregnancy. Previous study supporting this result is that significantly high titers of chlamydial antibodies found in the sera of women with habitual abortion [11]. Also, Qublan [12], postulated that an immune response to an epitope shared by a Chlamydia and a fetal antigen is responsible for recurrent miscarriage. There were, however, no data available to confirm the role of intervention in improving the outcome of pregnancy.

In this study, we found that the expression of IFN-γ proteins in circulation of women with abortion was significantly higher (p<0.001) than that of women with successful pregnancy. In addition, this high level of IFN-γ is a highly significant correlation (r=0.503, p<0.001) with C. trachomatis infection.

The pro-inflammatory cytokine, IFN-γ was targeted as a reflection for type 1 immune response in this study, because of its T_{H1} polarizing effect due to its potential role in generating T_{H1} cells, mediating their effectors functions and regulation of T_{H1}/T_{H2} balance [13].

Evidence from murine and human pregnancy showed that since T_{H1} type cytokines mediated pregnancy loss, a shift towards T_{H1}-type immunity during infection may help to explain pregnancy failure [14,15]. Thus, a considerable amount of evidence suggests that T_{H1} cytokine might well be implicated in adversely affecting pregnancy,directly by interfering with trophoblast survival and function, and indirectly by activating cell-mediated immune effecters[9].

Regarding IFN-γ, evidences had shed the light on the possible role of IFN-γ in pregnant women during *Chlamydia trachomatis* infection and showed that there was a concurrent increase in concentration of IFN-γ in placenta when there was a strong T_{H1} dominant response against infectious agents which resulted in abortion[16]. This could be a potential explanation that evolved to light the highly significant difference (P<0.001, table 4) in the mean percent of IFN-γ between positive and negative groups simultaneously, it was high when compared to the group of successful pregnancy. This result could be explained based on the fact that the presence of other infections cannot ruled out in the negative group which can cause abortion by the presence of high levels of IFN-γ[17]. Furthermore, the administration of one of the T_{H1} cytokines like IFN- γ, TNF-α or IL-2 to normal pregnant mice causes abortion [18]. IFN- γ and TNF-α inhibit the proliferation of human trophoblast cells in vitro and are toxic to human trophoblast cells [19,20].

Also, IFN- γ and TNF-α induce apoptosis in trophoblast cells by the increase of Fas expression and enhance trophoblast sensitivity to Fas-mediated apoptosis[21,22].

In conclusion, the data of this study strengthen the possibility that IFN-γ may explain the role of type 1 cytokines in the pathogenicity of abortion in the positive group for *Chlamydia trachomatis*.

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