THE POSSIBLE ROLE OF PROGESTERONE AND IL-4 IN TROPHOBLASTIC TISSUE OF ABORTED WOMEN COMPARED WITH NORMAL PREGNANCY.[+]

الدور المحتمل للبروجيسترون والانتر لوكين – ٤ في النسيج الاممي في حالات الاجهاض مقارنة مع حالات الحمل الطبيعي

Eman A. Abd [*]

Abstract :-

Objective: Estimation of the immunohistochemistry expression of progesterone and IL-4 in trophoblastic tissue of aborted women compared with normal pregnancy and to find out whether or not the expression of these two marker in low level is associated with abortion.

Methods: A technique utilizing immunohistochemistry was performed to detect and determine the immunohistochemistry expression of progesterone and IL-4 proteins using paraffin embedded sections of curettage samples obtained from 40 women, who were divided into two groups: 30 women with first trimester abortion (group 1) and 10 women with induced abortion (group 2).

Result: The levels of the immunohistochemistry expression of progesterone and IL-4 were found to be a significant increased in group 2 as compared with group 1 ($p<0.001$), with a significant positive correlation between these two parameters ($P<0.05$) in studied groups.

Conclusion: The data of this study demonstrated that progesterone might induce expression of IL-4 in trophoblastic tissue that lead to successful pregnancy; thus the low trophoblasts expression of progesterone and IL-4 in first trimester pregnancy might play a role in pathogenesis of pregnancy failure.

المستخلص :-

استعمال تقنية التحليل الكيميائي المناعي لقياس مستوى التعبير للبروجيسترون وانتر لوكين – ٤ في النسيج الاممي للنساء اللواتي يعانين من الاجهاض مقارنة مع حالات الحمل الطبيعي .

استخدمت طريقة التحليل المناعي الكيميائي لقياس وتشخيص تعبير البروجيسترون وانتر لوكين – ٤ باستخدام المقاطع المغمورة بالبارافين للنماذج المأخوذة من (٤٠) امرأة والتي قسمت الى مجموعتين المجموعة الاولى تضم (٣٠) امراة في حالة اجهاض في الاشهر الاولى من الحمل والمجموعة الثانية تضم (١٠) نساء في حالة حمل طبيعي واجهاض مفتعل .

كانت نتيجة الدراسة ارتفاع مستوى البروجيسترون وانتر لوكين – ٤ في المجموعة الثانية مقارنة مع المجموعة الاولى ويوجد فرق معنوي احصائي بين هذين المقياسين في المجاميع المدروسة .

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[*] **Asst. Lecturer / Health and Medical Technical College**

دلت النتائج المستحصلة ان البروجيسترون ممكن ان يلعب دور في حث تعبير انتر لكوين – ٤ في النسيج الاومي وهذا يقود الى نجاح الحمل لذلك ان انخفاض مستوى البروجيسترون والانتر لوكين – ٤ في الثلاث اشهر الاولى من الحمل ممكن ان يلعب دور في امراضية فشل الحمل .

Introduction

Progesterone is known to be essential for the establishment and maintenance of pregnancy including ovulation, uterine, and mammary gland development [1]. The major source of progesterone during pregnancy are the corpus luteum of the ovary and, in many species including humans and rodents, the placenta [2]. Progesterone prevent endometrial shedding, thereby protecting the developing fetus. [3, 4]

During pregnancy, a series of profound immunologic chance occurs, including changes associated with altered tryptophan metabolism and progesterone-mediated alterations in the balance of cytokine elaboration [5, 6]. One of the generally observed effects on immune responses during pregnancy has been a bias toward humoral responses, often at the expense of cell-mediated immunity and associated inflammatory sequelae [6, 7, 8, 9]. Progesterone has been associated with immune-histochemistry functions for many years and has been described as nature's immunohistochemistry [10]. Studies in sheep and hamsters illustrate that progesterone at concentrations found at the maternal- foetal interface can inhibit tissue graft rejection in utero [11]. Progesterone is able to promote LIF synthesis by the endometrium and to displace the Th1 and Th2 balance towards Th2 [12]. The Th2 cytokines (IL-4, 5, 6, 10 and 13) produced at murine fetal-maternal interface down-regulate Th1 responses responsible for acute allograft rejection, thus along with other mechanisms, promoting fetal survival [13,14]

Methods

Patients: the study included 40 women from hospital in Baghdad (Al- Kadhmiay, Al Ulwiya and Al- Noaman). Patients' ages ranged between (18-36) years with a mean of (27.5 – 30.1) year. They were separated into two groups.

Group1: 30 pregnant women who presented with spontaneous incomplete first trimester abortion. All gave a history of 1-6 previous consecutive miscarriages. None of them had any significant medical disease, family history of genetic disease, or anatomical uterine abnormality.

Group2: 10 pregnant women who had at least three normal previous pregnancies, undergoing elective termination of an apparently normal pregnancy in the first trimester for a maternal indication under the approved consent of two senior gynecologists and a physician.

Sera from women in these groups were negative for specific IgM and IgG for rubella virus, human cytomegalovirus, and *Toxoplasma gondii and negative for specific IgM for Herpes simplex virus.*

Samples: Trophoblastic tissue from each woman, two to three samples were taken from different sites of the uterus during evacuation operation; thus, 2-3 paraffin embedded blocks were prepared for each patient. Sections from each block were stained with heamatoxylin and eosin for histopathological examination (only the sections trophoblastic tissue were included in the study).

Immunhistochemistry: For Immunohistochemistry technique (IHC), Dako-Cytomation LSAB2 System-HRP code K0673 (DakoCytomation, USA) was used. Kit contents included: 3 % hydrogen peroxide in water (ready to use), biotin labeled affinity isolated goat anti-rabbit and goat anti-mouse immunoglobulins in phosphate buffer saline (PBS), containing stabilizing protein and 0.015mol/l sodium azide (ready to use), Strepavidin-HRP (ready to use) and 3.3'-diaminobenaidine (DAB) in a chromogen solution.

The monoclonal antibodies mouse anti-human progesterone (DAKO, Denmark) and Rat anti-human IL-4 [Serotec,Ltd,Oxford, UK].

Trophoblastic tissue sections were deparaffinized in xylene for 5 minutes and rehydrated through a series of ethanol dilutions;then the slides were put in a jar containing the antigen retrieval solution and placed in the autoclave, for 2 minutes under 121°C, after that the slides were washed in a distilled water jar for 5 minutes; then taped and wiped around sections, Then [2-3] drops of peroxides block were applied onto the tissue and incubated at room temperature for 30 minutes then drained and blotted as before. The 100 μl of a protein-blocking reagent were applied onto the tissue and incubated at room temperature for 5 minutes. Then 100 μl of the diluted primary antibody (1/20 diluted in antibody diluents) were applied on to the tissue and incubated at 37°C for 1 hour . After that, the slides were placed in PBS wash bath for 2 minutes then excess buffer were taped and wiped around section. 100 μl of diluted biotinylated link (Secondary antibody) (1/20 diluted in antibody diluents) was applied and incubated at 37°C for 30 minutes then drained and blotted as before. 100 μl of the strepaviden-HRP reagent was applied covering then incubated at 37°C for 30 minutes then drained and blotted as before. drops of DAB-Substrate chromogen solution were applied on each section covering the whole specimen, the slides were incubated in darkness at room temperature for 20 minutes then the reaction terminated by rinsing gently with distilled water from a washing bottle, The slides were counter stained with Mayr's hematoxlin stain or nuclear fast red stain and then washed as before after that dehydration, mounting and examination.

Evaluation of the Immunostaining: The expression of immunostaining of progesterone proteins was measured by the same scoring system, by counting the number of positive villi, which gave nuclear and/or cytoplasmic dark brown granules under the light microscope. The extent of the IHC signal in the villi was determined in 10 fields (X100 magnification). In each filed the total number of villi were counted and the extent of cytoplasmic staining of the trophoblast cells in a given villous was determined as a percent. The total staining score was divided by the number of whole villi per field in 10 fields [15], so the percentage of positively stained villi in the 10 fields was calculated for each case by taking the mean of the percentage of the positivity stained villi in the 10 fields.

Statistical analysis: The Student test (t-test) analysis program was used to calculate the values. Mean, and standard error were all used in the analysis and chi-square test of significant was comparism of the prevalence of progesterone and IL-4 depends on the scoring level ,in addition the relationship between the indicators was measured qualitatively by using the correlation (r). Values of $P<0.05$ were considered as statistically significant [16].

Results

The expression of progesterone was detected by Immuohistochemistry (IHC) technique. Table (1) and (2) show the mean percentage of progesterone and IL-4 immunostaining expression respectively in the villus trophoblasts in terms of mean ± SE and show a significant ($P<0.01$) increased expression of progesterone in group 2 compared with group 1. In table (3) chi-square test of significant was conducted to examine the association between progesterone and IL-4 proteins expression in trophoblasts tissue in the two groups of investigated women it was found that a significant association ($P < 0.01$) between them in the three scoring levels.

In addition, in this study a significant correlation ($P<0.05$) between progesterone and IL-4 in studied group, was found as shown in Table 3 .

The expression of progesterone and IL-4 was heterogeneous dark brown nuclear staining, of villus trophoblastic cells, as shown in Figure 1.

Table (1): Mean percent of the expression of progesterone (IHC assay) in the trophblasts of studied group.

Studied group	No	Mean ± Std. Error	Comparison of significant	
			P-value	Sig.
group 1	30	22.31 ± 1.56	0.000	Sig.($P<0.01$)
group s	10	75.30 ± 3.76		
Total	40			

Table (2): Mean percent of the expression of IL-4 protein (IHC assay) in the trophoblasts of studied group.

Studied group	No	Mean ± Std. Error	Comparison of significant	
			P-value	Sig.

group 1	30	25.10 ± 2.3	0.000	Sig.(*P<0.01*)
group s	10	69.51 ± 2.9		
Total	40			

Table (3): Comparison of the prevalence of progesterone and IL-4 proteins (IHC assay) in

trophoblasts depends on the scoring level.

variable	score[a]	Groups		Total (n=40)	Chi-Square P value
		1(n=30) No (%)	2(n=10) No (%)		
progesterone	1	17 (56.7)	0	17	0.000**
	2	13 (43.3)	5(50)	18	
	3	0	5(50)	5	
IL-4	1	19(63.3)	0	19	0.000**
	2	11(36.7)	3 (30)	14	
	3	0	7 (70)	7	

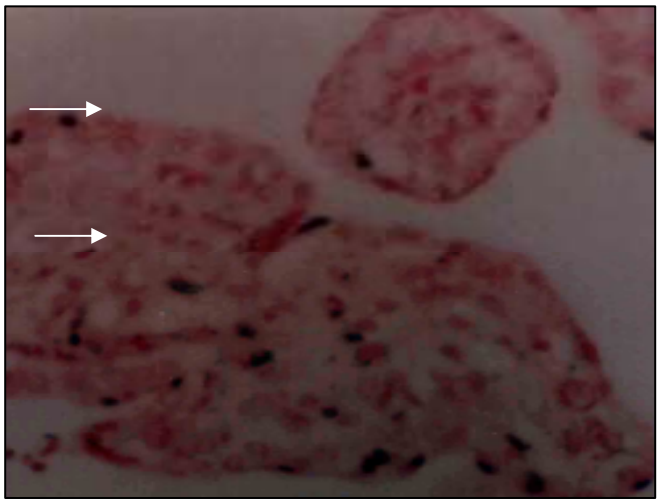
Score[a]: 1<25%; 2(25-74) %; 3(75-100) %

Table (4): Pearson correlation (r) between progesterone and IL-4 in studied group.

Variables		Correlation Coefficient r =	P value
Progesterone – IL - 4	group 1	0.483	< 0.05*
	group 2	0.626	< 0.05*

*=a significant correlation

A-



B-

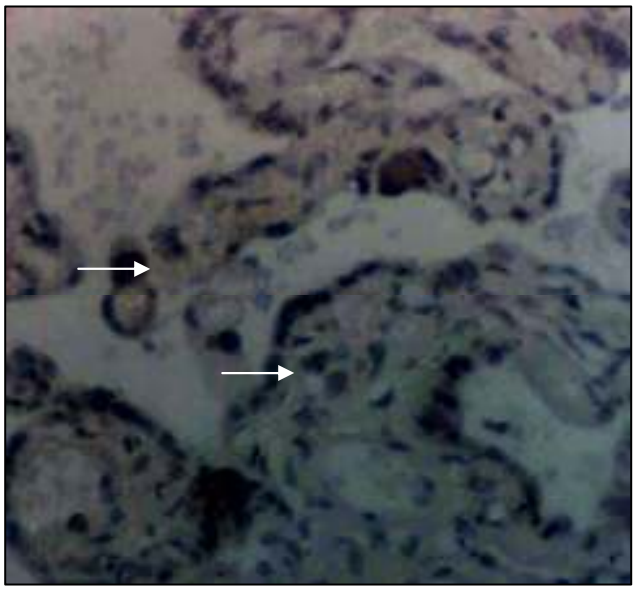


Figure (1): Immunohistochemical staining progesterone and IL-4 proteins in studied groups. Staining by DAB chromogen (dark brown) counterstained with muclaer fast

red (A) placental tissue from women shows positive progesterone immunostaining (X400). (B) Placental tissue from aborted women shows positive IL-4 (X400)

Discussion

This study has shown a lower production of progesterone in aborted women (group 1) compared with that of women with induced abortion (group 2). The possible explanation for these results by previous study that has been proposed that progesterone plays an important role in the maternal shift of the Th1/Th2 balance [17]. During implantation and gestation, progesterone appears to decrease the maternal immune response to allow for the acceptance of the pregnancy [18].

Progesterone is hormone of pregnancy that is derived from cholesterol steroids and produced mainly by the corpus luteum and the placenta, with some contribution from the adrenal glands. Progesterone is used to prepare uterine lining in infertility therapy and to support early pregnancy patient with recurrent pregnancy loss due to inadequate progesterone [19, 20].

Furthermore, in this study, we found that the expression of IL-4 proteins in trophoblastic tissue of women with successful pregnancy was significantly higher ($P < 0.001$) than that of aborted women. Previous study demonstrated that cytokines have been shown to influence all steps of reproduction, playing a fundamental role in pregnancy outcome.

Certain Th2 cell-derived cytokines such as IL-4, IL-6 and IL-10 seem to favors pregnancy success whereas Th1 cell-derived cytokines, such as IL-2 and interferon (IFN-γ), are harmful [21, 22]

In the current study, a significant correlation ($P<0.05$) between progesterone and IL-4 in tropholastic tissue of aborted women and successful pregnancy was found. This positive significant might be indicate that increased expression of progesterone leads to increased expression of IL-4 in trophoblatic of women involved in this study. Previous study demonstrated that the progesterone can induce T-cell differentiation towards the Th2 pathway, it has been associated with the induction towards the Th2 pathway, it has been associated with the induction of such a cytokine profile *in utero* [23]. Successful pregnancy has been associated with predominace of Th2-like cytokines at the maternal-foetal interface and a down-regulation of Th1 cytokines at the maternal-foetal interface and a down-regulation of Th1 cytokines [24]. In the progesterone, activated human lymphocytes, especially T cells, synthesize a 34-kDa molecule (progesterone-induced blocking factor) that inhibit NK activity and exerts an antiabortive effect in vivo [25]. Progesterone-induced blocking factor may be responsible in part for the progesterone effect on T cells [23]. Progesterone also plays a role in the induction of leukemia inhibitory factor, essential for embryo implantation, in the presence of IL-4, and the production of leukemia inhibitory factor and/or Th2 cytokines by decidual T cells contributes to the maintenance of pregnancy [26]. Therefore a part from the systemic changes in the maternal immune response, local immunmodulation at the feto-maternal interface via wide array of hormones and cytokines, and balance of the desirable immune response [27].

In conclusion, this study demonstrated that progesterone might induce expression of IL-4 trophoblastic tissue that lead to successful pregnancy ; thus the trophoblastic expression of progesterone and IL-4 in low level in first trimester pregnancy play a role in pathogensis of pregnancy failure.

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