Trophoblast-Expression of Interleukin-6 (IL-6) and Human Chorionic Gonadotropin (hCG) in Women with Recurrent Abortion Compared With Normal Pregnancy

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الخلاصة

تم تقييم التعبير المناعي للـ IL-6 وهورمون الـ hCG بطريقة التحليل المناعي النسيجي الكيميائي في النساء اللواتي يعانين من اجهاض تلقائي متكرر غيرمعروف السبب, وايجاد فيما اذا كان الـIL-6 ينظم افراز هورمون hCG .

استخدمت طريقة التحليل المناعي النسيجي الكيميائي لفحص وتحديد التعبير المناعي من بروتينات الـIL-6 و الـHCG باستخدام المقاطع النسيجية المغمورة بالبارافين لعينات الكورتاج المأخوذة من 50 سيدة, والتي قسمت الى ثلاث مجموعات:- الاولى 25 سيدة: مجموعة اجهاض تلقائي متكرر, الثانية 15 سيدة: مجموعة اجهاض لاول مرة, الثالثة 10 سيدات: مجموعة اجهاض مستحدث.

وجدت مستويات التعبير للـIL-6 و hCG باستخدام تقنية التحليل المناعي النسيجي الكيميائي ذو فرق معنوي عالي في المجموعة الثالثة مقارنة بالمجموعة الاولى والثانية ($P<0.01$) مع علاقة ارتباط موجبة بين المتغيرين ($P<0.05$) في المجاميع الثلاثة.

نستنتج من هذا البحث ان تعبير الخلايا الجنينية للـ IL-6 قد يلعب دور في تحفيز افراز هورمون hCG اثناء الحمل.

Abstract

Objective:

We studied the estimation of IL-6 and hCG expression in immunohistochemistry in women with unexplained Recurrent Spontaneous Abortion (RSA) and find out whether or not IL-6 regulates human Chorionic Gonadotropin release (hCG).

An immunohistochemistry technique was performed to detect and determine the immunohistochemistry expression of IL-6 and hCG proteins using paraffin embedded sections of curettage samples obtained from 50 women, who were divided into three groups: 25 women with RSA, 15 women with abortion for the first time, and 10 women with induced abortion.

The levels of the immunohistochemistry expression of both IL-6 and hCG were found to be highly significant increased in group 3 as compared with group 1 and group 2 ($P<0.001$), with a significant positive correlation between these two parameters ($P<0.05$) in three groups.

The trophoblasts expression of IL-6 might play a role in stimulation of the expression of hCG during pregnancy.

Introduction

Physiologically, the maternal immune system confronts the embryo/fetus with a host-defence reaction, based on the recognition of paternally derived fetal and placental antigens [1]. To avoid rejection of the semi-allogenic embryo/fetus, the maternal immune response is selectively suppressed in physiological pregnancies [2]. While helper T-cell (T_H-2) type immunity is believed to contribute to successful pregnancy, T_H-1 type immunity has been shown to be associated with idiopathic recurrent abortion [2, 3, 4]. Murine studies indicate that dominance of helper T-cell (T_H-1) type dependent cytokines, e.g. interleukin 1 (IL-1), interleukin 2 (IL-2), tumour necrosis factor (TNF)-α, and interferon (IFN)-α, are incompatible with successful pregnancy, whereas a dominance of T_H-2 cytokines, e.g. IL-4, IL-6 and IL-10 prevents fetal wastage [2]. Reports of elevated concentrations of T_H-1 cytokines and reduced concentrations of T_H-2 cytokines, including IL-6, among women with idiopathic recurrent abortion are in accordance with these animal models [5, 6, 7].

IL-6 is a multifunctional cytokine, produced by many different cell types, including immune cells, fibroblasts, endothelial cells, adipocytes and myocytes[8]. Besides the acute phase response, IL-6 is also known to play important roles in normal physiology [9, 10]. IL6 might be defining as a growth factor for trophoblast because of potency to release human chorionic gonadotropin (hCG) [11]. hCG influences several uterine factors, for example increases the expression of COX-2 gene, an enzyme involved in prostaglandin biosynthesis [12].LIF and vascular endothelial growth factor (VEGF) [13], suggesting a role in endometrial vascularization. In the baboon hCG was shown to cause physiological effects on the uterine endometrium in vivo, including an increase in glycodelin expression and secretion by the glandular epithelium, and differentiation of subepithelial stromal fibroblasts characterized by expression of the alpha smooth muscle actin, associated with the initiation of decidualization[14, 15]. This suggests that the primate blastocyst signal modulates the uterine environment prior to implantation [14].

In this study, we attempted to establish an association between the trophoblastic expression of IL-6 and hCG in women with recurrent abortion compared with normal pregnancy, to find out whether or not IL-6 regulates human chorionic gonadotropin release (hCG).

Materials and Methods

Patients:

The study included 50 women from three hospitals in Baghdad (Al-Kadhmiya, Al Ulwiya and Al- Noaman hospitals). Patients' ages ranged between (≥20-≤35) years with a mean of (23.9 − 28.5) year. They were separated into three groups:

Group 1: 25 pregnant woman who presented with spontaneous incomplete abortion. All gave a history of 3-6 previous consecutive abortion with no previous living baby. None of them had any significant medical disease, family history of genetic disease, or anatomical uterine abnormality.

Group 2: 15 pregnant women with no previous medical illness who presented with incomplete abortion for the first time.

Group 3: 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 all women in the three groups were negative for specific IgM and IgG for rubella virus, human cylomegalovirus, and Toxoplasma gondii and negative for specific IgM for Herpes Simplex virus ;Chlamydia trachomatis ;Syphilis ;antiphospholipid; anticardiolipin,; and antinuclear antibody.

Samples:From each woman, two to three samples were taken from different sites of the uterus during evacuation curettage 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 contained trophoblastic tissue were included in the study)

Immunohistochemistry:

For Immunohistochemistry technique (IHC), DakoCytomation 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'-diaminobenzidine (DAB) in a chromogen solution.

The monoclonal antibodies Rabbit anti–human interleukin-6 (Serotec, Ltd, Oxford, UK) and mouse anti-human human chorionic gonadotropin(hCG) (DAKO, Denmark).

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 peroxidase block were applied onto the tissue and incubated at room

temperature for 30 minutes then drained and blotted as before. The 100µl of a protein-blocking reagent were applied onto the tissue and incubated at room temperature for 5 minutes. The 100µl of the diluted primary antibody (1/20 diluted in antibody diluents) were applied onto 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 sections. The 100µl 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. The 100µl of the Strepaviden-HRP reagent was applied covering then incubated at 37°C for 30 minutes then drained and blotted as before. The 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 Mayer's hematoxylin stain or nuclear fast red then washed as before after that dehydration, mounting and examination.

Evaluation of the Immunostaining:

The expression of both the immunostaining of IL-6 and hCG 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 (X100magnification). In each field 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 [16], 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.

Statistics:

ANOVA test was used to determine the difference in the immunostaining of IL-6 and hCG among the three groups and in between each two groups, and the relationship between these two parameters was measured using the correlation coefficient (r), Values of $P<0.05$ were considered as statistically significant.

Results

The expression of IL-6 and hCG was detected by IHC technique. Table(1) show the percentages of IL-6 and hCG immunostaining expression respectively in the villus trophoblasts in terms of mean + SE. and show highly significant expression of IL-6 and hCG among the three groups and within the groups respectively.

In addition, the study demonstrated a significant correlation (P<0.05) between IL-6 and hCG, as demonstrated in Table (2).

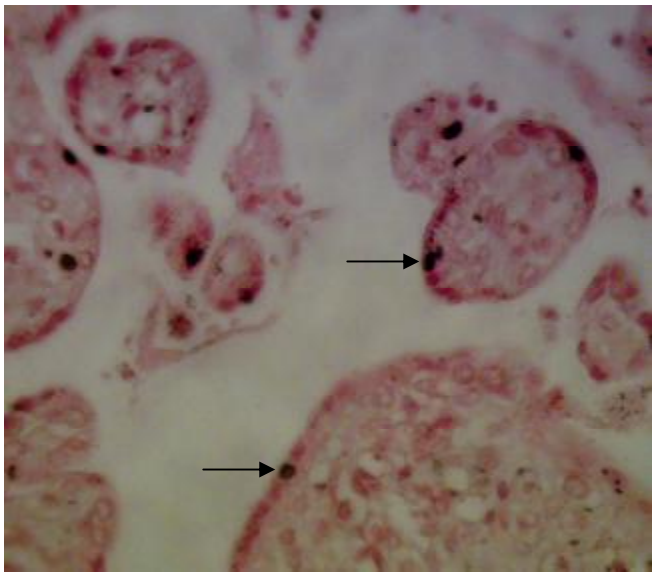
The expression of IL-6 and hCG was heterogeneous dark brown nuclear staining, of villus trophoblastic cells, as shown in Figure 1.

Variable	Group	n=50	Mean±SE	F test p value	Sig. between groups	
					Group	P value
IL-6	1	25	18.8±1.1	<0.01	1 □ 2	0.000**
	2	15	42.6±2.1		1 □ 3	0.000**
	3	10	75.9±3.1		2 □ 3	0.000**
HCG	1	25	±1.716.1	<0.01	1 □ 2	0.000**
	2	15	40.1±2.1		1 □ 3	0.000**
	3	10	69.3±2.5		2 □ 3	0.000**

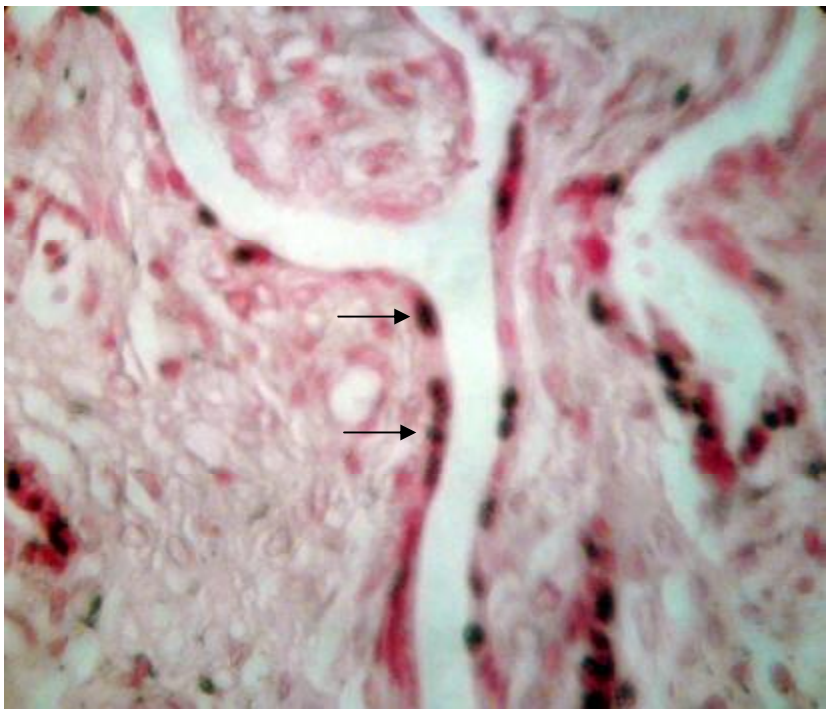
Table 1: Comparison between the mean percent of the expression of IL-6 and hCG protein (IHC assay) in the trophoblasts of studied groups.

P value	Correlation Coefficient r =	IL-6□ hCG
Group 1 (n=25)	0.510	<0.05
Group 2 (n=15)	0.230	<0.05
Group 3 (n=10)	0.416	<0.05

Table 2: Relation between the mean percent of IL-6 and hCG in trophoblasts among the studied groups.



(A)



(B)

Figure 1: Immunohistochemical staining (IHC) of IL-6 and hCG proteins in studied groups. Staining by DAB chromogen (dark brown) counterstained with nuclear fast red (A) Patient with RSA shows positive IL-6 immunostaining (X400). (B) Successful pregnancy shows positive hCG immunostaining (X400).)

Discussion

This study has shown a lower production of IL-6 and hCG in recurrent abortion compared with that of women with induced abortion or first time abortion.

Previous study found that IL-6 concentrations were lower in women with recurrent abortion (RSA) than in those women with induced or first time abortion; considering that IL-6 is a T_H-2 type cytokine and that normal pregnancy appears to be a T_H-2-biased condition [17].In this study the absence of high expression of IL-6 in the trophoblastic of women with RSA might reflect a bias away from T_H-2 type reactivity and a shift towards Th1-dominance.

Other studies demonstrated that IL-6 may induce prostaglandin synthesis by intrauterine tissues; thus, it seems to play physiological role in labor development. High levels of IL-6 have been detected in Pregnant women at term and in preterm at labor [7, 17,18]. Moreover, IL-6 is considered a T_H-2 cytokine; however, it may perform "T_H-1 type" or a "T_H-2 type" functions depending on the biological situation. Thus, IL-6 is involved in intrauterine infections and, in these conditions, its levels are even higher [19]. Some studies have evaluated women at the time of abortion, during pregnancy or at labor and these reports have shown lower levels of IL-6 in recurrent abortion patients compared to healthy women [20,21].

On the other hand, this study found that hCG expression were lower in trophoblastic of women with RSA than in those women with induced or first time abortion. This results in agreement with other study that found a large amount of hCG is rapidly produced by chorionic villi in the first trimester [22]. hCG is a highly evolved hormone that effectively elicits both endocrine and immune reactions in primates [23]. hCG stimulates placental steroid synthesis and the growth of the fetal adrenal gland. Also, hCG is involved in modulating the immunological response of the maternal tissues by immunosuppressive action on maternal leukocytes in the region of the invading trophoblast [24].Other study by [25], suggesting that hCG is a useful marker for the diagnosis of early pregnancy failure. Therefore, further research on the mechanism of transcriptional and translational regulation of immunity-related genes will help in understanding their roles in maintaining normal pregnancy.

In addition, the current study found a significant correlation ($p<0.05$) between IL-6 and hCG in three groups. Evidence supporting this suggestion includes the fact that secretion of IL-6 leads to a stimulation of the hypothalamic–pituitary–adrenal axis during inflammatory processes [26], promotes osteoclastogenesis and participates in the development of osteoporosis associated with estrogen withdrawal [27]. IL-6 is not constitutively expressed, but is highly inducible and produced in response to a number of inflammatory stimuli [28]. It has been demonstrated that IL1 induces IL6 production, and IL6 activates different intracellular signal transduction pathways in the placenta to stimulate human chorionic gonadotropin (hCG) release [29]. Because the capacity of hCG to support cytotrophoblast growth has been reported [30]. IL6 might be

defining as a growth factor for trophoblast because of potency to release hCG. It has also been demonstrated the elevated expression of IL6 and both receptors, gp80 and gp30, in placental tissue preterm in the absence of infection, thus making an association between these molecules and preterm labor preterm in the absence of infection [11].

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