Increased expression of estrogen receptors at the materno-fetal interface in patients with recurrent pregnency loss

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Abstract

Background: Estrogen hormone has been implicated in the pathogenesis of different genital tract pathologies and in counteracting the progress of normal pregnancy.

Objective: Localization and semi-quantization of estrogen receptors at the materno-fetal interface in patients with recurrent pregnancy loss (RPL).

Methods: Immunohistochemistry analysis of estrogen receptors using paraffin embedded sections of curate samples obtained from 40 women, who where divided into three groups: 24 women with RPL, 10 women with abortion for the first time, and 6 women with induced abortion.

Results: The mean value of the expression of estrogen receptors was (71.2 ± 2.3), which is significantly higher than that of the second group (52.2 ± 3.2), and the third group (43.7 ± 4.2), (p=0.001).

Conclusion: High expression of estrogen receptors in women with RPL may give a clue to its prominent role in the pathology of pregnancy loss.

Key wards: Estrogen receptor, RPL.

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Introduction

Spontaneous abortion is defined as the spontaneous loss of pregnancy prior to the 20th gestational week of pregnancy. Pregnancy losses which occur during this period of time are said to occur in about 15 percent of pregnancies. At the same time, the risk of miscarriage increases proportionately to the number of previous miscarriages experienced [(1)]. Many underlying hormonal abnormalities, ovulation defects and cyclic abnormalities can also be observed in patients with multiple miscarriages [(1, 2)].

Several causes for recurrent pregnancy loss (RPL) have been hypothesized, including endocrine disorders [(2, 3)], genetic [(4)], and uterine anatomical abnormalities [(5)].

Immunological factors are thought to account for many of the remaining 40-60% of unexplained miscarriages [(6)].

The interactions between immune-endocrine and reproductive systems are heightened during pregnancy as an adaptive mechanism and are regulated by a complex array of hormones and cytokines that control the survival of a semiallogeneic conceptus [(7)]. Multiple signals synchronize the development of the blastocyst and the preparation of the uterus. During early pregnancy estrogen stimulates proliferation and differentiation of endometrial stromal and epithelial cells. Downstream effectors of steroid-hormone actions include peptide hormones, growth factors, and cytokines [(8)].

Estrogen is implicated in many inflammatory and autoimmune diseases [(9-11)] and has been shown to up-regulate IFN in activated splenocytes [(12-14)].

In vivo studies of the role of estrogen and progesterone in the

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regulation of the uterine immune environment demonstrated a general pro-inflammatory effect of estrogen causing an influx of macrophages and neutrophils, which is antagonized by progesterone through its receptor [15-16].

Previous studies showed a very faint immunohistochemistry signal of the staining of estrogen receptors [17]. In this study, we attempted to detect the expression of estrogen receptor in women with RPL and compare it with that in normal pregnancy and women with pregnancy loss for the first time using monoclonal antibodies of estrogen receptor.

Patients, materials and methods

This study was conducted from November 2003 to April 2004. Patients were collected from Al-Kadhmya and Al-Ulwiya teaching hospitals, and then divided into three groups; **Group A:** 24 pregnant ladies presented with abortion during the first trimester, all of whom gave a history of previous 3-6 consecutive first trimester abortions, with no medical diseases, nor family history of genetic diseases or uterine anatomical anomaly, also all of them were confirmed by lab. Tests to be negative for acute infection with rubella, HCMV and toxoplasmosis. **Group B:** 10 pregnant ladies presented with abortion during the first trimester and had at least three previous normal pregnancies with no previous abortion, and no history of any medical illness, and **Group C:** 6 pregnant ladies with elective termination of pregnancy in the first trimester for a maternal indications under approved consent of two senior gynecologists and a physician (as control group). Curate samples of the materno-fetal interface were taken from all these women at the end of evacuation curate operation then embedded in paraffin and confirmed by a pathologist, and then subjected for immunohistochemistry technique using DAKO cytomation detection kit (Denmark).

Immunohistochemistry procedure: 5µm thickness tissue sections on positively charged slides were deparafinized in xylen then rehydrated in a series of ethanol concentrations. And then, 2-3 drops of peroxidase block were applied onto the tissue sections a step which is followed by application of the primary antibody (anti-estrogen receptor in a dilution of 1:30) (BioGenex-USA), then the secondary antibody was added, followed by application of the hoarse reddish peroxidase (HRP) conjugate, and then its substrate DAB chromogen. Sections were counterstained with hematoxyline, dehydrated and mounted to be finally examined under the microscope. For more details refer to the immunohistochemistry procedure in reference [18].

Evaluation of the immunohistochemistry signal: The expression of estrogen receptors was measured by counting the number of positive decidual and trophoblastic cells, which gave a dark-brown nuclear staining under the light microscope. The extent of the immunohistochemistry signal in the villi was determined in 10 fields (X100 magnification). In each field the total number of villi were counted and the extent of nuclear staining of the cytotrophoblast and syncytiotrophoblast in a given villous was graded as 3, (75–100%); 2, (25–75%); or 1, (<25%). The total staining score was divided by the number of whole villi per field in 10 fields. These scores (between 1 and 3) were added for each field, and a score between 10 and 30 was gained for each sample [19], and to be simplified as percent, the

percentage of positively stained villi was calculated for each case by taking the mean of the percentages of the positively stained villi in the 10 fields as advised by Hennessy (Personal communication, 2004). The scorer was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observers.

Negative controls were obtained by omitting the monoclonal antibody and using phosphate buffer saline to verify the signal specificity. Positive control signal was obtained using normal healthy ovarian tissue.

Statistics: ANOVA test was used to determine the difference in the expression of estrogen receptor among the three groups. Values of $p<0.05$ were considered as statistically significant.

Results

Table (1) shows the percentages of the expression of estrogen receptors in terms of mean ± SE, minimum and maximum values of the three groups, and it is obvious that the expression was higher in the recurrent loss group (mean=71.2 ± 2.3) than that of group B and C. (Table 2) shows the differences in the expression of estrogen receptor among the three groups and within the groups using ANOVA analysis. Estrogen receptors expression was heterogeneous dark-brown nuclear staining involving the trophoblasts, both cyto- and synsytiotrophoblasts in the three groups of women but it was more significant and obvious in the recurrent loss group (Figure 1).

Table 1: The expression of estrogen receptor among the studied groups

Estrogen Receptor	n	Mean ± S.E.$^{\Psi}$	Min. Value	Max. Value
Group A	24	71.2 ± 2.3	50	90
Group B	10	52.2 ± 3.2	35	70
Group C	6	43.7 ± 4.2	30	60

Total mean = 62.3 ± 2.4 %

$^{\Psi}$ Standard error

Table 2: The significance of differences in the expression of estrogen receptor in between the groups

Estrogen Receptor	*P* Value
Among the groups	0.001
Between group A and B	0.001
Between group A and C	0.001
Between group B and C	0.134

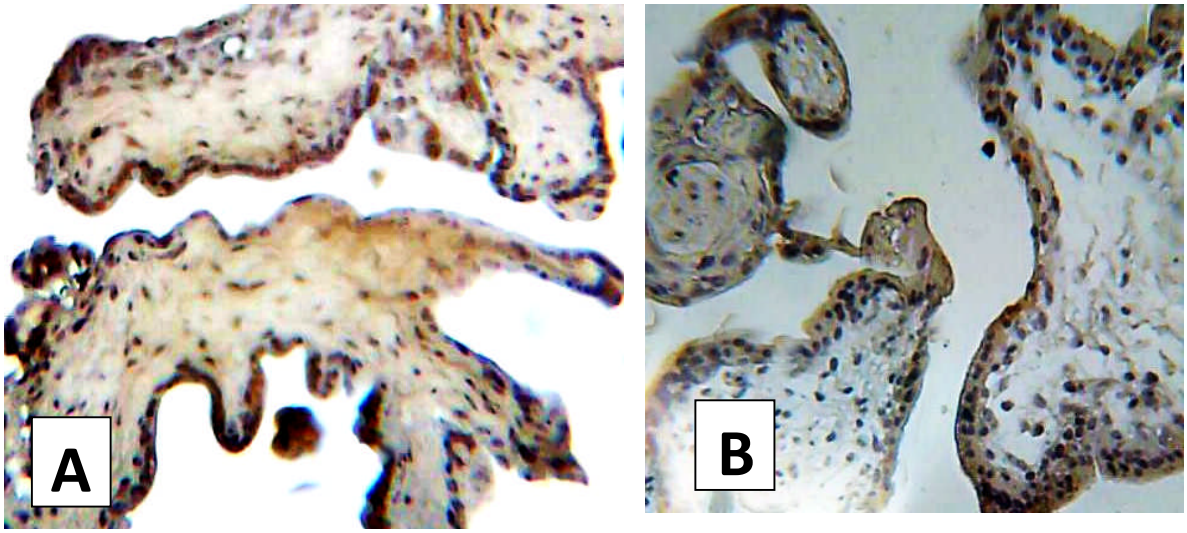

Figure 1: Detection of Estrogen Receptor by immunohistochemistry in women with pregnancy loss. (A & B) Expression of estrogen receptor in the trophoblasts in women with RPL and normal pregnancy respectively. Estrogen receptors expression was diffuse heterogeneous dark-brown nuclear staining involving the trophoblasts, both cyto- and synsytiotrophoblasts in the three groups of women but with darker and higher percentage of expression in the recurrent loss group. Magnification power of A and B (X400).

Discussion

It is well known that sex steroids have significant impact on the development of autoimmune diseases in both humans and rodents. In particular, estrogen has been suggested to be responsible for the strong female preponderance of the human rheumatoid arthritis, systemic lupus erythematosus, scleroderma, and Sjo¨gren's syndrome, but the role of estrogens in the female has not been fully characterized [20-22].

Sex hormones influence both humeral and cell-mediated immune response, and estrogen is one of potential factors in this immunological dimorphism [23].

The data of this study showed a significant increase in the expression of estrogen receptor in the tissue of women with RPL, in which estradiol has been shown to selectively enhance the development of IFN-γ-producing cells through an ER (estrogen receptor)-dependant mechanism [24]. In fact, estrogen is known to increase activity of the IFN-γ promoter and cause increase in the expression of IFN-γ mRNA in the stimulated murine spleen cells [25]. All these studies goes with the previous studies on these cases that showed a significant increase in the expression of the Th1 cytokine (IFN-γ) in women with RPL as compared with the control groups [26].

In addition another study showed that estrogen treatment up-regulates IFN-γ inducible-iNOS (nitric oxide synthase) gene expression, iNOS protein, nitric oxide, and cyclooxygenase-2 as an indirect consequence of activation of T cells [14]. Besides, estrogen may promote inflammatory conditions by altering the levels of chemokines, providing evidence for an additional mechanism by

which estrogens can regulate inflammation [27].

Recently, a study showed an inappropriate immune response to sex hormones especially estrogen and progesterone in RPL women as compared with the control group due to hypersensitivity to sex hormones [28].

On the contrary, a study compared the serum level of progesterone and estrodiol between a group of non-pregnant women with history of RPL during the follicular phase, and nulligravid females with tubal or male-factor infertility without miscarriage, showed comparable results in both groups with very few cases showing higher estrogen and lower progesterone levels in the study group [29]. But our data come from the local expression of the hormone at the materno-fetal interface meaning that we try to study the actual hormonal environment during pregnancy. Also apart from systemic changes in the maternal immune system, local immunomodulation at the materno-fetal interface via wide array of hormones and cytokines and immune effector cells also play a very critical role in maintaining the balance of a desirable immune response [30, 31].

In conclusion, increased expression of estrogen receptor in women with RPL could give a clue to its role as a pro-inflammatory stimulant augment the effect of Th1 cytokines participating in the pathology of RPL.

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