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Expression of Fas and FasL in Trophoblastic Tissue of Women with Spontaneous and Induced Abortion Using *insitu* Hybridization Technique

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Abstract

Background Apoptosis of trophoblastic cells may play a role in the pathogenesis of abortion, and one mechanism of apoptosis is Fas receptor and legand system.

Objective Estimate the level of Fas and FasL in trophoblastic tissue of aborted women.

Methods In this study, 25 women with spontaneous abortion and 5 women with induced abortion were included from attendants of Gynecology department at Al-Kadhimiya Teaching Hospital in Baghdad. In-situ hybridization (ISH) tests were done to detect the level of expression of Fas and FasL in trophoblastic tissue.

Results The highest percentage of expression of Fas and FasL was found in the trophoblastic tissue of females with spontaneous abortion (34.36% for Fas and 31.86% for FasL), while the expression of them was low in induced abortion group (9.2% and 9.4%, respectively).

Conclusion The data strengthen the possibility that the apoptosis of trophoblastic cells via enhanced expression of Fas-FasL system is an important mechanism of spontaneous abortion.

Key words Miscarriage, Trophblast, Apoptosis, Fas, FasL, ISH.

Introduction

Miscarriage, the loss of pregnancy before 20 weeks, is a devastating event with both physical and emotional components. The common causes of miscarriage are genetic error, abnormal hormonal levels (especially if progesterone levels are low), polycystic ovarian syndrome, structural problems, blood incompatibility, clotting disorders, environmental factors and infections which affect fetal development and in some cases result in miscarriage [(1)].

A number of studies have suggested that apoptosis plays a role in the normal development, remodeling, and aging of the placenta [(2,3)].

Analysis of apoptosis mechanism that leads to abortion may provide a new insight into pathogenesis of abortion. Since previous studies indicate that the apoptosis of placental could be as a result of immune responses, which could be mediated by Th-1 response, and most probably via enhance expression of Fas-FasL [(4)].

Apoptotic messages from outside the cell (extrinsic inducers), whereas apoptotic messages from inside the cell (intrinsic inducers) are a response to stress, such as nutrient deprivation or DNA damage. Both extrinsic and intrinsic

pathways have in common the activation of central effectors of apoptosis, a group of cysteine proteases called caspases, which carry out the cleaving of both structural and functional elements of the cell, resulting in the morphological changes of apoptosis [5].

One mechanism of inducing apoptosis is activation of the CD95 receptor and ligand system [6]. The CD95 molecule (synonyms: APO-1, Fas) is a type I transmembrane receptor which belongs to the nerve growth factor/TNF receptor superfamily [7,8]. CD95 is constitutively expressed in a wide range of normal human tissues. Moreover, expression of CD95 can be induced in various conditions [9]. CD95 ligand (CD95L) is a transmembrane protein which belongs to the TNF family [7]. CD95 ligand is expressed in various human cells and tissues, such as activated T lymphocytes, lung, liver or kidney [10,11]. Binding of CD95L to the extracellular domain of CD95 induces trimerization of the receptor then, the intracellular domain of CD95 recruits via an adaptor Fas associated death domain (FADD) the cytoplasmic caspase-8 [12].

In this study a try was made to investigate the level of expression of trophoblastic Fas and FasL (using in-situ hybridization method) as an indicator of apoptotic process in trophoblastic cells in abortion.

Methods

Twenty five pregnant female patients with spontaneous abortion, their age range from 18 to 44 years were included in this prospective study. They were attendants of Obstetrics and Gynecology department at Al-Kadhimyia Teaching Hospital in Baghdad, from 5th April 2007 to 30th September 2007. All were admitted to the hospital for evacuation. Another five females were also included, they were admitted for elective termination of pregnancy (induced abortion) due to maternal cardiac disease and they were considered as a control group.

From each patient and control included in this study, trophoblastic tissue was collected from the evacuated retained pieces during the procedure of curettage and placed in 10% formaldehyde. Paraffin embedded blocks were prepared and were sectioned, one section stained with haematoxyline and eosin, and only sections that contained trophoplastic tissue were included in this study.

In-situ hybridization technique using biotinylated long cDNA probe (318 bp for human Fas-gene detection and 250 bp for human Fas-L gene detection) together with Maxim's ISH detection kit was used.

Paraffin embedded sections of trophoblastic tissue were cut into 5 µm thickness, placed on positively charged slides and placed in a 65ºC hot air oven overnight, and cleared in two changes of xylene for 5 minutes each, rehydrated in two changes of absolute ethyl alcohol for 2 minutes each, then in fresh 95% ethyl alcohol for further 2 minutes. Slides then were placed in deionized water for 5 minutes then drained and blotted gently.

Deproteinization of the tissue was done by placing protienase K solution onto the tissue section at 37ºC for 15 minutes. 10-20 µL of the working (biotinylated probe) DNA probe/hybridization solution was placed onto the tissue sections, and then slides were placed in the oven at 70ºC for 10 minutes to denature the secondary structure of RNA.

In the next morning one to two drops of RNase A were placed onto the tissue sections and incubated at 37ºC for 30 minutes, and then the slides were washed in 1X proteinblock. One to two drops of the (biotinylated antibiotin Abs) were placed onto the tissue sections and incubated at 37ºC for 1 hr, then 1-2 drops of conjugate (red) (streptavidin-AP) were added for 20 minutes at 37ºC, and then 1-2 drops of the substrat, was placed onto the tissue section and incubated at room temperature for 15-20 minutes, and then the sections were

counterstained with neuclear fast red (NFR) stain for 30 seconds.

Dehydration of the sections was carried out by sequential dipping of the slides once in 95% ethanol, twice in 100% ethanol and then once in xylene. One to two drops of DPX were placed on xylene-wet sections, and the sections were quickly covered with coverslips and left overnight to dry. The slides were examined under light microscope.

A scoring system that includes an evaluation of the staining percentage of stained cells was employed for the expression of Fas & FasL, as following: Negative if < 5% of the cells were positively stained. Positive if ≥ 5% were positively stained.

Results

Among the two groups (spontaneous and induced abortion), the highest percentage of Fas were found in the spontaneous abortion group, while the lowest percentage were found in the induced abortion group, and the highest percentage of FasL were found in the spontaneous abortion group, while the lowest percentage were found in the induced abortion group, as shown in the table 1.

There is significant difference (p=0.016) in the mean value of Fas between spontaneous abortion group and induced abortion group, and there is significant difference (p=0.019) in the mean value of FasL between spontaneous abortion group and induced abortion group. There is high positive correlation (0.809) between Fas and FasL expression in trophoblastic tissue and it is highly significant statistically (p<0.001)

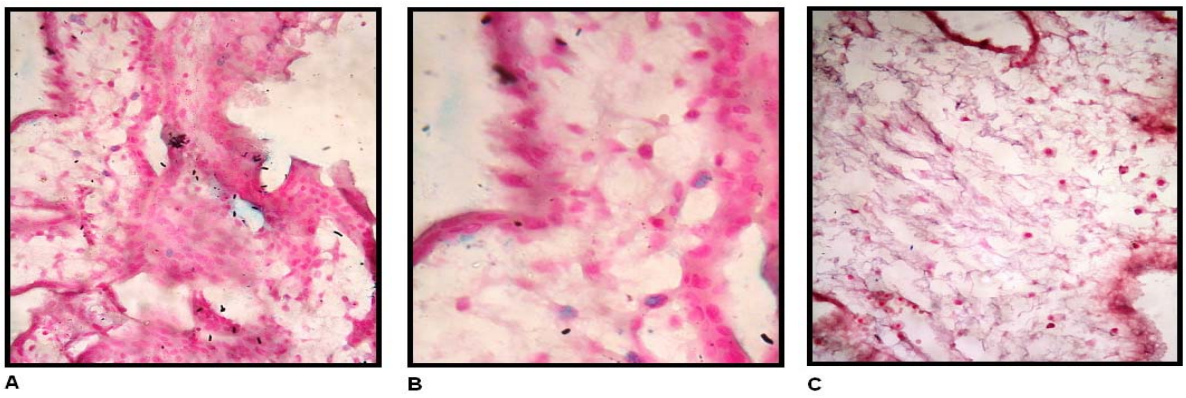

Figure 1: *In-situ* hybridization for human Fas in trophoblastic tissue sections. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red (pink). A: Positive case, magnification power (100X), B: Positive case, magnification power (400X) C: Negative case, magnification power (100X).

Table 1: Fas and FasL expression in spontaneous and induced abortion cases.

Abortion type	Fas			FasL		
	Mean±SE	N	p-value	Mean±SE	N	p-value
Spontaneous abortion	34.36±3.18	25	0.016	31.86±2.91	25	0.019
Induced abortion	9.20±1.56	5		9.40±1.03	5	

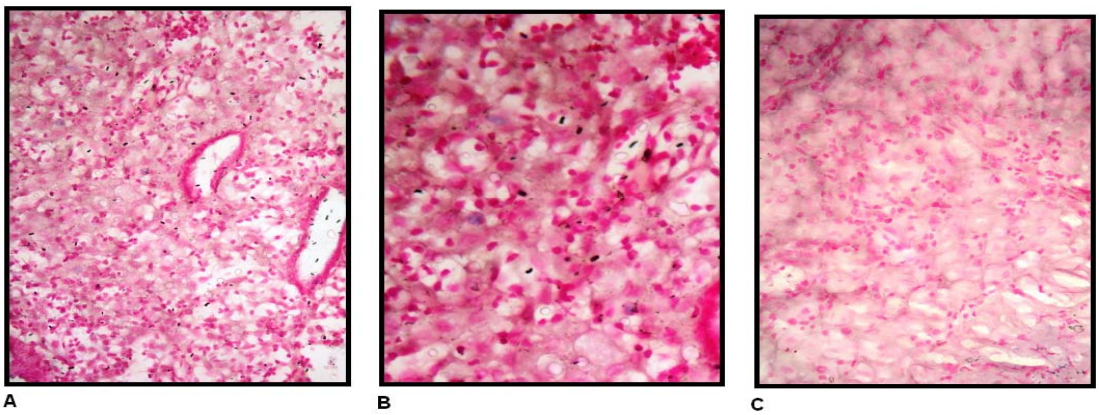

Figure 2: *In-situ* hybridization for human FasL in trophoblastic tissue sections. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red (pink). A: Positive case, magnification power (100X), B: Positive case, magnification power (400X) C: Negative case, magnification power (100X).

Discussion

Our results shed light on the delicate balance between immunoprotection and trophoblast death. The finding is that Fas ligation on trophoblastic cells induces proinflammatory cytokine production (which is IL-8 in our study), resulting in neutrophil chemo attraction agrees with the findings of a study done by David and co-workers, they investigated Fas-induced cytokine responses of normal human blood monocytes and monocyte-derived macrophages. Their principal finding was that Fas ligation on these cells induces predominantly proinflammatory cytokine production, resulting in potent neutrophil chemoattractant bioactivity. This represents the first evidence that Fas ligation activates human monocyte and macrophage proinflammatory cytokine responses. These observations are especially significant because they run counter to the prevailing notion that Fas ligation on phagocytes and Fas-induced phagocyte apoptosis result in predominantly anti-inflammatory effects that contribute mainly to the resolution of inflammation [13].

Our study demonstrate that Th-1 proinflammatory cytokine (IL-8) promotes Fas expression thereby increasing the sensitivity of trophoblast cells to Fas-mediated apoptosis and the enhanced sensitization to Fas results in trophoblast autocrine-induced apoptosis once FasL binds to and activates the Fas receptor, and this agrees totally with a study done by Sarit and co-workers, they demonstrate that Th-1 proinflammatory cytokines promote Fas expression, whereas Th-2 anti-inflammatory cytokines inhibit the expression of Fas, thereby decreasing the sensitivity of trophoblast cells to Fas-mediated apoptosis. The enhanced sensitization to Fas results in trophoblast autocrine-induced apoptosis once FasL binds to and activates the Fas receptor; they demonstrate that cytokines influence trophoblast sensitivity to apoptosis by regulating the expression and function of the Fas/FasL system [14].

We found that trophoblastic apoptosis occurred by the FasL/Fas pathway and suggest that FasL expressed on the trophoblasts and activated maternal lymphocytes can induce trophoblast Fas-mediated apoptosis and the increased Fas expression on trophoblasts may make them more sensitive to Fas-mediated apoptosis, this agrees with a study done by Dhruv and co-workers, they found that trophoblast apoptosis occurred by the FasL/Fas pathway, their findings suggest that FasL expressed on the trophoblasts

and activated maternal lymphocytes can induce trophoblast Fas-mediated apoptosis by autocrine or paracrine interactions.

In addition, the increased Fas expression on trophoblasts may make them more sensitive to Fas-mediated apoptosis. The increase in trophoblast apoptosis associated with chorioamnionitis provides support for their hypothesis that immune cells in placenta regulate trophoblast apoptosis via cytokines at the fetomaternal interface. One possible reason for the increase in trophoblast apoptosis associated with chorioamnionitis could be the changes taking place in cytokine composition in the placental microenvironment, by the activation of the placental and decidual immune cells, resulting in the release of inflammatory mediators , specifically cytokines and chemokines, into the placental microenvironment [15, 16].

This release of inflammatory mediators results in dense neutrophil and lymphocytic infiltration in the chorion, amnion, and placental villi. In addition to the immune cells, trophoblasts also produce cytokines. The proinflammatory cytokines are cytotoxic to trophoblasts, resulting in their apoptosis [17]. So we suggest that a possible mechanism for the increased trophoblast apoptosis associated with infection could be the activation of the FasL/Fas pathway of apoptosis. This is obvious from the results of increased expression of Fas/FasL in our research. These data have implications for understanding the mechanism of abortion and may help in preventing abortion if the baby normal.

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