Insulin receptor (IR) expression in human trophoblasts of recurrent pregnancy loss (RPL)

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ABSTRACT: Purpose: Insulin and glucose pathways play a key role to fetal viability and growth. The focus of the study is to investigate the potential differences of immunohistochemical expression of IR in trophoblastic and decidual cells between women who had recurrent pregnancy loss and women that underwent an abortion. Materials and methods: Trophoblastic and decidual tissues from fifty (50) women with elective abortion used as control group and from fifty (50) women with recurrent miscarriages were collected during gestational weeks 6 to 12. IR antibodies were used as immunohistochemical staining markers. Nuclear and cytoplasmic expression was evaluated. Results: No IR immunohistochemical expression was detected in both trophoblastic cells of the implantation site and deciduas basalis of the two study groups. Conclusion: The effort made to enhance our knowledge on the physiology and histology of IR expression in connection with pregnancy was halted because the results were inconclusive. While studying, though, the correlation of recurrent miscarriage with IR expression, it became evident that a lot of hormones and pathways form the weave of gestational pathology and its delicate harmony. Every piece of knowledge may clarify this still obscure field.

Key words*:Insulin receptor, recurrent pregnancy loss*

INTRODUCTION

The beta cells of pancreas that belong to the endocrine component of pancreas, secret insulin, the key hormone in the regulation of intracellular and blood glucose interchange[1,2,3,4]. During gestation, insulin and its receptor participate in placental endocrine regulation and fetal vitality[3,14]. Adding to its endocrine control, human placenta has multiple functions, including response to various fetal or maternal molecules[2,5,6]. This reaction is heavily dependent to insulin receptor (IR) availability and binding properties.

IR is a transmembrane tyrosine kinase receptor binding insulin, IGF-1 and IGF-2 and its activated form, encourages glucose uptake[2]. IR has two isoforms, IR-α and IR-β, encoded in one gene[7]. When insulin binds IR, glucose transporters are transferred to the membrane, so they are accessible to glucose. The part of the receptor that contains IR-α can be found mainly outside the cell and consists the binding component of the receptor[15]. Once activated by binding, the receptor is autophosphorylazed and thus bringing about the phosphorylazation of insulin receptor substrates, that helps manage the insulin intake through negative feedback (PI3-K)[2]. Activation of IR differentiates in effect depending in its location on deciduas basalis. In trophoblastic cells, it mainly stimulates cell proliferation and in the endothelium, it induces metabolic changes due to the activation of protein kinase B/Akt pathway[5].

All placental cells express insulin receptors, with varied densities among different tissues and throughout pregnancy[2,5,10,11,12,13,14].

MATERIALS AND METHODS

The study group was obtained from 50 women who miscarried between the ages of 20-47 years and during gestational weeks 6-12. Controls consisted of 50 healthy women between the ages 27-39 years, who had electively terminated their pregnancies during gestational weeks 6-12. The gestational age was calculated from the last normal period to the date of curettage.

Tissues were collected immediately after miscarriage or elective abortion and washed with distilled water. Then, they were studied under a microscope, so that specimens from decidua, villus chorion and parts of the embryo could be examined for formation abnormalities or placental lesions and excluded from the study.

Afterwards, specimens were stabilized in aqueous solution of neutral formalin 10% v/v for 12-24 hours and then placed in an automatic machine for further processing, including fixation, dehydration, xylene clarification and paraffin embedding. Then, paraffin-embedded blocks of specimens were cut in 3 mm sections, covered with tape and transferred to positive charged and properly prepared glass plates, which were kept in an oven, at 37- 40°C for 30 - 45 min. After this step, specimens were stained with haematoxylin- eosin solution (Harris) and examined with a microscope. The most suitable of them were gathered for immunohistochemical study.

Immunohistochemistry

In all specimens, decidua basalis was identified using the antibody cytokeratin (CK7), which is positive in trophoblastic cells. Furthermore, for discrimination between decidual and trophoblastic cells at the feto-maternal interface, duplicate sections were stained with a monoclonal antibody against prolactin, for the visualization of decidual cells. The unstained specimens were further processed using an automatic machine (Bond Max).

For the detection of immunohistochemical staining, specimens were firstly immersed in Post-Primary solution. After being washed, specimens were immersed in Polymere solution and then in chromogen diaminobenzidine (DAB) solution. Finally, specimens were stained with Haematoxylin- Eosin. Following the previous stages that

were performed by the automatic processor, specimens were rinsed in tap water and dehydrated with escalating densities of ethanol solution (70, 96 and 100% v/v consecutively) and xylene.
Then, they were covered with tape, placed in glass plates and immersed in Canada balsam
Microscopic evaluation was conducted on the cells of the intermediate trophoblast on decidua basalis and decidua parietalis of recurrent miscarriage and elective abortion material. Specimens were examined using an optical Zeiss TM microscope and photographs were taken using a Contax TM camera, attached to the microscope. In total, 100 specimens (50 from decidua basalis and 50 from decidua parietalis) were examined. Intensity of staining was evaluated as negative (-).

RESULTS

In trophoblastic cells of the implantation site the intensity of staining for IR immunohistochemical expression was negative to very lightly positive in both study groups.
In deciduas basalis cells, the intensity of staining for IR immunohistochemical expression was negative, in both study groups (Fig. 1-8)

DISCUSSION

The conception and maintenance of life throughout gestation has been the subject and focus of many studies for most of medical history. In the last decade, the role of hormones and hormonal receptors has been recognized as key for fetal survival and growth, but has yet to be fully outlined. In this study, an attempt was made in order to clarify if there is any correlation of the expression of insulin receptors (IR) with recurrent abortions. The result that pertain this particular interconnection is indecisive.
According to Desoye G et al[5], the levels of IR in different sites vary depending on the phase of gestation. Specifically, IR is elevated in synctytiotrophoblast, especially in syncytial sprouts and mesenchymal villi during the first trimester and has a higher affinity to insulin, and its levels and affinity slowly decline, resulting in sparse staining in term placentae[12,16,10]. Furthermore, the cytotrophoblast displays a dense expression of IR with a weaker one in microvilli that guides us to the following conclusion: insulin receptor is expressed whenever and wherever the need for growth arises[11, 13].
Let's not forget that the IR and type 1 insulin-like growth factor (IGF) receptor are co-regulated by the same gene. A study has shown that hybrid receptors with a variety of structures and diverse affinity for both hormones have been detected. This fact indicates that we could have a distorted view of hormonal selectivity and research on the subject may yet alter the known physiological pathways for both[17].
In our current study, the clinical aspect has not been considered. But, there are a lot of disorders that change both the IR expression and the outcome of gestation. The most prominent of these are Diabetes Mellitus (DM) and gestational diabetes mellitus (GDM). It is known that women with untreated or unsuccessfully treated DM type 1 in the first trimester have higher chances of fetal loss or neonatal complications, but the IR expression in these cases has not been widely studied[18].
As for GDM, according to studies contacted, the results vary, as one study shows a decrease of IR in placenta that was attributed to a negative feedback caused by high serum insulin levels[19] and another, more recent, presents an elevation of IR and other components associated with insulin signal transduction[20]. A third study is more specific. It compares a healthy-control group of pregnant patients to a group of GDM patients treated solely by diet and to another treated with insulin and it shows that the second group has elevated IR expression while the third has decreased compared to the control group[21]. Also, there seems to be a fluctuating expression of IR substrates, IR types and proteins that participate in insulin signaling, between pregnant women with obesity and variably treated diabetes[2]. Comparing villous and extravillous trophoblast in the first trimester of pregnancy between normal, terminated pregnancies and pregnancies complicated by either hydatidiform mole or miscarriage, there is definite elevation of IR expression in the latter two categories[14]. As for gestation complicated by preeclampsia, the expression is not altered, but the binding capacity of IR tends to decrease collated to a healthy control group[22].
Except from miscarriage, there can be other adverse effects of altered IR expression on the outcome of a gestation. In placentas taken from IUGR fetuses show an altered expression of IR substrates and an increased of activated IR when contrasted with placentas taken from AGA fetuses[23, 24]. Also, various studies have shown the effect that serum insulin and other hormones have on both IR expression and sustainability of gestation[23, 24].
It is quite apparent that there are a lot of factors and a lot of pathways that should be taken into account when investigating the interaction of IR expression with recurrent miscarriage, and a lot to be discovered still in the future, as our field of vision expands along with the development of technology.

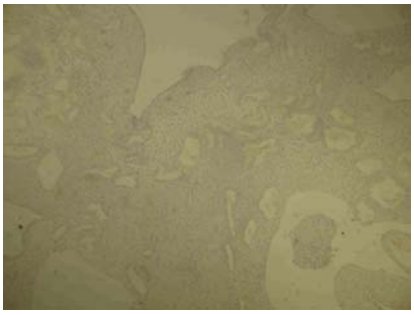

Fig 1. Conrtol group. Immunohistochemical staining of IR in deciduas basalis. X16

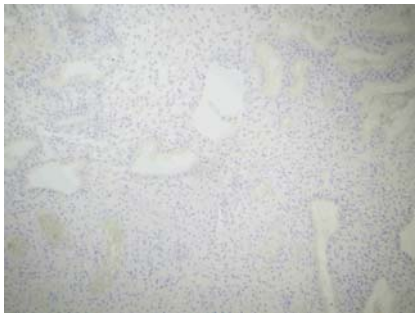

Fig 2. Conrtol group. Immunohistochemical staining of IR in deciduas basalis. X40

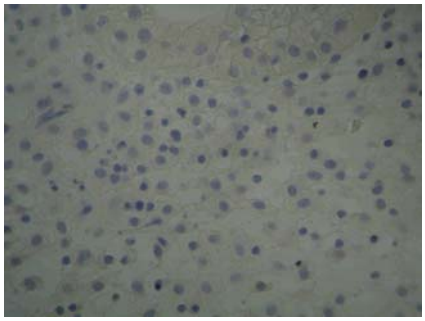

Fig 3. Conrtol group. Immunohistochemical staining of IR in deciduas basalis. X160

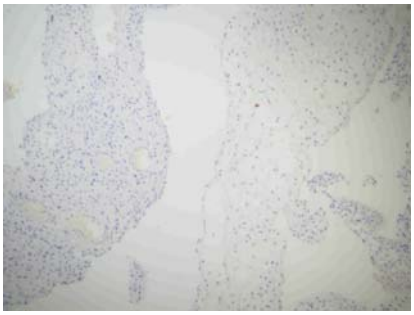

Fig 4. Conrtol group. Immunohistochemical staining of IR in implantation site. X40

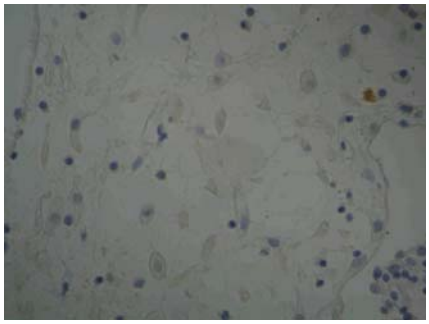

Fig 5. Conrtol group. Immunohistochemical staining of IR in implantation site X160

Fig 6. Miscarriage group. . Immunohistochemical staining of IR in deciduas basalis.4X16

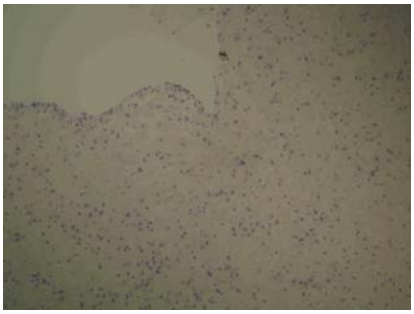

Fig 7. Miscarriage group. Immunohistochemical staining of IR in deciduas basalis. X40

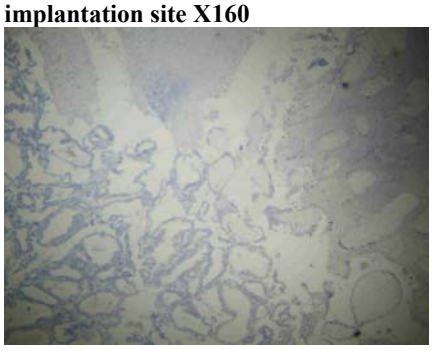

Fig 8. Miscarriage group. Immunohistochemical staining of IR in implantation site. X16

Η ανοσοϊστοχημική έκφραση του υποδοχέα της ινσουλίνης σε υλικά καθ'έξιν αποβολών.

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Εργαστήριο Ιστολογίας – Εμβρυολογίας, Ιατρική σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης

Περίληψη: Σκοπός: Η διερεύνηση των πιθανών διαφορών στην ανοσοϊστοχημική έκφραση του υποδοχέα της ινσουλίνης (IR) σε τροφοβλαστικά κύτταρα στη θέση εμφύτευσης του εμβρύου καθώς και σε κύτταρα φθαρτού, μεταξύ των γυναικών που είχαν επαναλαμβανόμενες αποβολές και των γυναικών που υποβλήθηκαν σε άμβλωση. Υλικά και μέθοδος: Δείγματα ιστού από τροφοβλάστη και φθαρτό από 50 γυναίκες με εκλεκτική άμβλωση (ομάδα ελέγχου) και από 50 γυναίκες με επαναλαμβανόμενες αποβολές συλλέχθηκαν κατά την $6^{η}$ έως $12^{η}$ εβδομάδα κύησης. Χρησιμοποιήθηκαν αντισώματα έναντι του IR και εκτιμήθηκε η ένταση της ανοσοϊστοχημικής χρώσης. Αποτελέσματα: Δεν ανιχνεύθηκε ανοσοϊστοχημική έκφραση του IR ούτε σε τροφοβλαστικά κύτταρα της θέσης εμφύτευσης ούτε σε κύτταρα φθαρτού, σε αμφότερες τις ομάδες μελέτης. Συμπέρασμα: Η προσπάθεια διαλεύκανσης του ρόλου του IR στην εγκυμοσύνη δεν ήταν εφικτή επειδή τα αποτελέσματα ήταν αμφιλεγόμενα. Ωστόσο, μελετώντας την πιθανή συσχέτιση των καθ'έξιν αποβολών με την έκφραση του IR, έγινε προφανές ότι πολλαπλοί μηχανισμοί, ανεξερεύνητοι σε μεγάλο βαθμό ακόμη, σχετίζονται με την παθολογία της εγκυμοσύνης

Λέξεις κλειδιά:Υποδοχείς ινσουλίνης, καθ' έξιν αποβολές

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