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ORIGINAL ARTICLE

Expression of ERK and Akt proteins in women with unexplained first-trimester recurrent miscarriage

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KEYWORDS

Recurrent miscarriage;
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Abstract *Objective:* Recurrent miscarriage (RM) is one of the most common clinical problems in reproduction. The aims of the current study were to evaluate the expression of ERK and Akt proteins in human trophoblastic tissue and to assess the significance of MAPK and PI3K-Akt signal pathways in the progression of unexplained recurrent miscarriage. *Study design:* A case-control study. *Setting:* Women Health Hospital, Assiut, Egypt. *Materials and methods:* All pregnant women presented with first-trimester inevitable miscarriage with a history of RM, defined as three or more spontaneous consecutive first-trimester miscarriages before 12 weeks' gestation, were included in the study (RM group). Age-matched healthy women who had at least one normal pregnancy with no history of miscarriage were included as a control group at the time of delivery. A sample of trophoblastic tissue was taken for Western blot test to evaluate the level of phospho-ERK and Akt (active forms) proteins in trophoblastic tissue. *Results:* The study included 20 women in each group. There were non-significant differences between both groups as regards maternal age, BMI, passive smoking and family residence. There were significantly lower levels of p-ERK and p-Akt in the RM group as compared to the control group ($p = 0.001$). *Conclusion:* The activation of ERK and Akt pathway plays a significant role in RM. The data suggest that decreased expression of p-ERK and p-Akt occurs less frequently during RM may play a role in this process. This suggests that p-ERK and p-Akt may be markers of RM.

Abbreviations: EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MCL-1, myeloid leukemia cell-1; MMP, matrix metalloproteinase; PI3K, phosphatidyl inositol 3-kinase

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1. Introduction

Recurrent miscarriage (RM), also referred to as recurrent pregnancy loss (RPL), is traditionally defined as three or more consecutive miscarriages occurring before 20 weeks gestation (1). It is one of the most common clinical problems in reproduction, with an establishment of a definite cause in only 50% of cases (2). Many etiological factors have been anticipated but none of them has been fully substantiated. RM has been directly associated with maternal thrombophilic disorders (3), parental chromosomal anomalies (4), structural uterine anomalies (5) and indirectly with maternal immune dysfunction and endocrine abnormalities (6).

Genetic factors can contribute significantly to the etiology of RM. The placenta is the first organ to form during human embryogenesis. The main function of the placenta during early gestation is to mediate implantation of the embryo into the uterus and to regulate the maternal immune response preventing rejection of the fetal semi-allograft during pregnancy (7). Because of the crucial role of the placenta for survival, it is very sensitive to disruption. Genetic factors that affect the development of the placenta are associated with poor pregnancy outcome. Abnormal expression of specific regulatory genes in the placenta and abnormal functions of imprinted genes may cause placental dysfunction and RM (8).

The MAP kinase (MAPK) signal transduction pathways play an important role in the regulation of proliferation in mammalian cells. The MAPK/ERK pathway (also known as the Ras-Raf-MEK-ERK pathway) is a chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell (9). They were involved in many pathological conditions, including cancer and other diseases. ERK1/2 is a member of the MAPK pathway which is implicated in the regulation of cellular proliferation and differentiation in multiple organs. The upstream Ras/Raf/MEK signaling flow through phosphorylation on residues threonine 202 and tyrosine 204 can activate the ERK1/2 forming the activated form of ERK1/2 (phospho-ERK1/2) that can subsequently phosphorylate different transcription factors to regulate cellular proliferation, differentiation, and apoptosis (10).

ERKs have been shown to have a pivotal role in growth-factor-dependent regulation of trophoblast growth and migration (11). The expression of ERK1/2 was detected in villous cytotrophoblasts, but the phospho-ERK1/2 was only present until the 12th week of pregnancy, suggesting that the activated form of ERK 1/2 has an essential role during early pregnancy (12). Human Chorionic gonadotrophin uses ERK pathway to facilitate trophoblast invasion due to inducing MMP-2 expression (13). Also, EGF-induced trophoblast migration requires ERK signaling cascades (14).

The Akt or PI3K-Akt pathway is a signal transduction pathway that promotes survival and growth in response to extracellular signals (15). Akt is the key molecule in Akt signal pathways. Activated Akt mediates downstream responses, including cell survival, growth, proliferation, cell migration and angiogenesis by phosphorylating a range of intracellular proteins (16).

MAPK and Akt pathways may play a prominent role in RM. So the aim of the current study was to evaluate the expression of ERK and Akt proteins in human trophoblastic tissue and to assess the significance of MAPK and PI3K-Akt signal pathways in the progression of unexplained RM.

2. Materials and methods

2.1. Study design, setting

The current study was a registered case-control study (ClinicalTrials. Gov; NCT02694367) conducted during the period between the 1st of July 2014 till the end of December 2015. The study participants were recruited from the emergency department of Women Health Hospital, Assiut University, Egypt. The ethical review board of Assiut Faculty of Medicine approved the study.

2.2. Eligible participants

All pregnant women presented with first-trimester inevitable miscarriage with a history of RM, defined as three or more spontaneous consecutive first-trimester miscarriages before 12 weeks' gestation, were included in the study **(RM group).** They were clinically examined and their investigations were reviewed for different known causes of RM including antiphospholipid syndrome, and endocrinal or uterine anatomical disorders. Informed written consent was obtained for participation after reading the patient information sheet.

We included in our study; pregnant women between 20 and 35 years of age with a regular marital life with the same partner and regularly menstruating before current pregnancy.

Women with polycystic ovarian syndrome, any endocrinal abnormalities such as DM, thyroid disorders or history of abnormal uterine cavity proved by sonohysterography or hysteroscopy before pregnancy were excluded from the study. Also women with positive consanguinity and those who refused to participate in the study were excluded.

Age-matched healthy women who had at least one normal pregnancy with no history of miscarriage had approached to participate in the study as a **Control group** at the time of delivery.

2.3. Intervention

A full history had been taken from each participant including age, BMI, residence, and exposure to passive smoking.

2.3.1. Sample collection and laboratory analyses

Trained nurses of the labor ward collected the samples at the time of evacuation in the study group or at the time of delivery in the control group whether it was vaginal or by cesarean section. The samples consist of trophoblastic tissue were washed several times with isotonic saline solution to get rid of blood and then collected on liquid nitrogen and frozen at −167 c for western blotting study.

The used reagents for western blot assay were as follows:

1. Primary antibodies: Anti phospho-ERK antibodies (reactivity; human, mouse, rat), Anti phospho-Akt antibodies (reactivity; human).
2. Secondary antibodies: Goat anti-mouse IgG-HRP secondary antibodies (reactivity; mouse), Mouse anti-rabbit IgG-HRP secondary antibodies (reactivity; mouse). All from Santa-Cruz Biotechnology Company.

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