ACTIVITY OF PHOSPHO EXTRACELLULAR SIGNAL-REGULATED KINASES p(ERK1 and ERK2) IN SERUM OF INFERTILIZED (STERILE) WOMEN IN SALAHDEEN GOVERNORATE/IRAQ

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Abstract

Background: Infertility is one of the most common female disease and it is defined as the inability of getting pregnant after trying for more than one year, without use of birth control means and while having normal sexual intercourse.

Objective: To know the activity of phosphoextracellular signal regulated kinase p(ERK1 and 2) in infertile women in serum and its correlation with age.

Patients and Methods: This study was carried out in Tikrit Teaching Hospital in salahdeen Governorate – Iraq, from December 2013 to February 2014. In this study, forty-six women (25 infertile married women and 21 apparently healthy women considered as control); their ages range from 16 t0 39 years, were collected to investigate their p(ERK1 and 2) activity in serum by using enzyme linked immunosorbent assay (ELISA) technique .

Results: Serum p(ERK1 and 2) activity was significantly decreased in infertile women, mainly in women more than 25 years of age.

Conclusion: Serum p(ERK1 and 2) activity were significantly less among infertile women than the control group. Women with age more than 25 years had less activity compared to infertile women less than 25 years of age. A significant statistical negative correlation was seen between serum p(ERK1 and 2) activity and the age.

Keywords: p(ERK1 and 2), women infertility, ELISA.

Introduction

Infertility could be defined as the inability of women to be pregnant after more than one year of marriage(1)(9). It is a disease of the reproductive system which affects both men and women with almost equal frequency, Although there is no universal definition of infertility(2). It is estimated that an average of 10% of the global reproductive-age population is unable to get pregnant or carry a pregnancy to term. Assisted reproduction includes all the methods used for fertilization, which is not achieved through sexual intercourse(1).

According to studies by the World Health Organization (WHO), about 8-10% of couples are facing many types of infertility problem. Globally, this means that 50-80 million people are facing the problem of infertility and getting normal life as integrated family(1). Numerous studies have showed that the proliferative capacity of cells declines with age, age-related

decline in the proliferative response to mitogenic stimulation is associated with decreased activities of extracellular signal-regulated kinase (ERK)(6). Extracellular signal-regulated protein kinases 1 and 2 (ERK1and 2) are members of the mitogen-activated protein kinase that can mediate cell proliferation and apoptosis. The Ras-Raf-MEK-ERK signaling cascade controlling cell proliferation has been well studied(5). In cell biology, Extracellular signal-regulated protein kinase phosphorylation (pERK) means that a signaling pathway comprised of proteins and enzymes involved in cellular processes, And thus the survival of the cell alive and apoptosis. ERK phosphorylation is one of four mitogen-activated protein kinase (MAPK) signaling pathways and this processes(The ERK phosphorylation cascade) is activated when a tyrosine (Tys) and/or threonine (Thr) kinase receptor molecule activates a protein kinase on the surface of a cell. When the ERK phosphorylation cycle does not function properly, inappropriate ERK activation may occur and abnormal and cancerous cells can develop. There are two ERK pathways — kinase-1 (ERK1) and kinase-2 (ERK2), also known as MAPK3 and MAPK1, respectively. ERK activation requires dual phosphorylation by Tys and Thr, starting with Tys phosphorylation(7).

Immunoassays involve tests using antibodies as reagents. Enzyme immunoassays make use of enzymes attached to one of the reactants in an immunoassay to allow quantification through the development of color after the addition of a suitable substrate/chromogen. As indicated, ELISAs involve the stepwise addition and reaction of reagents to a solid phase-bound substance, through incubation and separation of bound and free reagents using washing steps. An enzymatic reaction is utilized to yield color and to quantify the reaction, through the use of an enzyme-labeled reactant. Basic Systems of ELISA is The section describes the principle involved in the many configurations possible in ELISA. Three main methods form the basis to all ELISAs: Direct ELISA, Indirect ELISA, Sandwich ELISA(8). This study aims to identify the effect of the serum level of pERK1 and pERK2 on infertility in women.

Materials and Methods

This study carried out in Tikrit Teaching Hospital - Gynecology unit- Tikrit/ Iraq, between November 2013 to April 2014, in which 46 women (25 infertile and 21 control) their ages range from 16 to 39 years. The blood samples were collected from them in the second or third day of menstrual cycle. Enzyme linked immunosorbent assay (ELISA) technique was used to investigate p(ERK1 and ERK2) activity in blood samples. Infertile women included in this study were divided into two groups:
1. Group I: Women less than 25 years of age.
2. Group II: Women more than 25 years of age.

Four mls. of venous blood were collected into disposable plain tube , left for 15 - 30 minute at 37 C° for clot formation , then serum was separated by centrifugation at 3000 rpm for 10 minutes, the serum was stored at -8 °C and kept for analysis of ERK1/2 enzyme. Statistical analysis was performed by Minitab ver.11, the results were expressed as mean, SD and Chi square T-test. P value of less than0.05 was considered statistically significant.

Results

The results revealed a significant decrease in mean values of serum ERK1/2 activity in infertile women (5.23 ± 1.76) compared to mean values in apparently healthy women (8.07±1.28) as shown in Table (1) .

While classification of studied samples according to age showed a significant decrease in (mean ± SD) in serum ERK1/2 activity in infertile women more than 25 years of age

(7.611 ± 2.33) compared to those less than 25 years of age (8.417 ± 2.682).

The distribution of all study sample according to the difference in mean of serum p(ERK1and 2) between infertile women and healthy women were shown in table (2).

Discussion

Although extracellular phospho extracellular signal regulated kinase p(ERK1 and ERK2) may play a role in the pathogenesis of ovarian disease. In the present study, serum ERK1and 2 level were significantly decrease in infertile women. The results of my study were in agreement with JoAnne S. Richards, Dr. Heng-Yu Fan et al , and this could be attributed to that ERK1and 2 mutation can block ovulation and other activities involved with oocyte maturation or formation of corpora lutea(4).

The results of this study which showed a significant decrease in serum p(ERK1 and ERK2) activity in infertile women, mainly in women more than 25 years of age, and it was significantly less among infertile women than in apparently healthy group, and this study agreed with JoAnne S. Richards, Dr. Heng-Yu Fan et al, whose showed this enzyme low in serum of infertile women(4), and with Hutter D, Yo Y, Chen W et al, whose showed this enzyme decreases with age(6). The results which revealed that fertility in women begins to decline many years prior to the onset of menopause despite continued regular menstrual cycles(3). Evidence from several different model systems has indicated that cellular aging is associated with a loss in proliferative capacity of ovaries(6).

Further studies have showed that the proliferative capacity of cells declines with age. Using hepatocytes as a sample, showed that this age-related decline in the proliferative response to mitogenic stimulation is associated with decreased activities of both extracellular signal-regulated kinase (ERK)(6). To search in the

molecular basis for age-related defects in the ERK pathway ,the upstream signaling events that occur after epidermal growth factor (EGF) stimulation in young and aged hepatocytes was characterized. As previously noted for ERK, the activities of both MEK (the kinase immediately upstream of ERK) and Ras following EGF stimulation were significantly lower in aged hepatocytes. This results suggest that a decrease in the association between Shc and EGFR in aged cells underlies the age-related declines in the ERK signaling cascade and in proliferative capacity(6).

Conclusion

Serum p(ERK1 and ERK2) activity were significantly less among infertile women than in control group. Women who are aged more than 25 years had less pERK1 and 2 activity when compared to less than 25 years in age. Significant statistical negative correlation was seen between serum p(ERK1 and 2) activity and age.

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Table (1): The activity of ERK1 and 2 in patients and controls.

Groups	No.	Serum ERK1/2 activity (Mean±SD)
Infertile	25	5.23±1.76
Control	21	8.07±1.28
P value	P	0.05

Table (2): The activity of ERK1/2 in serum according to age.

Age	Infertile	Control
< 25 years	5.453±1.458	8.417±2.682
> 25 years	4.75±1.644	7.611±2.33