Original Article

SIRT6 IS CORRELATED WITH ESTRADIOL IN WOMEN WITH *IN VITRO* FERTILIZATION FAILURE

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ABSTRACT

Objective: *In vitro* fertilization (IVF) is an important tool and it is widely used in the treatment of infertility. However, the failure rate is still high. Thus the study of the factors affecting the rate of success of IVF cycles is very important field of study. In the present study, the possible relationship between the Sirtuin-6 (SIRT6), a stress-responsive protein deacetylase, and the outcome of IVF was studied. SIRT6 also was correlated with hormone levels in women with IVF failure.

Methods: Sixty women undergo IVF patients were participated in the study. Women group that had conceived from IVF are expressed as "pregnant group" while women who hadn't are expressed as "failure group." All groups had same preparations and same treatment regimen.

Results: Results revealed that there is an insignificant difference between pregnant and failure groups in serum SIRT6 level. The results showed a significant higher estradiol, and lower prolactin and antimullerian hormone in pregnant in comparing with failure group. Correlations studies indicated no significant correlation between SIRT6 and hormones in pregnant group, while there is a significant correlation between SIRT6 and estradiol hormone in the failure group.

Conclusion: It can be concluded that serum SIRT6 level does not differ between women who had success or failure IVF. However, in women with failure, SIRT6 is correlated with estradiol level.

Keywords: *In vitro* fertilization, Estradiol, SIRT6.

INTRODUCTION

Infertility is the inability of a couple to achieve pregnancy over an average period of one year (in a woman under 35 y of age) or 6 mo (in a woman above 35 y of age) despite adequate, regular (3-4 times per week) unprotected sexual intercourse [1]. Infertility may also be referred to as the inability to carry a pregnancy to the delivery of a live baby.

Infertility is a complex disorder with significant medical, psychosocial, and economic problems [2]. Hormonal imbalance is an important cause of anovulation. The disorder in the ovulation may lead to infertility. Hormonal irregularities that affect ovulation include hyperthyroidism, hypothyroidism, polycystic ovary syndrome and hyperprolactinemia [3]. Women with hormonal imbalance will not produce enough follicles to ensure the development of an ovule. Hormonal causes of female infertility involve ovulatory dysfunctions that may result from dysfunction of the hypothalamic-pituitary-ovarian axis, peripheral endocrine glands, non-endocrine organs, or metabolic disorders [4]. Therefore, the study of the hormonal state is essential in the diagnosis of infertility and treatment.

In vitro fertilization (IVF) is widely used and important tool in the treatment of infertility. IVF is a process by which an egg is fertilized by sperm outside the body. The process involves monitoring and stimulating a woman's ovulatory process, removing an ovum or ova (egg or eggs) from the woman's ovaries and letting sperm fertilize them in a liquid in a laboratory. The fertilized egg (zygote) is cultured for 2–6 d in a growth medium and is then implanted in the same or another woman's uterus, with the intention of establishing a successful pregnancy[5]. The cumulative percent pregnant was 20.7% after the first IVF cycle, with nearly half pregnant within three and over two-thirds being pregnant within six cycles [6]. The low incidence of pregnancy in the first few cycles requires a lot of money, time and medical intervention. Thus, the study of the factors affecting the rate of success of IVF cycles is a very important field of

study. In the present study, the possible relationship between the Sirtuin-6 (SIRT6), a stress-responsive protein deacetylase, and the outcome of IVF was studied in the present work. Furthermore, the correlation between SIRT6 and hormonal status in the women under IVF process is investigated. SIRT6 is a member of a highly conserved family of nicotinamide adenine dinucleotide (NAD$^+$)-dependent deacetylase that it has both de-acetylation and adenosine diphosphate (ADP)-ribosylation activities. SIRT6 are implicated in age-related disorders or longevity [7]. Studies in mice have revealed that Sirt6 is essential for post-natal development and survival. Sirt6 knockout mice, in which the gene encoding Sirt6 has been disrupted, exhibit a severe progeria, or premature aging syndrome, characterized by spinal curvature, lymphopenia and low levels of blood glucose [8]. SIRT6 plays diverse roles in regulating metabolism, cell proliferation, genome stability, and aging. Ovarian aging is thought to be characterized by a gradual decrease in both the number of follicles and the quality of oocysts. Ovarian reserve is indicated by the number of primordial follicles. Sirtuins including SIRT6 are present in the nucleus and cytoplasm of the oocysts and found to be a suppressor of the ovarian development [9]. However, the correlation of the SIRT6 with the result of IVF is not studied previously. The aim of the present study is to investigate the SIRT6 level in women with failure and success IVF. The second aim is to correlate the serum SIRT6 concentration with some related hormones in women undergo IVF procedure.

MATERIALS AND METHODS

I-Subjects

Sixty women undergo IVF patients participated in the study. Their age range was 27.35±7.61year. IVF cycles were conducted in the "Center of Fertility" in Al-Sadr Teaching Medical City in Najaf Governorate-Iraq during the period from June-August 2014. IVF process involves monitoring and stimulating a woman's ovulatory process, removing an ovum or ova from the woman's ovaries and

letting sperm fertilize them in a liquid in a laboratory. The fertilized egg (zygote) is cultured for 2–6 d in a growth medium and is then implanted in the same woman's uterus, with the intention of establishing a successful pregnancy. All the patients signed an informed consent form prior to the start of the study. The study was approved by the institutional review board at Kufa University, Iraq.

The study excluded the patients with any obvious major systemic diseases including diabetes mellitus, hereditary diseases, or other endocrine disorders. Women were divided into two groups (pregnant and failure) according to the results of the IVF after few weeks of IVF process. Blood samples were collected from women before the operation. Women group that had conceived are expressed as "pregnant group" while women who hadn't are expressed as "failure group." All groups had same preparations and same treatment regimen.

Methods

Serum hormones; Luteinizing hormone (LH), Follicle stimulating hormone (FSH), Estradiol (E2), Progesterone (PRG), Prolactin (PRL) were measured using ready for use ELISA kits supplied by Monobind®. Anti Müllerian Hormone (AMH) was measured using ready for use ELISA kits supplied by Ansh Labs, USA. SIRT6 ELISA kit was supplied from Cloud-Clone Corp., USA.

Statistical analysis

The distribution types of the variable results were examined by using the Kolmogorov-Smirnov test. Analysis results divided the variables into two types, namely, normally distributed variables and nonparametric variables, according to the statistical distribution. For the normally distributed variables, the results were expressed as mean±standard deviation. Pooled t-test was used for the comparison between the patients and control groups. Pearson's correlation coefficients (r) were used to estimate the correlation between parameters. For the nonparametric variables, the results were expressed as medians in addition to (mean±standard deviation). The Mann-Whitney U test was used for the comparison between the patients and control groups. Spearman's correlation coefficients (ρ, rho) were used to estimate the correlation between parameters. All statistical analysis was measured by using the SPSS Statistics Version 21 (2013) by IBM-USA.

RESULTS

1-Comparison between pregnant and failure groups

Fig. 1 showed the serum SIRT6 level in pregnant and failure groups. Serum SIRT6 showed the insignificant difference between groups.
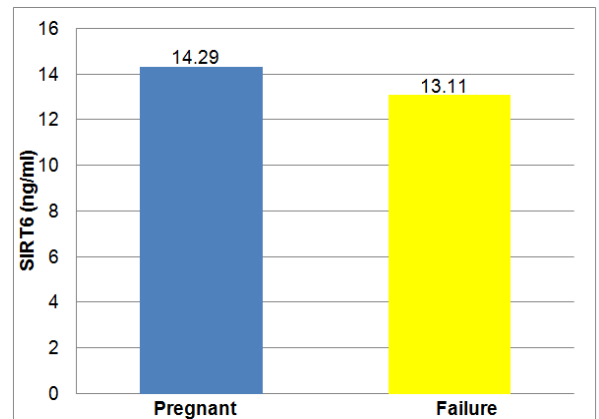

Fig. 1: Serum SIRT6 level in pregnant and failure groups

The results of hormones in pregnant and failure groups are presented in table (1). The results showed a significant higher estradiol (p=0.024), and lower PRL (p=0.002) and AMH (p=0.006) in pregnant in comparing with failure group.

Table 1: Descriptive correlations among parameters in pregnant and failure groups expressed as mean standard deviation

Parameters	Pregnant	Failure	Significance
LH (mlU/ml)	12.48±3.01	12.47±4.68	0.991
FSH(mlU/ml)	6.28±2.71	6.58±4.72	0.764
E2(pg/ml)	567.81±319.11	481.12±245.47	0.024*
PRG(ng/ml)	10.96±6.56	20.30±69.62	0.470
PRL(ng/ml)	8.94±4.39	15.72±4.89	0.002*
AMH(ng/ml)	2.53±1.38	1.46±0.69	0.006*
SAR6(ng/ml)	14.29±6.42	13.11±4.16	0.405

*Significant difference (p<0.05) between pregnant and failure groups.

Table 2: Correlations between SIRT6 and hormones in pregnant group

		LH	FSH	E2	PRG	PRL	AMH	BMI
SIRT6	r	-0.01	0.28	-0.14	0.02	0.16	-0.08	0.33
	p	0.94	0.14	0.43	0.91	0.40	0.64	0.08

Table 3: Correlations between SIRT6 and Hormones in the failure group

		LH	FSH	E2	PRG	PRL	AMH	BMI
SIRT6	r	0.09	-0.21	0.43*	0.25	-0.09	0.20	0.14
	p	0.65	0.25	0.02	0.18	0.60	0.30	0.46

*Significant difference (p<0.05) between pregnant and failure groups.

2-Correlations between SIRT6 and hormones in pregnant group

The results in table 2 showed no significant correlation between SIRT6 and hormones in pregnant group

3-Correlations among parameters in failure group

The results in table 3 showed a significant correlation between SIRT6 and E2 hormone in the failure group.

DISCUSSION

The results in table 1 are in agreement with results of a study that showed that the higher pregnancy rates achieved is associated with, the higher E2 levels [10]. The levels of E2 in both groups are higher than the normal range in untreated women. This is due to the controlled ovarian hyperstimulation. The majority of IVF cycles demonstrated an increase in E2 levels post-hCG associated with the

improved clinical pregnancy rates and live births [11]. It is clear that supraphysiologic levels of E2 are inevitably attained during ovarian hyper stimulation owing to the development of multiple ovarian follicles, each contributing significantly to E2 production which can reach levels up to 10 times or more those found during spontaneous cycles. The effect of such supraphysiologic E2 levels on the outcome of IVF has remained controversial [12, 13]. E2 increases endometrial proliferation and uterine perfusion and because of this characteristic, estrogen improves the possibility of pregnancy. Although supraphysiologic E2 levels during ovarian stimulation for IVF represent one of the major deviations undergone by the female endocrine environment compared with the natural cycle, their significance for pregnancy achievement in IVF has only been assessed retrospectively [14,15]. On the contrary, three studies suggested a detrimental role of high E2 levels on the day of hCG administration for pregnancy achievement [16, 17].

AMH belongs to the transforming growth factor-β (TGF-β) superfamily, and it is considered a local growth factor and a cellular differentiation factor [18]. In females, AMH is mainly secreted exclusively by the granulosa cells of ovarian early developing follicles from preantral and small antral follicles indicating AMH role in folliculo genesis [19]. AMH was an accurate marker for the occurrence of poor response to ovarian hyperstimulation with gonadotropins in IVF [20]. The correlation between AMH and IVF outcome. Several studies have revealed a significant positive correlation between AMH concentrations and pregnancy rate [21] and live birth rate [22,23]. However the results from the other studies [24] indicated that the predictive value for serum AMH in relation to clinical pregnancy rate, ongoing pregnancy rate and live birth rate is controversial [25]. There are some studies compare serum AMH and fluid follicle AMH on the predictive value of pregnancy rate, and the results are variable [26]. AMH is a predictor of IVF outcome [27]. AMH has been evaluated by several groups as a marker of ovarian response [18, 28]. AMH inhibits the initial recruitment of primordial follicles, through a paracrine effect (granulose cells-oocyte cross-talk) [29] and also inhibits the aromatase activity in granulose cells, thus reducing the production of E2 [30]. It is concluded that elevated AMH levels in either the serum or follicular fluid appeared to be predictive of clinical pregnancy [31].

Hyperprolactinemia is one of the major causes of infertility, brought about by inhibition of gonadotropin-releasing hormone (GnRH) or pulsatile GnRH secretion from the hypothalamus and impairment of LH output from the pituitary gland [32]. PRL within gonadotroph cells are controlled by dopamine, the main hypothalamic inhibitory regulator of PRL release *in vivo*, this specific actions of PRL within the gonadotroph and the cell signalling interactions that ultimately underlie hyperprolactinemia-induced infertility [32]; hyper-prolactinemia can occur in physiological and pathological conditions [33] such as psychotic stress, severe mental illness or other causes, and the reproductive dysfunction affecting about one-third of infertile women [34]. Hyperprolactinemia causes infertility by increasing the release of dopamine from the hypothalamus which inhibits GnRH and thus gonadal steroidogenesis and eventual infertility [35]. Thus, the elevation of PRL in the failure group in comparing with a pregnant group consisted with the previous evidence. PRL is known to play a significant role in regulating ovarian functions, including folliculo genesis, steroid genesis, ovulation, and corpus luteum function [36, 37]. PRL levels are generally high in patients with ovulation and steroidogenesis disorders. It is reported that in the hyperprolactinemia, the rate of cleavage embryo and pregnancy rate was low. In addition, serum PRL levels after hCG administration are higher in pregnant women than in non-pregnant women after IVF [38]. In a study of rabbit oocytes, the addition of PRL to the culture fluid was found to promote oocyte maturity [39]. One of the other actions of PRL is to stimulate the ovarian production of PRG. That is required in the process of preparation for embryo implantation, and it is dependent on a continued estrogen and PRG secretion by the corpus luteum, which is supported by a functional pituitary during the first half of pregnancy in rodents [40]. Deficiency in the amount of PRG produced after ovulation may result in a uterine lining that is less able to have an embryo implant. Some women with this problem may see their period come a short time after ovulation [41].

The insignificant difference in SIRT6 between women with success IVF and women with failed IVF in table 1 and Fig.1 indicated a lack of a direct action of SIRT6 on the fertilization process. SIRT6 is involved in genomic DNA stability and repair, and crucial in metabolism and aging [42]. In animal studies, it is found that mice that over express SIRT6 had a long lifespan and associated with reduced level of serum insulin-like growth factor-1s and increased insulin-like growth factor binding protein-1 [43]. While, SIRT6-deficient mice are small and they have severe metabolic defects, and they develop abnormalities that are usually associated with aging [44]. Over-expression of SIRT6 in mice had a protection effect against some metabolic impairment, including dyslipidemia [45]. Thus, the activation of SIRT6 might represent valuable therapeutic targets for aging and age-related diseases. However, a direct correlation between SIRT6 and all the above actions is not studied previously. In the present work, SIRT6 is not changed in the success or failure of IVF.

The correlation between E2 and SIRT6 may indicate a possible effect of SIRT6 and E2 on the IVF failure but not in women who had successful IVF as seen in table 2 and 3. SIRT6 may regulate estrogen levels through peroxisome proliferator-activated receptor alpha (PPARα) and liver X receptor alpha (LXRα). A possible effect of SIRT6 on estrogen levels through LXRα and PPARα may explain the feminization effect seen for the over-expressed SIRT mice, and the estrogen levels may be correlated with SIRT6 levels in mice. Female mice with boosted levels of SIRT6 may have resulted in only a minor increase on the estrogen levels and no significant effect on the lifespan. The estrogen levels in SIRT6 over expressed mice are currently experimentally tested. The effect of SIRT6 over expression on downstream genes may be similar to their levels with higher-than-average levels of estrogen which may drive female tissue tumors enriched in the correlated studies.

The anti-correlated studies which correspond to a reverse effect of SIRT6 on downstream genes may be similar to lower-than-average levels of estrogen [46]. Histological analysis showed that caloric restriction mice displayed a significantly greater number of primordial follicles and less atretic ovarian follicles. The expression levels of SIRT6 were significantly decreased in the ovaries of aged mice and mice treated with chemotherapy. SIRT6 showed a significantly positive correlation with the numbers of primordial follicles. These results indicate that SIRT6 are closely related to ovarian reserve and may be a marker of ovarian aging [47]. SIRT6 contain deacetylase activity [48] and has a DNA repair activity via interaction with different molecules [49]. Estradiol affects the biochemical components of the reproductive tracts and gonads by increasing lipids contents that have negative effects on the fertility [50]. SIRT6 also has same effects on the lipid metabolism. These facts may interpret the findings of the present study. Further investigation in a larger sample size is required to obtain more persistent conclusion about the role of SIRT6 in the IVF failure.

CONCLUSION

Serum SIRT6 levels is not differing between women who had success or failure IVF. However, in women with failure, Sirt6 is correlated with E2 level.

CONFLICT OF INTERESTS

Declared None

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