Anti-Mullerian hormone and Insulin-like growth factor-1 are predictive markers for ovarian reserve

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Abstract

Background: Ovarian reserve is defined as the existent quantitative and qualitative supply of follicles which are found in the ovaries that can potentially develop into mature follicles which in effect determines a woman's reproductive potential. Many tests of ovarian reserve are employed including clinical, endocrine static, endocrine dynamic and ultrasonographic markers.

Aims of study: To determine the age-related changes in AMH and IGF-1 levels that occurs in Iraqi women as markers of ovarian reserve and to determine the specificity and sensitivity of IGF-1 and FSH for ovarian reserve.

Subjects, material and methods: One hindered cases were collected dividing into two groups; first group includes fifty cases of child bearing age, healthy, fertile females with regular menstrual cycle while second group includes fifty cases of postmenopausal aging group, healthy with normal fertility history. Serum levels of Follicle stimulating hormone, Luteinizing hormone, Prolactin, Anti Müllerian Hormone and Insulin like Growth Factor-1 were estimated for all cases.

Results: The overall mean age of the respondents was 45.06 ± 16.68 years old with significant statistical difference between the mean age of pre and post-menopausal women. Results of Anti Müllerian Hormone showing a significant statistical difference between means of Anti Müllerian Hormone hormone for pre-menopausal women (2.89 ± 2.07 ng/ml) and post-menopausal women (0.0 ± 0.0 ng/ml). Measuring of Insulin like Growth Factor-1 showing significant statistical difference between pre-menopausal women (211.04 ± 63.81 ng/ml) and post-menopausal groups' women (120.70 ± 39.69 ng/ml). Similarly results of Follicle stimulating hormone reveal significant differences between means of Follicle stimulating hormone for pre-menopausal women (6.03 ± 1.53 ml U/ ml) and post-menopausal women (56.06 ± 17.07 ml U/ ml). There was no significant association between AMH and IGF1 hormones and IGF1 hormone has been failed to detect ovarian reserve and still the AMH is the gold standard test.

Conclusion: Significant changes occur in Anti Müllerian Hormone, I Insulin like Growth Factor-1 and Follicle stimulating hormone with progression of the age and Anti Müllerian Hormone still the stander ovarian reserve test in compare with Insulin like Growth Factor-1 and Follicle stimulating hormone.

 Key words: Ovarian reserve, AMH, IGF-1.

Introduction

Ovarian reserve is defined as the existent quantitative and qualitative supply of follicles which are found in the ovaries that can potentially develop into mature follicles which in effect determines a woman's reproductive potential [1]. The commonly employed tests of ovarian reserve are: Clinical markers (like age and menstrual pattern), endocrine static markers (as estradiol, FSH, Inhibin-B, and AMH), endocrine dynamic markers and ultrasonographic markers [2].

Basal FSH is the most widely used test to assess ovarian reserve. It is secreted by the anterior pituitary and acts on the receptors expressed by the granulosa cells of gonadotrophin responsive antral follicles. Increasing levels of basal FSH is the earliest sign of human reproductive aging [3]. The measurement of FSH in the early follicular phase of the menstrual cycle is an accurate indicator of ovarian function but is not a good predictor of time remaining to menopause [4].

AMH is expressed by the granulosa cells of the early growing, pre-antral and small antral follicles, but not by non-atretic, larger antral follicles or those that have become atretic, and may reflect or represent the population of smaller antral follicles more than the overall number [5,6]. The production of AMH starts following follicular transition from the primordial to the primary stage, and it continues until the follicles reach the antral stages, with diameters of 2-6 mm [6]. AMH acts as a paracrine rather than a systemic factor, and thus is not part of a negative feedback loop with involvement of gonadotropins. AMH is not influenced by the gonadotropic status and reflects only the follicle population [7]. The biological role of AMH is still unclear, but rodent data suggest that it acts as a modulator of follicle recruitment and ovarian steroidogenesis [8]. It inhibits recruitment of follicles from the primordial pool by modifying the FSH sensitivity of those follicles [7].

Growth hormone is made in the anterior pituitary gland, is released into the blood stream, and then stimulates the liver to produce IGF-1. IGF-1 then stimulates systemic body growth, and has growth-promoting effects on almost every cell in the body. IGF-I has stimulatory effects on cartilage growth, hematopoiesis, ovarian steroidogenesis, myoblast proliferation and differentiation, and many other body tissues [9]. Granulosa cells of several species possess receptors for IGFs and respond to IGFs; several endpoints related to growth and differentiations are stimulated. Ovarian follicular fluid contains abundant quantities of IGFs [10].

Menopause is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity [11].

Reproductive ageing is thought to be due to a gradual decrease in both the quantity and quality of the oocytes contained within the follicles present in the ovarian cortex [12]. With the decline in the number of primordial follicles, oocyte quality also diminishes, especially after the age of 31 years, when fecundity gradually starts to decrease. Several factors might be responsible for this age related decline in the quality of oocytes. These may relate to differences between germ cells at the time they are formed during foetal life, damage to oocytes during the course of a woman's life or changes in the quality of granulosa cells surrounding the primordial follicles [12]. Impaired perifollicular microcirculation resulting in low oxygen levels and a concomitant increase in anaerobic products in the follicular fluid [13]. Also, endocrine imbalance caused due to increase in levels of FSH and altered FSH: LH ratio is associated with decline in oocytes quality [14].

Subjects, material and methods

This study was conducted in the city of Karbala, from November 2012 to August 2013. All cases were collected at different sites depending on available data of age and fertility status, including Teaching Hospital for Gynecology and Obstetrics, Al-Hussain Teaching Hospital and own relatives. The practical side of the study was performed at the laboratory of clinical biochemistry department in College of Medicine / Babylon University and in Al-Hussain Teaching Hospital in Karbala.

This study was including 100 cases dividing into two groups; First group includes fifty cases of child bearing age, healthy, fertile females, and regular menstrual cycle with mean age (29.84 ± 6.66 years) while Second group includes fifty cases of postmenopausal aging group, healthy, normal fertility history with mean age (60.28 ± 6.74).

 About five milliliters of venous blood was aspirated and sera were separated and divided into 5 parts in labeled eppindorf tubes and given a serial number together with the patients names then frozen at –20 ^{0}C until time of usage.

Five parameters were estimated for all cases. FSH, LH and PRL were estimated using VIDAS technique (BioMerieux/France) with principle of combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). AMH estimated by ELISA technique (BioTek /USA) while IGF-1 estimated by LIAISON (DiaSorin S.P.A. Italy) for with one step sandwich CLIA (Chemiluminescence Immunoassay).

Data Analysis

Statistical analysis was carried out using SPSS version 18. Categorical variables were presented as frequencies and percentages. Pearson's chi square (X^2) test and fisher exact test were used to find the association between the categorical variables. ROC test was used to find the accuracy of using IGF1 as well as with FSH to detect ovarian reserve. Independent sample t-test was used to compare means between two groups. A p-value of ≤ 0.05 was considered as significant.

Result and discussion:

Distribution of Age: The overall mean age of the respondents was 45.06 ± 16.68 years old. There was significant statistical difference between the mean age of pre-menopausal women (29.84 ± 6.66 years) and the mean age of post-menopausal women (60.28 ± 6.74). This difference certainly related to the design of study regarding cases selection of both pre and post-menopausal age groups.

It is well understood that the ovarian follicular pool and hence fertility declines with age [17]. Age must always be the first marker to be considered in ovarian reserve assessment. Older women may benefit from ovarian reserve tests since they can help clinicians to find out acceptable chances of pregnancy through IVF [4].

Distribution of AMH: Results of AMH showing a significant statistical difference between means of AMH hormone for pre-menopausal women (2.89± 2.07 ng/ml) and post-menopausal women (0.0± 0.0 ng/ml). This statistical difference mostly related to normal physiological expression of AMH by granulosa cells of follicles in pre-menopausal period which start to diminish until their absence at menopausal age level [5].

Distribution of IGF1: Production of IGF-1 is stimulated by growth hormone which is produced throughout life. The highest rates of IGF-1 production occur during the pubertal growth spurt. The lowest levels occur in infancy and old age. Granulosa cells of several species possess receptors for IGFs and respond to IGFs [18]. These facts mostly explain the findings of the statistical difference in IGF-1 levels between pre-menopausal women (211.04± 63.81 ng/ml) and post-menopausal groups' women (120.70± 39.69 ng/ml).

Distribution of FSH: There were significant differences between means of FSH for pre-menopausal women (6.03± 1.53 ml U/ ml) and post-menopausal women (56.06± 17.07 ml U/ ml). These differences in statistical results are related to normal hormonal balance in pre-menopausal age of FSH level controlled by negative feedback of inhibin B and E2 which are produced by ovarian follicles while absence of such feedback lead to high FSH level in post-menopausal female [19].

Association of AMH with IGF1: There was no significant association between AMH and IGF1 hormones *p* = 1.000 (table 2). The sensitivity of IGF1 hormone to detect ovarian reserve was (49.5%), meanwhile, its specificity was (33.3%). The positive predictive value was (96.0%) that means 96% of ovarian reserve likely to have IGF1 hormone ≥ 150 ng/ ml, meanwhile, the negative predicative value was (6.0%), which means that the ovarian reserve unlikely to have IGF1 hormone < 150 ng/ ml.

These findings are consisting with many other studies regarding predictive value of AMH which showing that AMH is the best predictive factor for ovarian reserve despite many dynamic and static markers are still use, as studies of V.S Kalaiselvi et al (2012) and Ficicioglu et al (2006). The ROC curve has been done to determine the accuracy of IGF1 hormone in detecting the ovarian reserve comparing by AMH as in table 3. The accuracy of the test depends on how well the test separates the group being tested into those with and without the disease. Accuracy is measured by the area under the ROC curve. An area of one represents a perfect test; an area of 0.5 represents a worthless test. Table 3 shows that the area under the curve is 0.414, means that the IGF1 hormone has been failed to detect ovarian reserve and still the AMH is the gold standard test

Table 1: Mean differences of AMH, IGF-1 and FSH by pre and post-menopausal women

P value	t-test	S.D	Mean	N	Parameter	
<0.001*	9.891	2.07	2.89	50	Pre-Menopausal women	AMH
		0.0	0.0	50	Post-Menopausal women	
<0.001*	8.500	63.81	211.04	50	Pre-Menopausal women	IGF-1
		39.69	120.70	50	Post-Menopausal women	
<0.001*	20.644	1.53	6.03	50	Pre-Menopausal women	FSH
		17.07	56.06	50	Post-Menopausal women	

Table 2: Association of AMH with IGF1

Variable	AMH			P values
	≤ 6.5 ng/ ml (%)	> 6.5 ng/ml (%)	Total	
IGF1	48 (49.5)	2 (66.7)	50 (50.0)	
≥ 150 ng/ ml	49 (50.5)	1 (33.3)	50 (50.0)	1.000 *
< 150 ng/ ml	97 (100.0)	3 (100.0)	100 (100)	
Total				

Table 3: Area under the curve for detecting ovarian reserve by IGF1 comparing by AMH:

95% CI		P value	Std. Error	Area
Upper Bound	Lower Bound	0.613	0.163	0.414
0.734	0.095			

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