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The role of serum anti-Mullerian hormone in assessment of ovarian function in infertile women in Kirkuk.

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Abstract

Antimullerian hormone (AMH) is known as mullerian inhibiting substance (MIS), is produced by granulose cells from 36 week of gestation until menopause. AMH is produced by the ovarian follicles & its levels are used to assess the number of antral follicles in the ovaries. The AMH test is used more & more because the level of AMH do not change significantly throughout the menstrual cycle & can be measured anytime during the menstrual cycle. The aim of the study is to investigate the role of AMH in assessment of ovarian reserve in infertile women in Kirkuk. A cross sectional study was conducted in Kirkuk governorate from first of November to the end of January. This study includes 62 women (20 fertile women as control & 42 infertile women without polycystic ovary). Blood samples were taken at day 2 of menstrual cycle. Serum FSH concentration was measured by using ELISA. While, AMH was measured by using AMH/MIS enzyme linked immunosorbant assay kit (Immunotest material USA). There are no significant differences regarding BMI & age between infertile patients as compared with control fertile women. The serum FSH of infertile women was found significantly higher than that of control fertile women. The concentration of serum AMH of infertile women was significantly lower than that of fertile control women. In the infertile group, there is a significant inverse relationship between serum AMH concentrations & serum FSH concentration, ($r=0.3$, p $p\leq0.05$). Also, there is a negative inverse relationship between serum concentration of AMH & age of infertile patients, ($r=0.368$; $p\leq0.01$). Moreover, there is a positive relationship between serum FSH & age in infertile women ($r=0.43$; $p\leq0.01$).

دور هرمون إلانتي مولي رين (AMH في تقييم وضيفة المبايض لدى النساء العقيمات في كركوك)

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الملخص

أجريت هذه الدراسة المقطعية في مدينة كركوك على 62 امرأة (42 مرآة عقيمة و 20 امرأة طبيعية خصيبة كسيطرة) بمعدل أعمارا قل من 30 و لا يزيد عن 45 سنة للفترة من تشرين الثاني 2011- و لنهاية شباط 2012 . وذلك لتقييم تركيز هرمون أنتي مولي رين في ال نساء السليمات والنساء العقيمات إذا كان من الممكن استخدامه كعلامة لوضيفة المبيض في عقم النساء. تم قياس مستوى هرمون أنتي مولي رين وهرمون المحفز للجريب والهرمون الذكري بواسطة جهاز أل ELISA. أظهرت النتائج اختلاف واضح في تركيز هرمون إلانتي مولي رين ، الهرمون المحفز للجريب عندما تمت مقارنتهم بين ال نساء السليمات والعقيمات. اظهرت النتائج وجود زيادة معنوية في تركيز هرمون المحفز للجريب في النساء العقيمات مقارنة بالسيطرة . و كذلك هنالك انخفاضا معنوي في تركيز هرمون أنتي مولي رين في النساء العقيمات مقارنة بالسيطرة . كذلك لوحظ وجود علاقة عكسية بين هرمون أنتي مولي رين والهرمون المحفز للجريب في النساء العقيمات بينما كان هناك

علاقة عكسية بين هرمون أنتي مولي رين و عمر النساء العقيمات، أي كلما زاد عمر المرأة قل تركيز هرمون أنتي مولي رين في مصل الدم. إما في مجموعة النساء العقيمات هنالك انخفاض معنوي في تركيز هرمون أنتي مولي رين في النساء العقيمات ذوات الأعمار 40 - 45 سنة مقارنة مع النساء العقيمات بعمر 20-29 سنة. أثبتت هذه الدراسة بان هرمون إلانتي مولي رين يقل في النساء العقيمات و كذلك يقل كلما تقدمت المرأة في العمر. لذا يعتبر هرمون إلانتي مولي رين دليل على وضيفة المبيض في النساء العقيمات و السليمات أيضا.

Introduction

Anti-Mullerian hormone (AMH) was initially thought to be produced solely by the fetal male during sexual differentiation to promote regression of the Mullerian ducts (1). However, a new interesting role has emerged for AMH in the ovary. In human, AMH is produced by granulose cells from 36 week of gestation until menopause (2, 3). Anti-Mullerian hormone (AMH) is also called mullerian inhibiting substance (MIS). Since AMH is produced directly by ovarian follicles, so AMH levels correlate with the number of antral follicles in the ovaries. AMH is expressed in the growing preantral or small antral follicles in the over, but it is not expressed in ovulatory, atretic follicle, (3,4). It's action is to inhibit primordial follicle recruitment & decreases the responsiveness of growing follicle to FSH. When follicle reach the size at which becomes dominant one, this hormone is largely disappeared & this is important for dominant follicle to progress to ovulation ( ). AMH is produced by the ovarian follicles & its levels are used to assess the number of antral follicles in the ovaries (4). The AMH test is used more & more because the level of AMH do not change significantly throughout the menstrual cycle & can be measured anytime during the menstrual cycle (5). AMH is a member of the transforming growth factor-B (TGF-B) super family of glycoprotein that has been found to play important role in controls the development of primary follicles by inhibiting further recruitment of other follicles during folliculogensis (6). A number of different tests have been used to help in assessment ovarian reserve. These tests include age of women, day 3 FSH, inhibin B, antral follicle count, ovarian volume assessment & the clomid challenge test (5, 7, 8). The aim of the study is to investigate the role of AMH in assessment of ovarian reserve in infertile women in Kirkuk, Iraq.

Subjects & methods

A cross sectional study was conducted in Kirkuk governorate from first of November to the end of January. This study includes 62 women (20 fertile women as control & 42 infertile women). All control women & patients were from outpatients unit of gynecological department at Azadi hospital at Kirkuk. All infertile women were thoroughly investigate with there husband (apparently fertile). Infertile women with polycystic ovarian syndrome (PCOs) were excluded from this study. Body weight was measured to nearest 100 gm with light cloths & body height was measured to nearest cm. Body mass index (BMI) was calculated by weight in kilogram divided by height in meter square. Blood samples were taken at day 2 of menstrual cycle (MC). Serum FSH concentration at day 2 of Menstrual cycle was measured by using ELISA. While, serum AMH concentrations were measured by using AMH/MIS enzyme linked immunosorbant assay kit (Immunotest material USA). All data were presented as a mean & standard deviation (SD). Unpaired Student T test was used to compare between means. P value ≤ 0.05 was considered significant throughout the study.

Results

Infertile women of unexplained causes were 42 compared with 20 fertile women as control. There are no significant differences regarding age of women & BMI between infertile patients as compared with control fertile women, (Table 1). At day 2 of menstrual cycle, the serum FSH of infertile women was found significantly higher (6.58 ± 2.39), than that of control fertile women (5.224 ± 1.3, $p \leq 0.01$), Table 1. Moreover, there is a positive relationship between serum FSH & age in infertile women ($r=0.43$; $p \leq 0.01$). Table 1 show that concentration of serum AMH of infertile women was significantly lower in infertile women as compared with fertile control women ($p \leq 0.01$). In the infertile group, there is a significant inverse relationship between serum AMH concentrations & serum FSH concentration, ($r=0.3$, p $p \leq 0.05$). Also, there is a negative inverse relationship between serum concentration of AMH & age of infertile patients, ($r=0.368$; $p \leq 0.01$). Regarding AMH, the infertile group divided into 3 subgroups according to age, (20-29, 30-39 & 40-45 years). There is no significant difference between age group 20 to 29 years & group aged 30 to 39 years. However, there are significant differences between age group 20 to 29 as compare to age group 40 to 45 years ($t=3.88$, $P \leq 0.01$, table 2). Also, there is significant differences between age group 30 to 39 as compare to age group 40 to 45 years, ($t=3.52$, $p \leq 0.01$, Table 2). In other words, AMH concentrations decline significantly at age group 40-45 as compare to group 20-29 years. Table 3 show the correlation coefficient between ages, BMI, FSH & AMH. There strong inverse relationship between AMH & FSH in infertile group ($r= -0.3$; $p \leq 0.05$). Also, inverse relationship between AMH & age ($r=0.37$; $p \leq 0.01$). However, there is no significant relationship between AMH & BMI in infertile group.

Table (1):- Show the mean & standard deviation of age, BMI, FSH & AMH concentrations of infertile women & fertile control women.

Parameters	Fertile women	Infertile women	P value
Number of subjects	20	42	
Age (years)	32.9 ± 6.8	33.93 ± 7.5	NS
BMI (kg/m^2)	27.1 ± 3.39	28.26 ± 5.84	NS
FSH (mIU/ml)	5.224 ± 1.3	6.58 ± 2.39	≤0.01
AMH (ng/ml)	4.59 ± 0.4	3.197 ± 0.87	≤0.01

Table (2):- Distribution of serum AMH (mean & SD) according to age in infertile women

Age groups (years)	**Number (42)**	**AMH concentration (ng/ml)**	**P value**
a-20-29	16	2.366 ± 0.37	NS a & b; p≤0.01 between a & c
b-30-39	21	2.474 ± 0.40	p≤0.01 between b with c
c-40-45	15	1.946 ± 0.38	p≤0.01 between a & c & b with c

Table (3):- shows the correlation coefficient between AMH & age, BMI & FSH in infertile group.

Parameters	**AMH**		**FSH**	
	R= value	**P value**	**R= value**	**P value**
Age	0.37	≤0.01	0.432	≤0.01
BMI	0.14	NS	0.08	NS
FSH	0.3	≤0.05	------	-----
AMH	------	----	0.31	≤0.05

Discussion

In the present study, there is a significant decrease in serum concentration of AMH in infertile women as compared with fertile control women. A number of different tests have been used to help in assessment ovarian reserve. These tests include age of women, day 3 FSH, inhibin B, antral follicle count, ovarian volume assessment & the clomid challenge test (5, 7, and 8). FSH level vary with the day of menstrual cycle & its serum level affected by other hormone levels. A patient with elevated estrogen level for instance, may lead to false assumption of normal FSH & ovarian reserve (5).

Unlike serum FSH, AMH levels fluctuated very little during menstrual cycle & therefore can be takes at any times during the menstrual cycle (2, 3 & 9). Recently, it was found that AMH is better predicated a higher oocytes yields than normal as FSH. Under age 42 discrepancies between FSH & AMH. AMH remains similarly predictive of oocytes yields at all ages (10). FSH mostly reflects the last two weeks of follicular maturation when follicles become gonadotropin sensitive, while AMH is mostly representative of the young, post-primordial to pre-antral follicle pool going through earlier stages of folliculogensis (11). The old standard for ovarian reserve testing was the day 3 FSH level. However, the FSH level is not as reliable as the AMH level for 3 reasons. First reason is that FSH level varies according to the cycle dates. The second reason is FSH depend upon estradiol level (a high estradiol level will suppress a high abnormal FSH level in the normal range). While, the third reason is FSH varies from cycle to cycle, so is not always reliable or dependable (10, 11). However, AMH level is a much better marker for ovarian reserve. It is much

more stable than the FSH level & does not vary from cycle to cycle. AMH even better than FSH, it can be measured on any day of the cycle (12). There strong inverse relationship between AMH & FSH in infertile group ($r= -0.3$; $p\leq0.05$). Also, inverse relationship between AMH & age ($r=0.37$; $p\leq0.01$). However, there is no significant relationship between AMH & BMI in infertile group. More recently, two large studies examined the declined in serum AMH with age, they found a decline in serum AMH with advance in age (13,14). In the present study, it was found a decline in serum AMH in age group 40 to 45 years, & there is significant differences between age group 40 to 45 & both group 20 to 29 & 30 to 39 years. The present study concludes that AMH is significantly lower in infertile women s compared with normal fertile women of same age. Also, AMH is significantly lower in old age group of infertile women (40-45 years) as compare with young infertile group aged 20 to 29 year. The present study recommends that measurement of AMH should be done routinely at any day of menstrual cycle to see the ovarian reserve in fertile & infertile women. Also, AMH should be done for infertile women with PCOs. The present study also, recommends estimation of AMH in normoovulatory & anovulatory women & estimation of AMH in normal population of different age groups (15, 16).

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