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RESEARCH ARTICLE

Evaluation Of AMH Levels And Correlation With LH / FSH Ratio In Women Belonging To The Reproductive Age Group - A Cross-Sectional Study In A Tertiary Care Centre.

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ABSTRACT

Anti-Mullerian hormone, secreted by granulosa cells, conserve Ovarian reserve by inhibiting the primordial follicle activation. Age dependent variation in AMH levels and its correlation with LH/FSH ratio in predicting ovarian reserve is targeted in this study. A cross sectional study was conducted with 200 healthy females aged from 17-55 years with regular menstrual cycles. Hormonal parameters – AMH, FSH, LH, Prolactin and TSH analysis were done using ECLIA principle. Statistical analysis showed an age dependent decline of AMH levels with increasing age and there exists a strong association of variation in LH/FSH ratio with AMH levels. Hence both AMH and LH/FSH ratio are independent predictors of Ovarian reserve

Keywords: Anti-Mullerian hormone, LH/FSH ratio, Follicle-stimulating hormone, Luteinizing hormone, Ovarian reserve.

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INTRODUCTION

Ovarian reserve evaluation is essential for the fertility workup in the female and an aid to treatment. As age advances, the ovarian reserve diminishes and cannot be accurately predicted. The baseline hormones study, though used, have their own limitations. Studies are focusing on AMH as they are age-specific and readily available clinical marker of the ovarian reserve [1-6].

AMH is a dimeric glycoprotein consisting of two identical subunits linked together by sulphide bridges [7].

AMH prevents the development of the mullerian duct in the male. AMH action is executed by interaction with specific receptors on the surfaces of cells of target tissues [8]. AMH expression has been observed in ovary, brain, placenta and uterus. AMH baselines expressed by the granulosa cells during the reproductive years of the female and it inhibits excessive follicles limiting the formation of primary follicle. Changing AMH levels is seen in the onset of puberty in both sexes.

In healthy females AMH is undetectable in cord blood at birth, starts to rise from 3 months of age and remains fairly constant from childhood to early adulthood [9]. Then it declines and is not detectable after menopause.

AMH also has modulation effect of FSH sensitivity in granulosa cells unrelated to primordial follicle activation. Studies demonstrate that the loss of AMH, accelerates the activation of primordial follicles leading to premature depletion of the ovarian reserve similar to menopause. AMH indirectly affects fertility by altering primordial follicle activation rates.[10]. Serum basal FSH, LH, AMH are usually evaluated at the same time during menstruation.

The advantage of this marker is it is highly reproducible because of its little or no variation between cycles [11].

The LH / FSH ratio is quite high in patients with polycystic ovarian syndrome, when compared to normal ovaries. [12] As individual markers FSH could not significantly predict the ovarian reserve as they are elevated in the late period of reproduction. Hence, we can use LH/FSH ratio as a marker of ovarian reserve, as FSH increase as the ovarian reserve declines.

Hence, the present study focuses on the estimation of AMH and correlation of LH/FSH ratio in abnormally ovulating healthy females with regular menstrual cycle.

Aims and objectives

To estimate the AMH, FSH, LH, Prolactin and TSH levels in the study population and to study the age-related changes in the AMH levels and correlate with LH/FSH ratio in the study population.

METHODOLOGY

The study enrolled a random of 200 women aged 17 to 55 years with regular menstrual history from the general population. Patients with infertility, PCOD, Endometriosis, Amenorrhea, any hormonal therapy in the past 6 months, ovarian neoplasms, overt autoimmune disease along with chronic metabolic and endocrine disease are excluded from the study. After getting details about study subjects' menstrual and reproductive history, peripheral venous blood samples are collected on day 3 of the same menstrual cycle for AMH, FSH, LH, Prolactin and TSH levels assessment.

Statistical analysis

Quantitative variables were presented as mean ± SD and were compared using paired t test between the two variables- Anti-Mullerian hormone & LH/FSH ratio. ANOVA test is applied to compare the relationship

among all study variables. A p value of <0.05 was considered statistically significant. All data were entered in Microsoft excel spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 22.0.

RESULTS AND DISCUSSION

All statistical analysis were performed using SPSS software version 22.

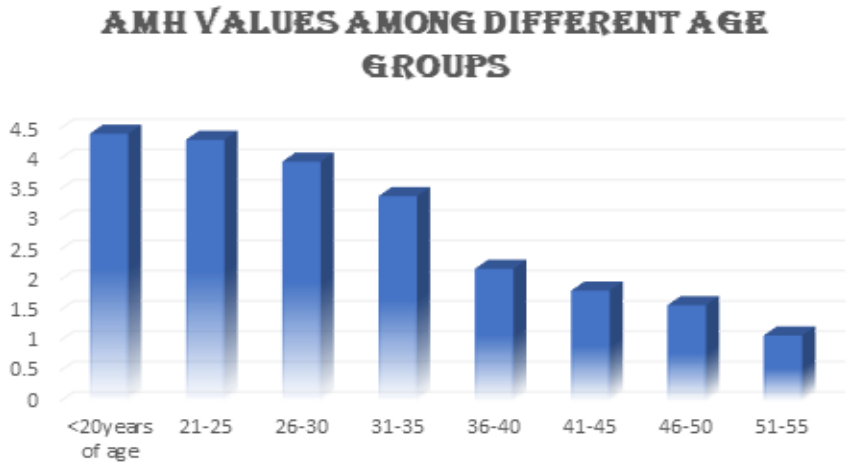
In Table 1, AMH decrease gradually with increasing age suggesting the age dependent decline in ovarian reserve. FSH and LH levels were higher under 30 years of age confirming the increased number of follicles, but these levels then show a decline and were high after 50 years of age. Sowers et al. [13] and Fang et al [14] showed that significant increase in FSH levels with increasing age is a result of disturbances in the ovarian function. Eddie Racoubian et al showed similar increase in FSH levels with increased age and no age related trend in LH levels [15]. Also, prolactin levels doesn't have trending increase or decrease with age, but they were very low after 50 years of age. TSH levels doesn't show any trend like other hormones with increasing age.

Table 1: Mean + Standard Deviation of AMH, LH/FSH ratio, FSH, LH , PROLACTIN , Testosterone (Total), TSH levels in the Study Population

Age Group in years	Number	AMH ng /ml	LH/FSH ratio	FSH mIU/mL	LH mIU/mL	Prolactin ng/mL	TSH µIU/mL
<20years of age	18	4.41+2.3	1.86+0.9	20.87+32.9	10.98+10.0	14.27+4.5	1.84+0.7
21-25	34	4.31+2.7	1.35+0.7	18.43+3.1	10.63+5.4	20.84+10.1	4.04+5.5
26-30	42	3.95+4.5	1.15+0.5	16.99+2.9	12.42+17.0	16.70+10.8	3.70+1.9
31-35	40	3.38+2.3	1.00+0.7	8.51+27.3	10.64+14.7	19.74+46.1	3.51+2.5
36-40	35	2.18+2.0	0.98+0.5	9.99+13.3	10.29+6.6	17.62+9.9	3.03+1.4
41-45	14	1.82+1.2	0.83+0.8	8.57+1.9	11.68+6.3	13.53+5.7	4.59+6.1
46-50	12	1.58+2.2	0.64+0.6	7.57+1.7	10.68+5.2	10.53+4.4	2.59+4.2
51-55	10	1.08+1.0	0.54+0.9	38.27+1.1	29.68+5.0	8.53+3.7	2.23+3.9

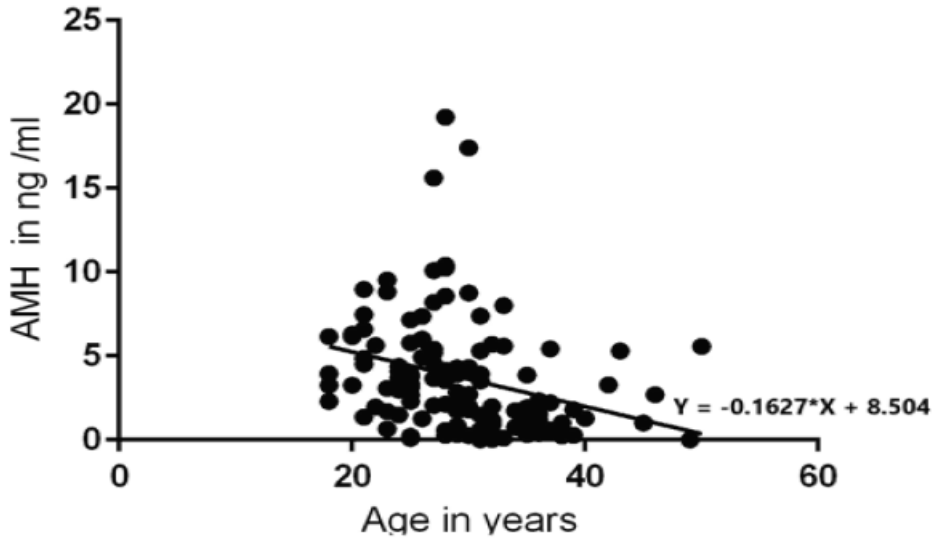
In Illustration 1 shows a gradual decrease in the Anti-Mullerian Hormone levels with increasing age among the different age groups in the study population suggesting the age dependent decline in the AMH levels. Bleil et al and Henderson et al showed this inverse relationship of age with AMH in their studies with respect to age and ethnic background [16,17].

Illustration 1: AMH levels amomg different age groups in the Study Population



In Illustration 2, Anti-Mullerian Hormone levels show a weak but negative correlation (r2 =-0.1627) with age indicating the decrease in AMH levels with increasing age in the study population.

Illustration 2: Linear Regression analysis of AMH levels with age in the Study Population



In Illustration 3, Anti-Mullerian Hormone levels show downward trending straight line (negative correlation (r2 =-0.07679) with LH/FSH ratio in the study population indicating that any increase in AMH levels will have a decrease in the LH/FSH ratio and vice versa.

Illustration 3: Linear Regression analysis of AMH levels with LH/FSH Ratio in the Study Population

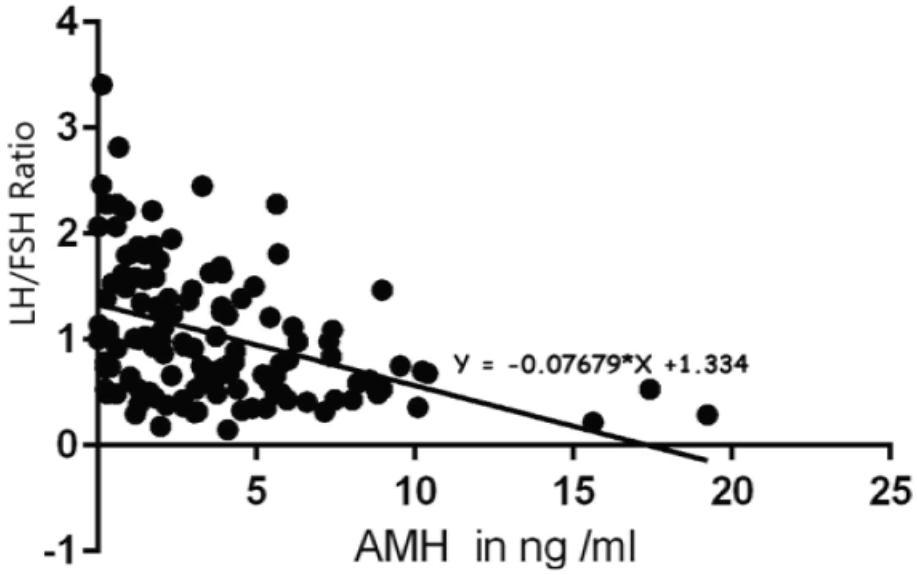


Table 2 shows the Pearson Correlation Coefficient of AMH Vs Other Hormonal parameters. AMH levels show a strong but negative correlation with LH/FSH ratio like linear regression analysis suggesting an opposite variation with each other. FSH and LH hormone levels doesn't have any significant variation trend with AMH levels with increasing age.

Table 2: Pearson Correlation Coefficient of AMH Vs Other Hormonal parameters:

Age Group in years	Pearson Correlation Coefficient of AMH Vs		
	LH/FSH ratio	FSH mIU/mL	LH mIU/mL
<20years of age	-0.54	0.60	0.39
21-25	-0.51	-0.09	0.37
26-30	-0.49	-0.22	0.09
31-35	-0.36	-0.31	-0.21
36-40	-0.34	0.05	-0.29
41-45	-0.42	-0.35	-0.32
46-50	-0.36	-0.25	-0.22
51-55	-0.29	-0.31	-0.39

Table 3 shows the statistical significance of the parameters - AMH, LH/FSH ratio, FSH, LH, Prolactin and TSH in the study population. There is significant difference in the averages of these variables suggesting that Anti-mullerian hormone has significant variation with the other hormone levels like FSH, LH, Prolactin and TSH. Surprisingly, there is no significant variation of Anti-mullerian hormone with FSH, LH, Prolactin and TSH levels under 20 years of age. This could probably be due to inadequate samples in that age group or due to any high outlier value of AMH among the patients. Limitations of our study include smaller sample size. On overcoming these, our study can likely be representative of the general female population.

Table 3: ANOVA test results among different age groups of the Study population

ANOVA test results of the hormonal parameters – AMH, LH/FSH ratio, FSH, LH, Prolactin &TSH Levels								
Age Group in years	<20years of age	21-25	26-30	31-35	36-40	41-45	46-50	51-55
p value	p>0.05	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significance	**Not Significant**	**Significant**	**Significant**	**Significant**	**Significant**	**Significant**	**Significant**	**Significant**

CONCLUSION

From Our study, we conclude that Anti-mullerian hormone levels vary inversely with increasing age. AMH and LH/FSH ratio are independent predictors of ovarian reserve in the management of infertility & artificial reproductive techniques and hence we can rely on LH/FSH ratio in situations where AMH is not readily available.

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