ORIGINAL ARTICLE

CORRELATION OF ANTI-MÜLLERIAN HORMONE WITH LUTEINIZING HORMONE TO FOLLICLE STIMULATING HORMONE RATIO IN PATIENTS WITH SUSPECTED INFERTILITY

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Background: Anti-Müllerian Hormone (AMH) is produced by growing ovarian antral follicles. AMH is well correlated with the quantity of primordial follicles in the ovary. This study aimed to see any association of Anti-Müllerian Hormone with Leutinizing Hormone (LH) to Follicle Stimulating Hormone (FSH) ratio in patients suspected of infertility. **Methods:** It was a cross-sectional study performed in Department of Chemical Pathology/Biochemistry Laboratory Services of Liaquat National Hospital and Medical College Karachi from 1st April 2020 to 31st March 2021. Females aged of 18–50 years with suspected infertility were included. Women having other gynaecological diseases were excluded. Non-probability consecutive sampling was done. Blood was taken during the follicular phase of the menstrual cycle for estimation of AMH, FSH, and LH by Electrochemiluminescence Immunoassay. Data was analysed on SPSS-21. Mean and standard deviation were calculated, and Pearson's correlation and One-way ANOVA was applied for variables. **Result:** A total of 103 patients were included in our study, out of which 62 (60%) had abnormal AMH and 41 (40%) had normal AMH levels. With abnormal AMH, we found a mild negative correlation with FSH and LH which are significant (p=0.001 and p=0.01 respectively). Regarding LH/FSH ratio, there was a mild positive and significant correlation with AMH (p=0.03). **Conclusion:** With low AMH levels, FSH and LH are increased and LH/FSH ratio is reduced. These findings can be used as markers of ovarian reserve in patients with infertility.

Keywords: Anti-Müllerian Hormone, Follicle Stimulating Hormone, Leutinizing Hormone, Infertility

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INTRODUCTION

Anti-Müllerian Hormone (AMH) is produced by growing ovarian antral follicles and is a member of transforming growth factor β super family.[1] In the embryonic period of both sexes, AMH plays a significant role in the development of the reproductive organs.[2] It is present in the serum of women of reproductive age and is secreted by the granulosa cells of the ovarian follicles and appears to help in early follicular development.[3] It inhibits primordial follicle recruitment and reduces the sensitivity of Follicle Stimulating Hormone (FSH) in the small antral follicles.[2,4] AMH secretion by the antral follicles is stimulated by FSH.[5] The value of AMH is well correlated with the quantity of primordial follicles in the ovary and shows the number of dormant follicles present.[6]

Ovarian reserve is the number of primordial follicles present in the ovary at the time of birth.[7] The number of primordial follicles do not increase after birth, rather they keep on decreasing throughout the reproductive life of a woman as certain follicles grow and then undergo apoptosis with each menstrual cycle till menopause is reached.[7] AMH can be used as a surrogate marker of functional ovarian follicle reserve. With increasing age, there is a decline in reproductive capacity of a woman. This is due to a decrease in the ovarian reserve which is because of attenuation of the size and quality of the pool of primordial follicles. As the primordial follicle pool is exhausted, new follicles cannot be recruited, leading to menopause.[8] Advancing age also shows a decrease in AMH levels.[6]

As compared to other markers of ovarian reserve like FSH and Estradiol (E2), AMH has the advantage that it is stable throughout the menstrual cycle and samples can be taken at any time, whereas FSH and E2 can only be detected on the first five days of the menstrual cycle.[7] AMH levels are high in Polycystic Ovarian Syndrome (PCOS), but women with a high AMH level without PCOS are very fertile.[9] In conditions like PCOS where there is high Luteinizing Hormone (LH) and normal to low FSH, AMH is positively correlated with LH, while it is not negatively correlated with FSH.[10] This study aimed at determining the correlation of AMH with the ratio of LH to FSH to see if it can predict infertility.

MATERIAL AND METHODS

This was a cross-sectional study performed in the Department of Chemical Pathology/Biochemistry Laboratory Services of Liaquat National Hospital and Medical College Karachi from 1st April 2020 to 31st

March 2021 after approval by the Ethical Review Committee in a certificate dated 26 Dec 2019.

Adult women aged 18–50 years who presented to the Outpatient Department with suspected infertility were included in the study after their informed consent. Non-probability consecutive sampling was done. Women having other gynaecological diseases were excluded. Sample size was calculated to be 62 with PASS-11 taking power of test=80%, level of significance=5% and correlation=0.348.[11] The patients were divided according to into age groups of ≤30 years (n=42, 40.8%), 31–40 years (n=50, 48.5%), and >40 years (n=11, 10.7%) respectively.

The patients' demographics were recorded. Five ml of blood was taken during the follicular phase of the menstrual cycle for the analysis of AMH, FSH and LH in a yellow gel tube, the samples were centrifuged and the supernatant was collected for analysis. Serum was analysed with Electro-chemiluminescence Immunoassay on Elecsys e411 (Roche Diagnostics). The reference intervals used for AMH, FSH and LH were 0.9–9.5 ng/ml, 3.5–12.5 mIU/ml, and 2.4–12.6 mIU/ml respectively.

Data was analysed using SPSS-21. Mean±SD were calculated, Pearson's correlation and one-way ANOVA was applied for the variables, and $p \leq 0.05$ was taken as statistically significant.

RESULTS

A total of 103 patients were included in the study out of which 41 (40%) had normal and 62 (60%) had abnormal AMH level. With abnormal AMH, there was a mild but significant negative correlation with FSH and LH (p=0.001 and p=0.01 respectively). Regarding LH/FSH ratio, there was a mild positive but significant (p=0.03) correlation with AMH. There was a mild negative non-significant (p=0.074) correlation of age with abnormal AMH (Table-1).

There was no significant correlation of normal AMH with FSH and LH levels, LH/FSH ratio, or age (Table-2).

Comparison of AMH levels in different age groups revealed that the AMH decreases with increasing age (p=0.00) (Table-3).

Comparison of LH/FSH ratio in different age groups showed no significant differences (Table-4).

Table-1: Correlations of abnormal AMH with different parameters

Parameter	Mean±SD	Pearson Correlation	*p*
AMH (ng/ml)	0.3229±0.33140	1	
FSH (mIU/ml)	25.1805±29.53871	-0.398	0.001
LH (mIU/ml)	18.7463±17.97142	-0.321	0.011
LH/FSH Ratio	1.2681±1.69621	0.267	0.036
Age (Years)	35.7742±6.47421	-0.228	0.074

Table-2: Correlations of normal AMH with the different parameters

Parameter	Mean±SD	Pearson Correlation	*p*
AMH (ng/ml)	3.1366±1.71449	1	
FSH (mIU/ml)	6.8871±3.02440	-0.075	0.643
LH (mIU/ml)	8.5954±5.50258	-0.085	0.596
LH/FSH Ratio	1.3273±0.77368	-0.043	0.791
Age (Years)	28.5854±5.22961	-0.224	0.159

Table-3: Comparison of AMH in different age groups

Age Groups	n (%)	AMH (ng/ml) Mean±SD	*p*
≤30 years	42 (40.75)	2.3067±2.01782	0.000
31–40 years	50 (48.5)	0.9978±1.37877	
>40 years	11 (10.6)	0.1682±0.21517	
Total	103 (100)	1.4429±1.77030	

Table-4: Comparison of LH/FSH ratio in different age groups

Age Groups	n (%)	LH/FSH Mean±SD	*p*
≤30 years	42 (40.7)	1.2729±1.00779	0.729
31–40 years	50 (48.5)	1.3712±1.77081	
>40 years	11 (10.6)	1.0018±0.54637	
Total	103 (100)	1.2917±1.39865	

DISCUSSION

Using FSH and LH is quite common to predict the ovarian reserve as it is easy to obtain the samples, even though they have a poor predictive value.[11] Lee *et al* found that the LH/FSH ratio decreases with age and this ratio may be a better marker of ovarian reserve as compared to FSH alone. They also found a mild positive correlation of LH/FSH ratio with AMH which is similar to our study.[11]

Melado *et al* studied the variations in AMH levels in healthy women and found that there are significant inter-cycle variations in AMH levels during the course of a menstrual cycle and also that there was a significant positive correlation between the AMH levels and LH during the LH surge.[12] There is also a study which shows that AMH may have some role in Gonadotrophin Releasing hormone (GnRH) release which is the regulator of FSH and LH.[13] Another study described a negative correlation of AMH levels with age and FSH levels which are similar to our study.[14] Chung HJ *et al*[15] showed that AMH levels decrease significantly with age, also AMH and LH/FSH ratio showed a significant positive correlation. There was a negative correlation between AMH with FSH or LH. They suggested that AMH can be replaced by LH/FSH ratio in small clinics that cannot perform AMH test.[15] Our findings are in agreement to that study.

Jamil *et al* studied AMH and they say that AMH is a good predictor of ovarian response but its levels lack proper prediction of pregnancy outcomes. They opined that AMH indicates quantity of the follicles but not the quality. They concluded that patients with

low AMH levels may conceive whereas patients with high AMH may fail to conceive.[16] Another study carried out in Iran shows that AMH levels can be used to predict ovarian response to stimulation in patients with endometriosis.[17] Le MT *et al*[18] measured AMH levels and FSH/LH ratio in PCOS patients and found that the combination of different factors like AMH, FSH/LH ratio, Body Mass Index and other clinical characteristics may have a better role in diagnosing PCOS.[18] Another study to see the AMH levels and response to *in vitro* fertilization found that in patients aged <40 years, higher AMH and lower FSH had more chances of pregnancy.[19]

CONCLUSION

With low AMH levels, FSH and LH are increased and LH/FSH ratio is reduced. These parameters can be used as markers of ovarian reserve in patients with infertility in conditions where AMH test is not available or expensive to use.

REFERENCES

1. Abbara A, Eng PC, Phylactou M, Clarke SA, Hunjan T, Roberts R, *et al*. Anti-Müllerian hormone (AMH) in the diagnosis of menstrual disturbance due to polycystic ovarian syndrome. Front Endocrinol (Lausanne) 2019;10:656.
2. Kruszyńska K, Słowińska-Srzednicka J. Anti-Müllerian hormone (AMH) as a good predictor of time of menopause. Menopause Rev (Prz Menopauzalny) 2017;16(2):47–50.
3. Bednarska-Czerwinska A, Olszak-Wasik K, Olejek A, Cerzerwinski M, Tukiendorf AA. Vitamin D and anti-Müllerian hormone levels in infertility treatment: The change-point problem. Nutrients 2019;11:1053.
4. Saadullah SM, Sulaiman DM. Anti-Müllerian hormone level in patients with polycystic ovary syndrome. Med J Babylon 2018;15:295–9.
5. Urrutia M, Grinspon RP, Rey RA. Comparing the role of anti-Müllerian hormone as a marker of FSH action in male and female fertility. Expert Rev Endocrinol Metab 2019;14(3):203–14.
6. Segawa T, Omi K, Watanabe Y, Sone Y, Handa M, Kuroda M, *et al*. Age-specific values of Access anti-Müllerian hormone immunoassay carried out on Japanese patients with infertility: a retrospective large-scale study. BMC Women's Health 2019;19(1):57.
7. Sun H, Mao H, Cai J, Zhao Y. Research progress on anti-Müllerian hormone clinical applications and immunoassay development. Front Lab Med 2018;2:14–8.
8. van Houten EL, Themmen AP, Visser JA. Anti-Müllerian hormone (AMH): Regulator and marker of ovarian function. Ann Endocrinol (Paris) 2010;71(3):191–7.
9. Yue CY, Lu LK, Li M, Zhang QL, Ying CM. Threshold value of anti-Müllerian hormone for the diagnosis of polycystic ovary syndrome in Chinese women. PLoS One 2018;13(8):e0203129
10. Shembekar CA, Upadhye JJ, Shembekar MC, Welekar SH. Anti-Müllerian hormone (AMH) as predictor of ovarian reserve. Int J Reprod Contracept Obstet Gynecol 2017;6(9):4006–10.
11. Lee JE, Yoon SH, Kim HO, Min EG. Correlation between the serum luteinizing hormone to follicle stimulating hormone ratio and the anti-Müllerian hormone levels in normo-ovulatory women. J Korean Med Sci 2015;30:296–300.
12. Melado L, Lawrenz B, Sibal J, Abu E, Coughlan C, Navarro AT, *et al*. Anti-Müllerian hormone during natural cycle presents significant intra and intercycle variations when measured with fully automated assay. Front Endocrinol 2018;9:686.
13. Pasquali R. New perspectives on the role of anti-Müllerian hormone (AMH) in women. Ann Transl Med 2018;6(Suppl 2):S94.
14. Barbakadze L, Kristesashvili J, Khonelidze N, Tsagareishvili G. The correlations of anti-Müllerian hormone, follicle-stimulating hormone and antral follicle count in different age groups of infertile women. Int J Fertil Steril 2015;8(4):393–8.
15. Chung HJ, Park MH. Can AMH Level be replaced by LH/FSH ratio? Am J Clin Pathol 2013;140(Suppl 1):A036.
16. Jamil Z, Fatima SS, Ahmed K, Malik R. Anti-Müllerian hormone: Above and beyond conventional ovarian reserve markers. Dis Markers 2016;2016:5246217.
17. Safdarian L, Ghalandarpoor Attar SN, Aleyasin A, Aghahosseini M, Sarfjoo FS, Hosseinimousa S. Investigation of anti-Müllerian hormone (AMH) level and ovarian response in infertile women with endometriosis in IVF cycles. Int J Reprod BioMed 2018;16(11):719–22.
18. Le MT, Le VNS, Le DD, Nguyen VQH, Chen C, Cao NT. Exploration of the role of anti-Müllerian hormone and LH/FSH ratio in diagnosis of polycystic ovary syndrome. Clin Endocrinol (Oxf) 2019;90:579–85.
19. Chen YP, Wu WH, Wu HM, Chen CK, Wang HS, Huang HY. Effects of anti-Müllerian hormone and follicle stimulating hormone levels on in vitro fertilization pregnancy rate. Taiwanese J Obstet Gynecol 2014;53(3):313–6.

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