ORIGINAL ARTICLE

Comparison of Serum Anti-Mullerian Hormone and Antral Follicle Count as Predictor of Infertility in females of Karachi

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ABSTRACT

Back ground: Fertility in women is determined by ovarian reserve, which is currently defined as the number and quality of the follicles left at any moment in the ovary. Anti-Mullerian Hormone (AMH), has been suggested as a representative of the ovarian reserve because in females, AMH is exclusively produced by granulosa cells of preantral (primary and secondary) and small antral follicles.
Aim: To estimate serum level of Anti-mullerian hormone in study subjects and to compare it with antral follicle count (AFC) as a predictor of infertility.
Methods: A comparative cross sectional study was conducted on total ninety female subjects (sixty infertile and thirty fertile) recruited conveniently through outpatient department of two tertiary care hospitals of Karachi. Serum AMH of each participant was recorded on day 3 of menstrual cycle and on the same day antral follicle count was measured through transvaginal ultrasonography.
Results: Both the AMH and AFC were reduced in infertile group as compared to control but reduction in mean AMH levels with age was found statistically significant than AFC.
Conclusion: It was concluded that as compared to AFC, AMH is considered as more suitable hormone, to identify the potential reproductive capacity of a female.
Keyword: Anti-Mullerian Hormone (AMH), Antral follicle count(AFC), ovarian reserve, infertility.

INTRODUCTION

Infertility is defined as failure of the couple to conceive after 12 months of contraceptive-free intercourse if the female is under the age of 34 years, whereas the couple is considered infertile after 6 months in females over the age of 35 years[1,2]. According to WHO primary infertility refers to couples who have never conceived, whereas secondary infertility refers to couples failed to conceive after one year of following previous pregnancy and not using any contraceptives[3].A woman's fertility decreases with increasing age, naturally starts to decline in late 20's. A woman is born with all the eggs and with time, the supply diminishes. The remaining eggs also age along with the rest of the body[4].Fertility in women is determined by the ovarian reserve, which represents both the quantity and quality of the ovarian follicle pool. Besides oocyte quality, the number of primordial follicles that are left in the ovary is also an important parameter for ovarian reserve. Therefore, a marker that reflects all follicles that have made the transition from the primordial follicle pool to the growing pool may be a good indirect marker of the quantitative aspects of the ovarian reserve[5]. Anti Mullerian hormone (AMH) also known as Mullerian Inhibiting Substance (MIS), is a member of transforming growth factor-β (TGF-β).It is a peptide homodimeric of molecular weight of 140 kDa consisting of two identical glycoprotein subunits, connected by disulfide bridges[6]. Anti-Mullerian Hormone (AMH), has been suggested as a representative of the ovarian reserve [7,8] because in females, it is exclusively produced by granulosa cells of preantral (primary and secondary) and small antral follicles. Its production starts after follicles differentiate from the primordial to the primary stage and continues until the follicles have reached the antral stage with diameters of 2-6 mm[7]. Since the number of the small antral follicles is related to the size of the primordial follicle pool, now there is increasing evidence that AMH in contrast to other markers can be used as a cycle independent marker[9].

The antral follicle count (AFC) is defined as the number of follicles smaller than 10 mm in diameter detected by Transvaginal scan (TVS) in the early follicular phase. AFC ultrasonographically is the physical evaluation techniques for ovarian reserve prediction[10].

Studies abroad have proven AMH as better predictor of infertility among female than AFC. We found no such study conducted in Pakistan yet. In our setting, AFC is still taken as routine test for infertility. We conducted this study to compare the efficacy of

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AMH and AFC in diagnosis of infertility in developing country like Pakistan. AMH was hypothesized as better predictor than AFC. As AFC detected by TVS is a painful procedure, therefore AMH as a biomarker would make the diagnosis of primary infertility much easier.

MATERIALS AND METHODS

It was a comparative cross-sectional study conducted during a period of one year in 2010. The study was carried out on outpatients selected from department of Gynae/Obs of two public tertiary care hospitals include Jinnah Post Graduate Medical Centre and Abbassi Shaheed Hospital of Karachi. Total 90 subjects were included in the study by consecutive sampling and a written informed consent was taken from all the participants after explaining them about the nature and purpose of the study.

All the subjects were classified into two main groups, fertile (control) and infertile on the basis of history. Of the total, 30 subjects were placed in the fertile group and 60 subjects in the infertile group. Both the groups were matched with respect to biophysical parameters i.e. age, weight, height, BMI, blood pressure, pulse, temperature and respiratory rate as shown in table 1 in order to control any possible confounding effects of these variables.

On the morning of day 3 menstrual cycle, about 6 ml of blood was drawn from venepuncture after all aseptic measures for the measurements of Serum level of AMH from each woman who fulfilled the criteria. On the same day of the menstrual cycle, trans-vaginal ultrasonography was performed to evaluate the number of Antral Follicles. The serum AMH was determined by sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). Observations were entered and analysed in SPSS version 11. Mean and standard deviation for both the AMH and AFC were calculated and compared by Student t – test and the p –value < 0.05 was considered as significant.

RESULTS

Mean values of AMH and AFC were calculated among both the infertile and fertile groups as shown in table 2. While comparing, value of both the predictors were found to be reduced among infertile group but the difference in serum AMH level was found highly significant statistically. To overcome the confounding effect of age, we stratified the infertile participants into two groups, one group was 20 – 29 years of age while second was 30 39 years. Table 3 and figure 1 show the comparison of parameters of AMH and AFC in these age groups. Statistically significant difference ($p<0.05$) was observed in the level of serum AMH in 30 - 39 years age group while no such difference was noticed in AFC.

Table 1: Biophysical parameters of infertile and fertile women

Parameters	**Infertile (n=60) Mean±SD**	**Fertile (n=30) Mean ± SD**
Age (years)	25.4±0.65	26.7±0.91
Weight (Kg)	59.3±1.42	60.7±2.44
Height (m)	1.56± 0.01	1.58±0.01
BMI (kg/m^2)	24.3±0.55	24.8±0.95
BP (systolic in mmHg)	108.7±0.87	111.3 ± 0.82
BP (diastolic in mmHg)	70.8±0.83	73.6±1.45
Pulse rate / minute	82.9±0.80	82.1±0.90
Respiratory rate/ minute	19.8±1.12	18.9±0.18
Temperature (^{0}C)	98.1±0.03	98.0 ± 0.01

**t test was applied and no significant difference observed among both groups in any variable*

Table 2: comparison of serum AMH and AFC among infertile and fertile groups

Variables	**Infertile (n=60)**	**Fertile (n= 30)**
Serum AMH(ng/ml)	2.52 ± 0.25**	3.72 ± 0.34
AFC	14.4 ± 0.60	15.4 ± 0.51

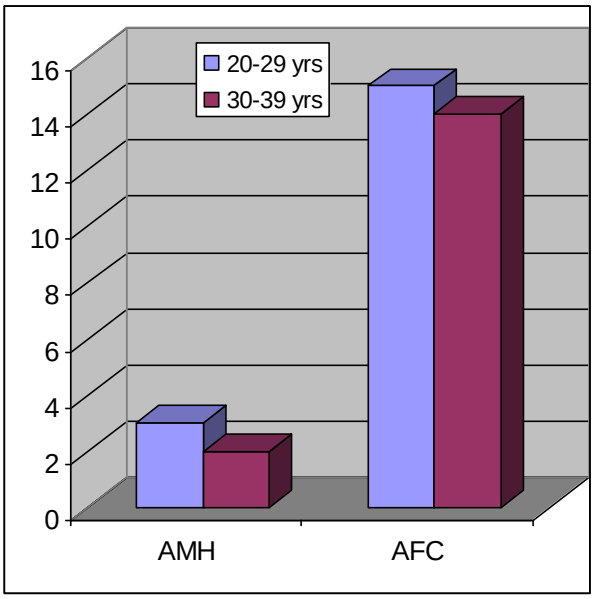
T test applied ; ** P < 0.01

Table 3: AMH and AFC with age distribution in infertile group (Values are expressed as mean±s.e.m.)

Parameters	**20-29 years (n=49)**	**30-39 years (n=11)**
ng/AMH ml	2.77±0.29	1.44±0.23*
AFC	14.7±0.69	13.2±1.06

*p<0.05 Statistically significant

Fig.1: AMH and AFC with age distribution in infertile group



DISCUSSION

Fertility in women is determined by the ovarian reserve, AMH has been suggested as a representative of the ovarian reserve[8]. Under these circumstances the current study works undertaken to assess the relationship of AMH with AFC in isolation of primary infertility. Our result showed that the AFC is reduced in infertile group than fertile group, these finding are in accordance with studies conducted by Broekmans and Haadsma[11,12] et al but the finding in our study is not significant as it was in the above mentioned studies may be because the Haadsma in his study has stratified the size of antral follicles and we take the overall mean of the AFC. Fertile women contained significantly more antral follicles than infertile according to Van Rooij et al[13].

Current study showed that serum AMH is reduced in infertile group as compared to fertile group. The same was reported by de Vet et al[14], they demonstrated that serum AMH level on cycle day 3 decrease progressively along with age and become undetectable after menopause.

Our result showed statistically significant reduction in mean AMH level with age whereas AFC reduced insignificantly with age which are in agreement with the finding of Shin et al [15]. This suggests that peripheral AMH levels are valuable parameter to monitor the relative follicles exhaustion due to the ovarian aging consistently. Knauff et al[16] noticed that day 3 AMH levels are positively related with number of oocytes retrieved after control ovarian hyperstimulation (COH) taken together, these results indicate that circulating AMH levels reflect the number of selectable follicles during early follicular phase.

CONCLUSION

AMH is useful as a diagnostic as well as prognostic tool, but because of limited number of data that require further investigation in studies with larger numbers of participants that evaluate the confidence interval to determine the correlation of this marker with infertility.

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