Short Communication

Study on Anti-Mullerian Hormone (AMH) Levels in Women Patients

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Abstract

The study analyzed the Anti-Mullerian Hormone levels by Elisa kit method. Serum sample was collected from the 24 patients attending Regenix Super Specialty Laboratory, Chennai, India. The Ultra-Sensitive Anti-Müllerian hormone/Müllerian inhibiting substance (US AMH/MIS) enzyme linked immunuosorbent assay (ELISA) kit was used to test AMH levels. It was noted that women patients in the age group 20 to 25years having higher value (>3.0 ng/mL) may be due to the PCOS (Polycystic ovarian syndrome) condition. Woman patients in the age group 26 to 30 years having lower value (<1.0 ng/mL) may look for *in vitro* fertilization for getting pregnant. It was also noted that woman patients in the age group 31 to 40 years also had low value (<1.0 ng/mL) of AMH levels. The measurement of AMH level by Elisa kit method may be used as an important marker for women patients having infertility problems. The diagnostic tool may be further improved for its reliability and sensitivity by the manufacturers in near future.

Keywords: Anti-Mullerian hormone, Elisa kit method, polycystic ovarian syndrome, *in vitro* fertilization, reliability.

Introduction

AMH produced by granulosa cells (GC) of the ovary; AMH, also known as Mullerian-inhibiting substance, is a dimeric glycoprotein that belongs to the transforming growth factor-β family (Wilson *et al.,* 1983). Over the last decade, a large number of studies examining the multiple roles of Anti-mullerian hormone (AMH) have been published. It is virtually undetectable but increases gradually until puberty and remains relatively stable through the reproductive period (Barbakadze *et al.,* 2009). It is widely accepted that the reduction of AMH levels in serum is the first indication for decline in the follicular reserve of the ovaries and can be measured in the blood at any time in the menstrual cycle due to its stability. AMH is a marker for ovarian reserve and naturally lower in older women (>40 year) and higher in women with Polycystic ovaries (PCO) and polycystic Ovary Syndrome (POCS) (Teede *et al.,* 2014). It was reported that Follicle stimulating hormone (FSH), Estradiol (E2) levels and antral follicle count (AFC) have been used for evaluation of ovarian reserve to determine suitable treatment strategy for female infertility by age, which becomes very essential in recent years (Raeissi *et al.,* 2015). Traditionally, age, Follicle Stimulating Hormone (FSH), E2 levels and antral follicle count (AFC) at have been used for evaluation of ovarian reserve the early follicular phase.

Levels of FSH and E2 were considered to be the determining biochemical markers for assessment of low ovarian reserve for many years and FSH level was found above the norm only in cases when the ovary function is largely decreased however, it is still the most commonly used test although its reliability is weak and also FSH association with significant inter- and intra-cycle variability is documented (Dumont *et al.,* 2015). The present research problem was aimed to collect the serum sample of women patients attending Regenix super specialty Lab, Chennai and analyze the Anti-Mullerian Hormone levels by Elisa kit method.

Materials and methods

Specimen collection: Serum sample is collected from the 24 patients attending Regenix Super Specialty Laboratory and used for determination of AMH levels.

Ultra-Sensitive AMH ELISA test: The Ultra-Sensitive Anti-Müllerian hormone/Müllerian inhibiting substance (US AMH/MIS) enzyme linked immunuosorbent assay (ELISA) kit was used to test AMH levels. In the first step calibrators, controls and unknown samples are added to AMH antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated AMH antibody solution.

Table. 1. AMH levels of the women patients.

Patients	Age in years	Anti-Mullerian Hormone Levels (ng/mL)
1.	20	4.1
2	20	4.6
3.	20	8.6
4.	22	4.7
5.	22	4.7
6.	22	1.2
7.	24	5.2
8.	25	5.6
9.	25	4.9
10.	25	4.6
11.	25	5.9
12.	25	4.6
13.	25	7.6
14.	25	8.91
15.	25	5.6
16.	26	3.8
17.	27	3.1
18.	28	4.1
19.	28	6.1
20.	29	6.1
21.	30	3.5
22.	31	0.65
23.	34	0.98
24.	37	0.97

After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of AMH in the samples and calibrators. The AMH levels were expressed in ng/mL.

Results and discussion
Serum sample was collected from the 24 women patients attending Regenix Super Specialty Laboratory with PCOS and infertility problems. AMH levels were measured in the serum sample by ELISA kit method. It was noted that women patients in the age group 20 to 25years having higher value (>3.0 ng/mL) may be due to the PCOS (Polycystic ovarian syndrome) condition. Woman patients in the age group 26 to 30 years having lower value (<1.0 ng/mL) may look for *in vitro* fertilization for getting pregnant. It was also noted that woman patients in the age group 31 to 40 years also had low value (<1.0 ng/mL) of AMH levels (Table 1).

Conclusion
To conclude, the measurement of AMH level by Elisa kit method may be used as an important marker for women patients having infertility problems. The diagnostic tool may be further improved for its reliability and sensitivity by the manufacturers in near future.

Acknowledgements

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