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Establishing age-specific reference intervals for anti-Müllerian hormone in adult Chinese women based on a multicenter population

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ABSTRACT

Aim of this study: Anti-Müllerian hormone (AMH) is useful for the assessment of ovarian reserve and treatment of individualized in vitro fertilization (IVF). The aim of this study is to establish AMH reference interval for adult Chinese women on the Beckman Beckman DxI 800 platform.

Material and methods: From May to September 2013, serum from 1169 apparently healthy adult females from five representative cities in China (Beijing, Hangzhou, Guangzhou, Dalian and Urumqi) were collected, and AMH was analyzed on the platform of Beckman DxI 800 automated chemiluminescence immunoassay. Multiple regression analysis was used to investigate the effects of region, sex, age, body mass index (BMI), systolic blood pressure (SBP), exercise on AMH. Age specific reference interval for AMH was established.

Results: The main factor affecting AMH levels was age ($B = -0.756$, $P < 0.001$). The AMH reference intervals for adult Chinese women aged 19–24 years, 25–29 years, 30–34 years, 35–39 years, 40–44 years, 45–49 years and ≥ 50 years were 0.74–16.06, 0.67–11.64, 0.50–9.99, 0.09–8.33, 0.04–4.09, 0.01–1.46 and < 0.01–0.18 ng/ml, respectively. The linear, quadratic and cubic models could either provide good fit regression model to describe the decline of AMH with age ($R^2 = 0.40$).

Conclusion: This study firstly established age-specific reference intervals for AMH in Chinese women based on multicenter population.

1. Introduction

Anti-Müllerian hormone (AMH) is a glycoprotein in the transforming growth factor-β (TGF-β) superfamily. The gene that encodes it is located on the short arm of chromosome 19 [1]. AMH is expressed by interstitial cells of the testes after testes formation in males and can cause Müllerian duct degradation, whereas AMH is expressed by ovarian granulosa cells in females beginning at 36 weeks of gestation. AMH plays an important role in human development [2]. The granulosa cells begin to secrete AMH in the preantral follicle phase. Both in vivo and vitro studies have demonstrated that AMH is mainly produced by 4- to 8-mm growing follicles [3] and plays a decisive role in selecting the dominant follicle during the follicular phase of the menstrual cycle [4]. In clinical practice, AMH can be used for the diagnosis or differential diagnosis of premature ovarian failure (POF), polycystic ovary syndrome and ovarian granulosa cell tumors. Additionally, it can be used for assessments of the ovarian reserve and the influences of ovarian surgery and chemotherapy on ovarian function [5–8]. During individualized in vitro fertilization (IVF) therapy, AMH can also be used to determine the reactivity of follicular cells after stimulation and assist the prediction of IVF live births [9–11].

Based on the important roles of AMH, Beckman has developed more sensitive and accurate chemiluminescence method for detecting AMH than the Gen II ELISA method, and it may become the mainstream

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method of AMH measurement [12]. The functional sensitivity of the Beckman's chemiluminescence method is 0.01 ng/ml, whereas the functional sensitivity of the ELISA Gen II method is 0.05 ng/ml [13]. Accurate clinical diagnosis for AMH related diseases by clinicians has relied on accurate and representative AMH reference intervals, and now there is a better method. Although, AMH reference intervals have been established based on some western and Asian populations, or on other platforms [14–18], there may be some differences in AMH among different regions, ethnic groups, and platforms. Thus, it is necessary to establish AMH reference intervals based on the Chinese population on the platform of the Beckman chemiluminescence method. However, to our knowledge, until now, no AMH reference intervals have been established based on the Chinese population on the Beckman Access platform, and it is necessary to establish age-specific reference intervals for AMH.

In 2013, we collaborated with the IFCC Committee on Reference Intervals and Decision Limits (C-RIDL). As the designated research institution in China for global reference interval studies, we organized and implemented a multi-center study in a Chinese Han population according to C-RIDL research programs and standard operating procedures [19]. The subjects of this study were apparently healthy people who were enrolled during our collaboration with the IFCC, and they were used to establish Chinese population reference intervals. Based on this population, we established representative age-specific AMH reference intervals for adult Chinese women and explored the sources of variation (SVs) that possibly influenced AMH, including region, sex, age, BMI, systolic blood pressure (SBP), and exercise.

2. Methods

2.1. Study population

The study population was a part of the International Federation of Clinical Chemistry (IFCC) Global Multicenter study of reference intervals in China. A total of 1310 apparently healthy female adults from the North China region (Beijing), Northeast region (Dalian), South China region (Guangzhou), East China region (Hangzhou) and Northwest region (Urumqi) of China were recruited. In each city, 60 subjects were selected from each age group, which included subjects aged 19–29 years, 30–39 years, 40–49 years and 50–64 years, and 20 subjects were selected from the age group of 65 years and older. Approximately 260 subjects were selected from each region. The inclusion and exclusion criteria are described in a previous article [20].

The inclusion criteria were set a priori as follows: living in the selected region for > 1 year; free from acute or chronic infections, digestive diseases, kidney disease, metabolic and nutritional disease, rheumatic diseases, endocrine disease, and circulatory system diseases; and had not undergone surgery, or received medication, blood donations, or transfusions within the previous 6 months. The heights and weights were measured by a trained research nurse, and body mass index (BMI) was calculated as the weight divided by the height squared. Information related to the above inclusion and exclusion criteria as well as the baseline subject characteristics were obtained using self-administered questionnaires. Additionally, only female adults were included in this study. After excluding abnormal samples, such as those with hemolysis, icterus or lipemia, serum samples from 1169 subjects were measured.

2.2. Laboratory analysis

2.2.1. Sample collection and processing

Preparation before sampling, blood sample collection and specimen processing were conducted according to IFCC/C-RIDL protocols [19]. During the menstrual cycle, the change in AMH is very limited. Therefore, specimens collected on any day of the menstrual cycle can fully reflect the ovarian reserve [21,22]. To ensure that the results of this study were representative in a clinical setting, we decided to collect specimens on a random day of the menstrual cycle. We used separation gel coagulant tubes (Vacuette) to collect 9 ml of blood. At 15–30 min after the sample collection, the samples were centrifuged (1200 *g*, 10 min) to separate the serum. The serum was aliquoted into cryovials and stored at − 80 °C before analysis. The frozen sera from different areas were transported on dry ice under refrigeration to a laboratory at Peking Union Medical College Hospital for centralized testing.

2.2.2. Analysis of AMH and other biomarkers

AMH detection was conducted using an automated chemiluminescence immunoassay analyzer (Beckman Coulter UniCel DxI 800) and the corresponding reagents, calibration materials and quality control materials. Before measuring the samples, three quality control products were consecutively analyzed four times per day for five continuous days to evaluate the precision of the method (inter run-CV:1.1–3.9%; total CV: 2.4–5.2%). The imprecision of this study is consistent with previous reports and the sensitivity of this method is 0.01 ng/ml [13]. We subsequently measured the samples after first measuring the quality control products to ensure quality control. All of the specimens were tested in the laboratories of Peking Union Medical College Hospital. Dixon's rule was applied for outlier data exclusion [23].

Alanine transaminase (ALT), creatinine (Cr), triglycerides (TG), total cholesterol (TC), and glucose (Glu) were measured using the Beckman AU Series Automatic Biochemical Analyzer and Beckman AU reagents.

2.2.3. Ethics

This study was approved by the Ethics Committee of Peking Union Medical College & Chinese Academy of Medical Sciences, Peking Union Medical College Hospital. All the subjects were informed of the intended use of the sera and provided written informed consent.

2.3. Statistical analysis

In this study, all of the data were analyzed using SPSS 19.0 software (SPSS Inc., USA) and Microsoft Excel 2010 statistical software (Microsoft Corporation, USA). The Kolmogorov-Smirnov test was used to assess whether the investigated parameters were normally distributed. Comparisons among different groups were performed using the Kruskal-Wallis method, and comparisons between two groups were conducted using the Bonferroni method to adjust the alpha level. Multiple regression analysis (MRA) was performed to identify the sources of variation (SVs) that possibly influenced the AMH levels, including age, region, sex, BMI, SBP, and exercise [24]. In the analysis of regional differences, through the use of dummy variables, Beijing was set as the reference region. A given explanatory variable was considered to be of practical importance when its standardized partial regression coefficient, which corresponds to the partial correlation coefficient, was > 0.15, which corresponds to $P \approx 0.0$ with a large dataset size [24]. The regression model was used to describe the decline of AMH with age, and the F-test was performed to evaluate the effectiveness of the regression model. $P < 0.05$ indicated a significant difference.

3. Results

3.1. General clinical data of the subjects

This study included a total of 1169 adult women from five areas: Beijing, Hangzhou, Guangzhou, Dalian and Urumqi. General information about the subjects is presented in Table 1. The average age was 42.3 ± 14.0 years, and the average BMI was 22.3 ± 2.9 kg/m^2. There were no significant differences in age or BMI among these urban populations ($P > 0.05$). There were 1152 AMH results after the outlier data were excluded according to Dixon's rule [23].