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ORIGINAL ARTICLE

The relationship between anti-Müllerian hormone serum level and body mass index in a large cohort of infertile patients

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Abstract

Purpose To evaluate the relationship between serum Anti-Müllerian hormone (AMH) level and body mass index (BMI) in infertile patients.

Methods Medical records of patients with infertility evaluated between January 2013 and February 2018 in the Reproductive Medicine Department of a private hospital were reviewed. Patients with the following criteria were excluded from the study: polycystic ovary syndrome, primary ovarian insufficiency, AMH values > 10 ng/mL, current oral contraceptive users and previous ovarian surgery or endometriosis, and anovulation of other causes, except decreased ovarian reserve.

Results A total of 2204 infertile patients were included (mean age 34.58 ± 4.3 years, mean BMI 22.35 ± 3.6 kg/m^2, and mean serum AMH 2.44 ± 2.17 ng/ml). In the entire group of patients, serum AMH level was positively correlated with BMI after adjustment for age (beta = 0.059, $p < 0.005$). When the association between serum AMH level and BMI was analysed in subgroups of patients, after adjustment for age, we found a positive correlation between the two parameters in patients ≤ 35 years old (< 0.05), of normal weight ($p < 0.05$) and with normal ovarian reserve ($p < 0.05$). After adjustment for age, BMI ≥ 25 kg/m^2 was significantly associated with higher AMH values in comparison to normal weight patients.

Conclusions In infertile patients, AMH is positively correlated with BMI, especially in patients younger than 35 years, of normal weight and with normal ovarian reserve. Moreover, the presence of mild excess adiposity seems to be associated with higher AMH values. Our data contradict the previous studies showing a negative impact of excess adiposity on AMH serum levels.

Keywords Anti-Müllerian hormone · Body mass index · Infertility · Obesity · Adiposity

Introduction

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor beta family [1], which is produced by the granulosa cells of small, growing follicles. AMH acts on the ovaries by inhibiting the initial follicle recruitment and the FSH-dependent growth of the follicles and selection of pre-antral and small antral follicles. Serum AMH levels are proportional to the number of developing follicles in the ovaries. Therefore, AMH was proposed as a marker for ovarian reserve [2]. Due to the decreased variability of serum AMH levels during the ovarian cycle, AMH measurement became widely used for testing the ovarian reserve, especially in infertile patients [3]. This hormone has a particular utility in this category of patients because it was demonstrated to have a predictive value for the response to assisted reproductive technologies [4]. Nonetheless, the serum AMH level can be modified by some conditions, thereby potentially affecting its predictive value for the ovarian reserve. Obesity was reported as a factor that could decrease the serum AMH level. This aspect was initially observed in women of advanced reproductive age [5]. Further studies, which analysed a wider range of age categories, reported divergent results, confirming the negative relationship between AMH and body mass index

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(BMI) [3, 6–8] or reporting no relationship [9–11]. Moreover, the severity of excess adiposity, which can negatively influence the AMH production and the category of patients most susceptible to excess adiposity, is incompletely clarified. Currently, there is wide acceptance that nutritional status influences the follicle development through specific energy sensing mechanisms [12]. Therefore, it is possible that the adiposity influences the AMH production not only in obese patients but in other categories of BMI as well. However, the data regarding the relationship between adiposity and serum AMH level in patients without obesity are scarce. Nonetheless, the connection between AMH and adiposity could be different in infertile patients in comparison to the general population. Taking into account the main utility of AMH measurement in infertile patients, it is of great importance to study the relationship between AMH and adiposity in this category of patients. Moreover, the possible contribution of age as a modifier of the connection between AMH and adiposity is incompletely elucidated. However, a detailed evaluation of the variation of the relationship between AMH and adiposity, depending on BMI category, ovarian reserve and age in infertile patients, was not previously performed. Therefore, the aim of the present study was to analyse the relationship between BMI and AMH serum level according to age category, severity of adiposity excess and ovarian reserve in a large sample of infertile patients.

Materials and methods

We performed a retrospective study, which included 2204 female patients evaluated for infertility between January 2013 and February 2018 in the Medlife Maternal–foetal and Reproductive Medicine Department. The study was approved by the Local Research Ethics Committee, and all patients gave written informed consent for the clinical evaluation and blood collection. According to the protocol for the evaluation of infertility of the Department, data about age, menstrual history, ovarian surgery, endometriosis diagnosis or treatment, oral contraceptive use, and previous investigations for infertility were collected at the first presentation. Weight and height were measured with the patients in light clothing. BMI was calculated using the formula of weight in kilograms divided by square of the height in metres. After the first consultation, the patients were advised to perform the following investigations, unless such investigations were already available and not older than 3 months: 1. transvaginal ultrasound on the day 8–20 of the menstrual cycle for the evaluation of ovulation (considered to be present if a dominant follicle or a corpus luteum was identified) and for the uterine and ovarian aspect; 2. Hysterosalpingography or hysterosonography for fallopian tube patency; 3. Semen analysis of the male partner; and 4. Hormonal evaluation of the female partner (blood tests for thyroid function, prolactin, total testosterone, and sex hormone binding globulin (SHBG) and AMH. Blood samples for hormone measurement were obtained by venepuncture in the morning on any day of the menstrual cycle. A commercial ELISA kit was used for AMH measurement (AMH Gen II ELISA kit from Beckman-Coulter, USA), an enzymatically amplified two-site immunoassay. The AMH was measured in serum obtained after centrifugation of the blood sample and stored at 2–8 °C for 48 h or frozen at −20 °C, if the assay was not completed within 48 h after centrifugation. The AMH limit of detection was 0.08 ng/mL. For women with AMH levels below the assay limit of detection, the values were imputed using 0.04 ng/mL, the midpoint between 0 and the assay limit of detection.

Infertility was defined as lack of conception after at least one year of unprotected intercourse. Polycystic ovary syndrome (PCOS) was diagnosed according to Rotterdam Consensus criteria [13] if the patient presented with at least two of the following three criteria: 1. Chronic oligo-ovulation or anovulation (considered to be present in patients with oligo or amenorrhoea or no ultrasound sign of ovulation); 2. Clinical or biochemical hyperandrogenism (defined as hirsutism and/or increased total testosterone or free androgen index calculated as total testosterone in nmol/LX100/SHBG in nmol/L); and 3. The ultrasound aspect of polycystic ovaries [13]. Endometriosis was diagnosed by laparoscopy in patients with suggestive clinical signs and symptoms. Decreased ovarian reserve was considered to be present in patients with AMH < 1.1 ng/ml [14]. Body weight categories (underweight, normal weight, overweight, obesity, and severe obesity) were evaluated according to WHO criteria [15].

The medical records of the patients were reviewed and only patients with the following data available for the study were selected: age, height and weight of the patient, serum AMH levels (measured within three months from the first evaluation in the Laboratory Department of Medlife), and full evaluation for infertility according to the Department's protocol.

Patients with the following criteria were excluded from the study: 1. polycystic ovary syndrome; 2. patients with primary ovarian insufficiency defined as amenorrhea of at least 12 months and increased values of FSH and LH; 3. AMH values above 10 ng/mL; 4. current oral contraceptive users; 5. previous ovarian surgery or endometriosis; and 6. anovulation of other causes, except decreased ovarian reserve.

Statistical analysis

Data are expressed as the mean and standard deviation or percentage as appropriate. The comparisons between groups

Table 1 Characteristics of the study group and age-subgroups

	Total ($n = 2204$)	≤30 years ($n = 326$)	30–35 years ($n = 878$)	35–40 years ($n = 734$)	>40 years ($n = 266$)
Age (years)	34.58 ± 4.3	27.9 ± 2.08	32.64 ± 1.43	37.25 ± 1.41	41.85 ± 1.43
BMI (kg/m^2)	22.35 ± 3.6	21.8 ± 3.46	21.83 ± 3.43	22.65 ± 3.83	23.6 ± 4.27
AMH (ng/ml)	2.44 ± 2.17	3.78 ± 2.42	2.74 ± 2.22	1.88 ± 1.78	1.24 ± 1.47

were performed using analysis of variance test (for multiple groups) or Student's *t*-test for independent samples (for two groups) for continuous variables. Correlations were tested with Pearson analysis. Adjustment for age was performed with multivariate linear regression with AMH as the dependent variable and BMI and age as independent continuous variables. A model of multivariate linear regression with AMH as the dependent variable, age as a continuous variable and BMI as a dummy variable (with normal weight patients as the reference category) was created in order to analyse the relationship of AMH with categories of body weight. A *p*-value below 0.05 was chosen to indicate statistical significance. All statistical analyses were performed using SPSS for Windows version 20.

Table 2 The relationship between AMH and BMI in subgroups of patients according to age

Age category	Number of patients	BMI (kg/m^2)		Age (years)	
		ß coeff[a]	*p* value	ß coeff[a]	*p* value
≤30 yo	326	0.132	0.028	−0.130	0.019
>30 and ≤35 yo	878	0.088	0.011	−0.179	<0.0001
>35 and ≤40 yo	734	0.023	NS	−0.150	<0.0001
over 40	266	−0.020	NS	−0.205	0.002

The results of the multivariate linear regression analysis with AMH as the dependent variable and age and BMI as continuous independent variables [a]ß coefficient for BMI as an independent variable in the multivariate model after adjustment for age

Results

Characteristics of the study group

The 2204 patients in the study group had a mean age of 34.58 ± 4.3 years, mean BMI of 22.35 ± 3.6 kg/m^2 and mean AMH value of 2.44 ± 2.17 ng/mL (Table 1). Most of the patients were in the age group 30–40 years old ($n =$ 1612, 73.1%), while patients 30 years old and younger ($n =$ 326, 14.8%), and over forty ($n = 266$, 12.1%), represented only 26.9% of the patients in our study group. Only 12 patients (0.5%) were 45 years and older. Most of the patients were of normal weight ($n = 1624$, 73.68%) (BMI ≥ 18.5 and <25 kg/m^2) and only a small proportion of patients were overweight ($n = 339$, BMI ≥ 25 and < 30 kg/m^2)) and obese ($n = 95$, BMI ≥ 30 kg/m^2) ($n = 434$, 19.69%). Only two patients in our study group had BMI over 40 kg/m^2. Two thirds of the patients ($n = 1432$, 64.97%) had normal ovarian reserve, while 1/3 ($n = 772$, 35%) had decreased ovarian reserve showed by an AMH value below 1.1 ng/ml.

The relationship of AMH with BMI according to age group

Analysing the entire group of patients, we noticed that although BMI was not significantly correlated with AMH serum level in bivariate analysis, in a multivariate linear regression model, both age (beta −0.386, $p < 0.0001$) and BMI (beta 0.059, p 0.004) were independently associated with AMH.

When the association between AMH serum level and BMI was analysed according to age group, we found that in patients in age group ≤ 30 years old and 30–35 years old AMH and BMI were positively correlated ($r = 0.139$, $p =$ 0.021 and $r = 0.077$, $p = 0.027$, respectively) and the association was maintained after adjustment for age (Table 2). In turn, in patients over 35 years of age, AMH and BMI were not correlated in bivariate analysis or after adjustment for age in a multivariate linear regression model (Table 2). However, the age of the patients was negatively associated with serum AMH level in all age groups after adjustment for BMI (Table 2).

The relationship between AMH and BMI according to AMH category

Patients with decreased ovarian reserve (serum AMH level < 1.1 ng/mL) ($n = 772$) were significantly older and had slightly, but significantly, higher BMI in comparison with patients with normal ovarian reserve (serum AMH level ≥ 1.1 ng/mL) ($n = 1432$) (Table 3). In patients with decreased ovarian reserve, AMH and BMI were negatively correlated (r −0.102, $p < 0.01$), but in multivariate analysis, only age was independently associated with AMH (beta - 0.187, $p <$ 0.0001) (for BMI beta −0.026, $p =$ NS) (Table 3). In patients with normal ovarian reserve (AMH ≥ 1.1 ng/mL) (*n*

Table 3 Patient characteristics and the association between AMH and BMI according to AMH category

	AMH < 1.1 ng/mL (n = 772)	AMH ≥ 1.1 ng/mL (n = 1432)	p value[a]
Age (years)	36.66 ± 3.97	33.47 ± 4.18	<0.001
BMI (kg/m^2)	22.54 ± 3.81	22.19 ± 3.69	<0.05
AMH (ng/mL)	0.53 ± 0.29	3.45 ± 2.05	<0.0001
r coeff for BMI[b]	−0.102*	0.026**	
ß coeff for BMI[c]	−0.026**	0.053***	

[a]p value for the comparison between the two groups of patients according to AMH cut-off value of 1.1 ng/mL (Student's t test)

[b]r coefficient for the correlation between BMI and AMH (Pearson test)

[c]ß coefficient for BMI as a continuous independent variable in the multivariate linear regression model with AMH as the dependent variable and adjustment for age

*$p < 0.01$; **p = non-significant; ***$p < 0.05$

Table 4 Characteristics of patients and the association between AMH and BMI according to BMI category

	BMI < 18.5 kg/m^2 (n = 227)	BMI ≥ 18.5 and < 25 kg/m^2 (n = 1543)	BMI ≥ 25 kg/m^2 (n = 434)	p value[a]
Age	33.8 ± 4.2	34.43 ± 4.2	35.57 ± 4.9	<0.001
BMI	17.69 ± 0.72	21.34 ± 1.7	28.29 ± 3.11	<0.0001
AMH	2.61 ± 2.22	2.4 ± 2.1	2.42 ± 2.36	NS
r coeff for BMI[b]	−0.058*	0.002*	−0.035*	
ß coeff for BMI[c]	−0.067*	0.056**	−0.002*	

NS non-significant

[a]p value for the comparison between the three groups of patients according to BMI category (ANOVA)

[b]r coefficient for the correlation between BMI and AMH (Pearson test)

[c]ß coefficient for BMI as a continuous independent variable in the multivariate linear regression model with AMH as the dependent variable and adjustment for age; *p = NS; **$p < 0.05$

= 1432), no relationship between AMH and BMI was detected in bivariate analysis, but in multivariate analysis, both age (beta – 0.264, $p < 0.0001$) and BMI (for BMI beta 0.053, $p < 0.05$) were independently related to circulating AMH.

The relationship between AMH and BMI according to BMI category

When patients were divided according to BMI category, we noticed that age increased with increasing BMI (Table 4). However, the serum AMH levels were similar across BMI categories (Table 4). In underweight patients (BMI < 18.5 kg/m^2, $n = 227$), BMI was not correlated with AMH, and in multivariate regression, only age was independently associated with AMH (Table 4). In normal weight patients (BMI ≥ 18.5 and <25 kg/m^2, $n = 1543$), BMI and AMH were not correlated in bivariate analysis, but BMI was positively associated with AMH after adjustment for age (beta 0.056, $p < 0.05$) (Table 4). In overweight and obese patients (BMI ≥ 25 kg/m^2, $n = 434$), AMH and BMI were not correlated in bivariate and multivariate analysis (Table 4). We found no difference in serum AMH level in the three categories of patients according to BMI (Table 4). However, after adjustment for age in a multivariate linear regression model with AMH as the dependent variable, BMI ≥ 25 kg/m^2 (beta 0.041, $p < 0.05$) was positively associated with AMH value, while BMI < 18.5 kg/m^2 was not an independent predictor.

Discussion

To the best of our knowledge, this is the first study that reports a positive association between BMI and serum AMH levels in infertile patients. The presence of overweight and obesity was related to higher AMH levels in comparison to normal and underweight patients. Moreover, when subcategories of patients were analysed, we found that a positive linear association was present for patients with normal ovarian reserve and normal weight and who were younger than 35 years of age. It is possible that the lack of correlation of the two parameters in the other categories of patients may be due to the small sample size, which was insufficient to detect a weak (although significant) correlation such as the one observed in our study. Another possible explanation is a different relationship between AMH and adiposity according to age, severity of excess adiposity and ovarian reserve. This hypothesis is supported by the reports showing a different pattern of AMH secretion according to the ovarian age [16] and the presence of obesity [17].

Our results contradict the previous studies, which found negative [5, 6, 18, 19] or no association at all [10, 20–22] between the two parameters. This discrepancy in the results of these studies could be due to the heterogeneity of the populations analysed. For instance, the study of Buyuk et al. [18] showed that in a population of infertile women (mean age 38 years), high BMI (above 25 kg/m^2) has a negative impact on serum AMH in patients with decreased ovarian reserve but not in patients with normal ovarian reserve [18]. However, in our patients with decreased ovarian reserve (AMH below 1.1 ng/ml), the negative correlation between AMH and BMI was lost after adjustment for age. We interpret this finding as a consequence of the increase of BMI with age (demonstrated by the positive correlation of the two parameters, $r = 0.255$, $p < 0.0001$, data not showed), which confounded the relationship between BMI and AMH. Therefore, the negative impact of adiposity on

AMH production in patients with decreased ovarian reserve is not confirmed in our study. However, in the study of Buyuk et al [18], decreased ovarian reserve was defined by an FSH serum level in early follicular phase exceeding 10 mUI/mL, which is not as reliable as AMH as a marker of ovarian reserve [23]. Moreover, we should take into account that the BMI of the patients with decreased ovarian reserve in our study is 22.63 ± 3.77 kg/m^2, which is significantly lower than the BMI in the study of Buyuk et al. (25.3 ± 5.1 kg/m^2) [18].

Moreover, the severity of excess adiposity could be of relevance when the relationship between BMI and AMH is analysed. For example, the study of Bernardi et al. [19] evaluated the impact of adiposity in 1654 African–American women aged 23–35 with a wide range of BMI (15.9–79.4 kg/m^2) and found that BMI was negatively correlated with circulating AMH. However, in a multivariate regression model, only BMI of 40 kg/m^2 and over was independently associated with lower AMH values. In turn, in the study of Halawaty et al., which excluded women with BMI > 35 kg/m^2, no difference was found in FSH, AMH, antral follicle count, and ovarian volume in premenopausal women with and without obesity [10]. These data suggest that the negative relationship between AMH and BMI could be obvious only in populations comprising a significant proportion of patients with severe obesity (BMI over 40 kg/m^2). However, in our group, the proportion of patients with severe obesity was very low (only two patients) and insufficient to detect a possible negative impact on serum AMH levels. Moreover, we noticed that in patients with BMI over 25 kg/m^2, there was no correlation between BMI and AMH, suggesting the lack of a linear relationship between the two parameters in patients with moderate excess adiposity. The time of the exposure to excess adiposity could also be of relevance in the interaction between adiposity and AMH production, since it was reported that the presence of obesity at the age of 18 is independently associated with lower AMH value later in life [19].

It was also showed that BMI can be negatively related to AMH serum levels in women of advanced reproductive age [5, 6]. In the study of Freeman et al. [5], AMH serum levels were negatively correlated with BMI and were 65% lower in obese women (BMI above 30 kg/m^2) (mean age 45.8 years old) [5]. In contrast, in patients with a similar age (mean age 46.1) and BMI ≤ 35 kg/m^2, no difference was found in all the markers of ovarian reserve, including AMH in premenopausal women with and without obesity [10]. However, the severity of excess adiposity was higher in the first study (mean BMI 37.6 kg/m^2) than in the study of Halawaty et al. [10], suggesting once again that the severity of obesity could modulate this relationship. Nevertheless, the number of patients in these studies was quite small, their results requiring confirmation in larger studies. In our study, the relationship between AMH and BMI in women older than 45 years could not be analysed because our retrospective review of the medical data found only 12 patients above this age. However, in patients over 40 years old, no relationship was found between AMH and BMI.

One of the possible explanations for the positive association of BMI and AMH serum levels observed in our patients is a hormonally mediated relationship. Thus, it is known that circulating androgens [17], insulinaemia and insulin resistance increase along with BMI [24]. The same parameters were shown to be positively associated with serum AMH levels. In the study of Cui et al [25], serum AMH level was positively correlated with total testosterone in the general female population. Moreover, Nardo et al [26], found that circulating AMH is positively related to both androgens and insulinaemia/insulin resistance in PCOS and non-PCOS subfertile patients of normal weight. It was also demonstrated that increased androgen levels in the follicular fluid increase the AMH production from the granulosa cells [27]. In turn, insulin can stimulate androgen production in human thecal cells [28]. Therefore, increasing insulin in parallel with BMI can indirectly stimulate, through increased intraovarian androgen synthesis, the AMH production by granulosa cells.

It was assumed that the decreased production of AMH in obese patients is a consequence of increased ovarian aromatase activity and high oestrogen to androgen ratio, which suppress local AMH production [3, 27]. It was also suggested that adiponectin could be the mediator of adiposity effect on aromatase activity, as adiponectin was shown to inhibit aromatase activity in the ovary [29]. Therefore, it was assumed that low adiponectin levels found in obese patients [30] are responsible for increased aromatase activity. However, other studies contradict these results, reporting that adiponectin increases oestradiol and aromatase expression in granulosa cells of human ovaries [31]. Accordingly, it is possible that in humans, serum adiponectin level has a negative impact on ovarian AMH production, mediating a positive association between adiposity and circulating AMH. Similarly, in mice, very low adiponectin levels were associated with decreased oestradiol levels [32]. However, it is difficult to say how the results of these experimental studies are translated into clinical practice, as the differences in adiponectin serum levels between normal weight and overweight/obese patients, although significant, are small in clinical terms, as showed by the study of Zeng et al. [33]. On the other hand, a study in mice showed that only very low serum adiponectin levels found in null adiponectin gene mice were associated with perturbed ovarian steroidogenesis, while in heterozygotic animals, these parameters were similar to those in wild-type animals [32].

The strengths of our study include the largest sample size of infertile patients collected to date in which the relationship between AMH and BMI was analysed. All the blood samples for AMH measurement were uniformly analysed in the same laboratory using one particular AMH assay (Gen II Beckman Coulter). Exclusion criteria were applied in order to obtain homogeneity of the study population. Therefore, current contraceptive users were eliminated, since the circulating AMH values were reported to be significantly decreased in this category of women (by 30–50%) [34, 35]. Other categories of patients (ovarian surgery, endometriosis, and ovarian insufficiency) were also excluded because low ovarian reserve of these patients is probably due to anatomical/structural factors with possible negligible influences from adipose tissue. Similarly, patients with polycystic ovary syndrome were excluded because AMH production is increased in these patients and possibly regulated differently from other categories of infertile women. Moreover, our study group comprised only infertile patients; therefore, our results can be applied to patients with infertility. Among the limitations of our study is the relatively small sample size in some subgroups of patients like those with obesity. Furthermore, patients with severe obesity and advanced reproductive age (over 45 years) were poorly represented in our study, making it impossible to draw conclusions regarding the relationship between BMI and AMH in these categories of women.

Regarding the clinical implications of our findings, the positive relationship between AMH serum levels and BMI is rather weak, although significant, and probably without significant impact in clinical terms. However, the negative impact of increased adiposity on circulating AMH level reported by the previous studies was not confirmed in our study. Thus, our findings suggest that a low AMH level in a patient with moderate body weight excess (BMI below 40 kg/m^2) cannot be explained by increased adiposity. Therefore, these patients should be advised to start the treatment for infertility as soon as possible instead of postponing treatment until body weight is optimised.

In conclusion, our study showed that in infertile women without severe obesity, there is a positive impact of increasing BMI on AMH serum level. We also found a linear positive relationship between BMI and AMH in patients aged 35 and below, with normal weight and with normal ovarian reserve. However, the previously reported negative relationship between adiposity and serum AMH level in special subgroups of patients (with low ovarian reserve or older women) was not confirmed in our study. Nevertheless, definitive conclusions regarding the relationship between AMH and BMI in patients with severe obesity and advanced reproductive age (over 45 years) cannot be drawn due to the small number of these types of patients in our study group.

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Compliance with ethical standards

Conflict of interest D.A. has received travel grants from Merck, Merck Sharp & Dohme and Ferring Pharmaceuticals. A.A. has received travel grants from Ferring Pharmaceuticals, Pfizer and a speaker honourarium from Sandoz Pharma Services.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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