ORIGINAL ARTICLE

Anti-Müllerian hormone concentration as a biomarker of pregnancy success or failure

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Abstract

OBJECTIVE: This study was conducted to determine serum anti-Müllerian hormone (AMH) concentration influence on pregnancy outcome.
STUDY DESIGN: In this study we investigated sixty one infertile women (aged 27 to 44 years) who were diagnosed and treated between 2011 and 2013. We determine ovarian reserve measured by AMH concentration. Patients were divided in three groups according to their serum AMH concentration (<1 ng/ml; 1–2.5 ng/ml; >2.5 ng/ml respectively). We investigated the relationship between clinical pregnancy rate and AMH concentration. In addition, anti-thyroid antibodies (anti-TG and/or anti-TPO) positivity and insulin concentration were correlated with AMH level and pregnancy outcome in the study groups.
RESULTS: We found no statistical differences between AMH concentration regarding number of pregnancies (42.3%; 41.1 %; 38.9% respectively in study groups; $p>0.05$). The miscarriage rate was highest in women with AMH>2.5 ng/mL (27.3%, 0%, 86% respectively in study groups; $p>0.05$). We found that anti-thyroid positivity is more frequent in women with lower AMH concentration (23.1%; 11.7%; 5.5% respectively; $p>0.05$) and patients with lower serum AMH had higher serum insulin concentration ($p<0.05$).
CONCLUSIONS: It seems that AMH concentration might not reflect oocyte quality and the chance of pregnancy, but increased AMH concentration may be associated with negative pregnancy outcome. Moreover, it cannot be excluded that presence of anti-thyroid antibodies and increased insulin serum concentration may be connected to diminished ovarian reserve measured by AMH concentration.

Abbreviations:

AMH	- anti-Müllerian hormone
TTP	- time to pregnancy
FSH	- follicle stimulating hormone
AFC	- antral follicle count
E2	- estradiol
TGF-β	- transforming growth factor β
Anti-TPO	- anti-thyroid peroxidase
Anti-TG	- thyroglobulin antibodies
IVF	- *in vitro* fertilization
OGTT	- oral glucose tolerance test
hCG	- human chorionic gonadotropin

INTRODUCTION

Infertility represents a group of disorders with considerable socioeconomic and clinical impact. The problem affects 10–15% of Polish couples. The choice of treatment depends largely on the patient's age, duration of the attempts at pregnancy – time to pregnancy (TTP) and infertility causes. Ovarian reserve and assessment of the likelihood of becoming pregnant are important elements of the diagnostic workup. Traditional biomarkers, such as FSH (follicle-stimulating hormone), AFC (antral follicle count), E_2 (estradiol) and inhibin B are considered to be quantitative indicators of the number of oocytes retrieved (Eldar-Geva *et al.* 2005; Miao & Huang 2009). These biomarkers appear to be inadequate for predicting the outcome of pregnancy. There is a growing need for clinically relevant and practical biomarkers, which will provide a qualitative assessment of oocytes, fertilization rate and clinical pregnancy rate.

Recently, anti-Müllerian hormone (AMH) has been evaluated as a potential novel clinical marker of ovarian reserve and pregnancy outcome. AMH is a glycoprotein belonging to the transforming growth factor β (TGF-β) family. It is produced by the granulosa cells of growing primordial follicles (Hagen *et al.* 2012). In women of childbearing age, AMH inhibits the recruitment of primordial follicles and reduces the response of growing follicles to FSH, thus participating in the selection of a dominant follicle (Hampl *et al.* 2011). Serum AMH concentration increases with age, reaches maximum levels between the age of 21 and 30, then gradually decreases. It reflects the size of a growing primordial follicle population, thus making AMH a very sensitive marker of ovarian reserve and early-stage ovarian aging (Kevenaar *et al.* 2006; de Vet *et al.* 2002). The most established role for AMH measurement is prior to *in vitro* fertilization, because AMH levels can be predictive of ovarian response, namely poor and hyper-response (Nelson *et al.* 2012).

However, it is currently not known if serum AMH levels also reflect oocyte quality and the chance of successful live birth pregnancy. Recent studies have shown that AMH may predict ovarian reserve and the subsequent success of IVF (Lekamge *et al.* 2007; Wunder *et al.* 2008; Barad *et al.* 2009). However, other reports did not find any value for predicting pregnancy outcomes (Smeenk *et al.* 2007; Lee *et al.* 2008).

AMH concentration and the resulting ovarian reserve are influenced by multiple factors. There is growing evidence suggesting thyroid autoimmunity and high insulin levels as the potential causes of diminished ovarian reserve and infertility.

Our interest was in evaluating potential influences of serum anti-Müllerian hormone (AMH) concentration, insulin concentration and anti – thyroid antibody positivity on pregnancy outcome.

MATERIAL AND METHODS

Material

For inclusion into the study group we qualified sixty-one (n=61) infertile women (age range 27–44 years) who were diagnosed and treated between 2011 and 2013. Diagnosis of infertility was made according to WHO as a failure to achieve clinical pregnancy after 12 months or longer of regular unprotected sexual intercourse. All information was obtained from the patients' medical records. Pregnancy outcome data were obtained by a telephone. All patients expressed oral informed consent for the use of their medical records provided that their information will be kept confidential and anonymous. This study design was approved by the Institutional Review Board of the Military Institute of Medicine in Warsaw.

Methods

Serum AMH concentration was measured on various days of the cycle for the purpose of determining ovarian reserve. Patients were divided into 3 groups according to their AMH concentration: group 1 with AMH level <1 ng/ml, group 2 with AMH level 1–2.5 ng/ml and group 3 with AMH >2.5 ng/ml. In order to assess the impact of AMH concentration on pregnancy outcome, we established AMH concentration of 1–2.5 ng/ml as a normal value.

The initial routine infertility workup also included thyroid function tests and serum insulin concentration. All patients were evaluated for serum anti-thyroid antibodies: anti-thyroid peroxidase (anti–TPO) and thyroglobulin antibodies (anti-TG). Patients with upper levels of normal range were considered anti-thyroid antibody positive. Furthermore, each patient underwent a 75 g oral glucose tolerance test (OGTT). Insulin levels were measured at 0, 1 and 2 hours.

The mean calculated follow-up time from the diagnosis and treatment until pregnancy outcome assessment was 13 months. For the purposes of pregnancy outcome we defined pregnancy as urine or serum betahCG positivity. Miscarriage was defined as spontaneous termination of pregnancy before week 24.

In this study we assessed the correlation between AMH concentration and pregnancy outcome. Additionally, anti–thyroid antibody (anti-TG and/or anti-TPO) positivity and insulin concentration were correlated with AMH concentration and pregnancy outcome in the study groups.

Statistics

Statistical analysis was performed using Statistica version 10.1. Data were summarized by descriptive statistics (mean ± SD for continuous variables and frequency of percentages for categorical variables). The Mann-Whitney U test and Spearmen rank correlation were used to analyze the association between age, pregnancy rate, miscarriage, insulin concentration, anti-thyroid

antibody positivity and AMH concentration. A value of $p<0.05$ was considered as a statistically significant.

RESULTS

Between 2011 and 2013 a total of 61 women met the criteria of infertility and were qualified to our analysis. Of the 61 women n=26 (42.6%) was included in the group 1 with AMH level <1 ng/ml, n=17 (27.8%) women was included in the group 2 with AMH level 1–2.5 ng/ml and n=18 (29.5%) women was included in the group 3 with AMH level >2.5 ng/ml. The mean patients age was 36±3.88 years (37.4, 34.5, 35.4 respectively in each study group). Across all groups the average level of AMH concentration was 2.88ng/ml (0.39±0.27, 1.71±0.46 and 6.35±7.15 respectively in each study group).

In our analysis we confirm the statistically significant, negative correlation between patient age and AMH value (p=0.014).

Twenty-five n=25 (41%) out of 61 women included in the study became pregnant. As seen in table 1 the pregnancy rate were similar in each study group (42.3%, 41.1%, 38.9%, respectively). We found that clinical pregnancy rate does not depend on AMH concentration (p=0.7). Out of n=25 pregnancies that took place, n=9 (36%) ended in miscarriage. We reported 27.3% miscarriages in group 1, 0% in group 2 and 86% in group 3 respectively. The miscarriage rate was highest in women with AMH>2.5 ng/mL. However, the difference was not statistically significant in all study groups (p=0.19). The mean AMH level in patients with normal pregnancies was significantly lower than in patients whose pregnancies ended with miscarriage (1.25 ng/mL vs. 5.7 ng/mL). Outcome data are shown in Table 1.

We found that anti-TPO positivity is more frequent in women with lower AMH concentration (23.1%, 11.7%, 5.5%, respectively), however the difference is not statistically significant in all study groups (p=0.07). Moreover there is no correlation (p=0.6) between the presence of anti-TG antibody and AMH concentration (15.4%, 23.5% and 11%, respectively in study groups). There is no statistical differences between anti-TPO/anti-TG positivity and pregnancy outcome. Table 2 depicts the pregnancy outcome data in women with anti-TPO/anti-TG positivity.

In study group 1 and 2, the patients with higher serum AMH had lower serum insulin concentration measured at 1 hour after glucose load (p=0.03). We did not find any significant differences between insulin levels at 0 and 2 hours following glucose load, and the AMH levels in all study groups. Outcome data are shown in Table 3.

DISCUSSION

In this study, we analyzed AMH serum concentration in 61 infertile women to better understand the relationship between the AMH level and pregnancy outcome. Conflicting reports exist in the literature regarding the relationship between serum AMH levels, oocyte quality and pregnancy outcome.

Our study of infertile women has demonstrated no statistically significant association between AMH concentration and the occurrence of pregnancy. Pregnancy rate was similar in all studied groups. Similarly Eldar-Geva *et al.* (2005) also reported no distinct correlation between AMH level, oocyte quality, fertilization rate and clinical pregnancy rate. Pereira *et al.* (2013) show that patients with extremely low AMH concentration demonstrate low, but moderate ongoing pregnancy rates. There are some reports suggesting that AMH values have no impact on the outcomes of IUI/IVF and particularly on pregnancy rates (Lamazou *et al.* 2012). On the other hand, current literature convincingly demonstrates the relationship between AMH level and pregnancy outcome. For instance, Brugo *et al.* (2013) show that pregnancy rate increases significantly with AMH level. Carvalho *et al.* (2012) reported comparable correlation between AMH level >2.7 ng/ml and an increased rate of implantations and pregnancies. Moreover, according to Koshy *et al.* (2013), women with lower AMH concentrations have a higher probability of treatment cancellation, failure to proceed to embryo transfer and a low chance of achieving viable pregnancy.

Tab. 1. Pregnancy outcome in each study group.

AMH ng/mL	Clinical pregnancy			Miscarriages		
	N	%	*p*-value	N	%	*p*-value
<1 ng/mL	11	42.3	0.53	3	27.3	0.6
1–2.5 ng/mL	7	41.1	0.76	0	0	1
>2.5 ng/mL	7	38.9	0.78	6	86	0.54

Tab. 2. Pregnancy outcome in women with anti-TPO/anti-TG positivity.

AMH ng/mL	Anti-TPO positivity				Anti-TG positivity			
	N	%	Preg.	Misc.	N	%	Preg.	Misc.
<1 ng/mL	6	23.1	1	1	4	15.4	0	0
1–2.5 ng/mL	2	11.7	2	0	3	23.5	3	0
>2.5 ng/mL	1	5.5	0	0	1	11	1	1

N - number of patients.

Tab. 3. The mean insulin level (μIU/mL) after OGTT.

AMH ng/mL	Insulin 0h	Insulin 1h	Insulin 2h
<1 ng/mL	7.25 (0.63)	64.02 (0.03)	37.36 (0.78)
1–2.5 ng/mL	7.8 (0.91)	69.21 (0.03)	56.55 (0.19)
>2.5 ng/mL	8.14 (0.13)	50.55 (0.46)	40.23 (0.29)

In parentheses () there are *p*-values.

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A novel finding of the current study relates to the AMH concentration and miscarriage rate. We found increased miscarriage rates (86%) among women with higher AMH concentration, however the difference was not statistically significant. In contrast, Lekamge *et al.* (2007) reported that patients with low AMH levels had a higher incidence of miscarriage in comparison to those with high levels of AMH. Morel *et al.* (2013) for instance, showed that failure to conceive could not be predicted by the levels of AMH. Tremellen & Kolo (2010) found no relationship between AMH levels and the chances of live birth or miscarriage. We supposed that our results should be evaluated further on a larger group of patients.

Our findings regarding the correlation between AMH and anti–TPO positivity are partly consistent with existing literature reports. Kelkar *et al.* (2005) showed that ovarian zona pellucida and thyroid tissue seem to share similar antigens. Zona pellucida may in turn be a target for anti-thyroid antibodies (Twig *et al.* 2012). The presence of anti-thyroid antibodies may facilitate identification of women at risk of ovarian failure independently of the levels of reproductive hormones (Edassery *et al.* 2010). In consistency with these results, Montelone *et al.* (2011) reported that oocyte fertilization and pregnancy rates were lower in women with thyroid autoimmunity than in negative controls, while early miscarriage rates were higher. Also Samy *et al.* (2006) showed that the presence of ovarian autoantibodies is associated with lower pregnancy rate. These findings support our data suggesting that thyroid autoimmunity may lead to diminished ovarian reserve. We found an association between AMH concentration and anti-TPO positivity but the difference was not statistically significant. However, in our study we did not observe a higher miscarriage rate among women with anti-thyroid antibody positivity.

There is a large number of reports suggesting that hyperinsulinemia may impair female reproduction (Cano *et al.* 1997; Vlaisavljević *et al.* 2009). Lower fertilization and implantation rates, and an increased risk of spontaneous pregnancy loss is observed among women with confirmed insulin resistance. It has also been reported that insulin resistance causes oxidative imbalance in the ovarian microenvironment, in an unexplained mechanism, leading to reduction in the quality of oocytes and their early progression into apoptosis (Ou *et al.* 2012). Thus, we examined the relationship between AMH and insulin concentration. Our observations of patients with higher serum AMH concentration and significantly lower serum insulin levels suggest a relationship between insulin concentration and ovarian reserve measured by AMH concentration.

The results of our study provide preliminary evidence that AMH concentration might not reflect oocyte quality and the chance of pregnancy. Moreover, it seems that increased AMH concentration may be associated with negative pregnancy outcome. Furthermore it cannot be excluded that presence of anti-thyroid antibodies and increased insulin serum concentration may be connected to diminished ovarian reserve measured by AMH concentration. Limitations of this study include retrospective design and rather small cohort. These factors prevented us from identifying AMH as a biomarker for pregnancy success or failure in women with infertility. However, these preliminary results may serve as a basis for larger prospective studies with long follow-up time.

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