The relation between Anti Mullerian hormone and hormone receptor status of breast cancer: a cross-sectional study

Sadaf Alipour¹, Bita Eslami¹, Ramesh Omranipour¹, Azin Saberi¹, Amirmohsen Jalaeefar¹, and Leila Haji Maghsoudi²

¹Tehran University of Medical Sciences
²Alborz University of Medical Sciences

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Abstract

Background: Estrogen is the strongest risk factor for breast cancer, especially hormone receptor positive subtypes. Anti-Mullerian hormone (AMH) can stimulate apoptosis, reduces breast tumor growth, and also has several actions in the steroid biosynthetic pathway. Therefore, there may be a correlation between AMH and breast cancer. Aims: In this study, we evaluate the correlation between AMH levels and the estrogen receptor (ER) or progesterone receptor (PR) status of breast cancer. Methods: This retrospective study was performed at two University Hospitals between August 2018 and April 2019. Serum AMH level, ER and PR status of the tumors were extracted from medical records. Results: Totally 100 premenopausal women with biopsy proven breast cancer were included in our study. AMH level was slightly higher in patients with ER positive than those with ER negative tumors, however, the difference was not significant (3.33 ± 3.15 vs 2.71 ± 2.06, p-value =0.69). In categorized AMH level, however, we could not find any significant differences between ER positive and negative as well as PR positive and negative patients. Conclusion: This study shows that there might be an association between serum AMH and breast cancer subtype regarding hormone receptors. We propose larger studies to be performed in order to verify this important issue further.

Introduction

The estrogen hormone works as a chemical messenger in the body and is essential for normal sexual development and female organ function. Excess exposure to estrogen raises the risk of cancer, especially estrogen receptor positive breast cancer. About 80 percent of breast cancers are hormone receptor-positive. These types of cancer need estrogen, progesterone or both hormones to develop. (1)

Estrogen can lead to carcinogenesis via several mechanisms. First, it stimulates cell divisions in the breast tissue (mitosis) as a “mitogen”; and can cause cancers due to replication errors (mutation). Secondly, estrogen has certain metabolites that act as Third, hormones like relaxin can be activated by estrogens and thereby stimulate cell division. It has been shown that relaxin (RLX) has a powerful effect on growth and differentiation of breast cancer cells. Anti-Müllerian hormone (AMH, also called Müllerian inhibiting substance, MIS) is a member of the transforming growth factor-β (TGF-β). (3) It is produced in the granulosa cells of the ovary. As follicles grow from primary to small antral follicles, AMH is secreted and inhibits the transition from primordial to the primary follicle. (4) Serum AMH level is clinically used in adult women as a measure of ovarian reserve. AMH also has several actions in the steroid biosynthetic pathway. (5) Some studies have shown an inverse correlation between AMH and breast cancer. In these studies the majority of tumors were estrogen receptor (ER) and progesterone receptor (PR) positive. (6, 7)

Estrogen is considered a well-established risk factor of ER-positive breast cancer, and as AMH can influence the level of estrogen by its effect on the steroid biosynthesis pathway, this raises the question of whether
there is any correlation between AMH levels and ER or PR of breast tumors. This study aimed to evaluate the relation between serum AMH levels and breast cancer subtype regarding ER and PR status

Materials and Methods

This retrospective study was performed at two Hospitals (Cancer Institute and Arash Women’s Hospitals) affiliated to Tehran University of Medical Sciences (TUMS), Tehran, Iran; between August 2018 and April 2019. Data of the present study including age, weight, height, age at menarche, parity, duration of breast-feeding, serum AMH level, and ER and PR status of the tumors were extracted from medical records. The inclusion criteria were a biopsy-proven breast cancer, female sex, and age less than 45 years, and having regular menstrual cycles (defined as the absence of menopause). The exclusion criteria were a previous or current history of infertility. Body mass index (BMI) was calculated by the formula of weight divided by height squared (kg/m²).

Blood collection and AMH measurement

In the previous study, a 5 ml sample of blood was collected from the cubital vein of all patients by nurses. Then, samples were sent to the reference laboratory in cold packs within 2 hours of collection. AMH was measured by the AMH enzyme-linked immunosorbent assay kits (Beckman Coulter, AMH gene II assay, Brea, CA, USA) at one laboratory. The limit of detection (LOD) was 0.08 ng/ml intra-assay and inter-assay variations were 7.7%.

Ethical considerations

This study was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.IKHC.REC.1401.262). Appropriate written informed consent was obtained from all participants prior to blood sampling.

Statistical Analysis

The statistical analyses were performed using IBM SPSS 26 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Data are presented in mean and standard deviation for continuous variables and number and percentage for categorical variables. The normality of AMH level before treatment was evaluated using the Kolmogorov- Smirnov test. AMH levels between ER and PR positive and negative tumors were compared by Mann-Whitney U-test. AMH level was categorized into three models considering previous studies’ categories (less than 0.7 and ≥ 0.7 ng/ml; 1, 1-3, >3 ng/ml; <1.2 and ≥ 1.2 ng/ml). Comparison between categorical variables was conducted using the Chi-square test. A P-value less than 0.05 was considered statistically significant.

Results

Totally 100 women with biopsy proven breast cancer were included in our study. The mean age of all the participant was 35.76 ± 4.99 (range: 23-45 years old). Table 1 presents the characteristics of all participants, AMH levels and hormone receptor status of tumors. The Kolmogorov-Smirnov test showed the AMH levels were not distributed normally (p-value= 0.023).

AMH level was higher in patients with ER- positive than those with ER- negative tumors, however, the difference was not significant (3.33 ± 3.15 vs 2.71± 2.06, p-value =0.69). Also, AMH levels in women whose tumors were positive for PR (3.23 ± 3.14) did not statistically differ from those with PR-negative (3.08 ± 2.41) breast cancer (p-value= 0.78). Table 2 compares the categorized AMH levels before treatment considering ER and PR status.

Discussion

In the current study, the association between AMH levels and estrogen or progesterone-receptor (positive or negative) status of breast cancer was evaluated in women of ages under 45 years. We did not find any association between the AMH level and the status of hormone receptors of cancer.
Estrogen plays a vital role in a wide variety of physiological processes, such as regulating energy, metabolism, stress response, mineral balance, and sexual development. Estrogen also plays a crucial role in the occurrence of breast cancer, especially estrogen receptor positive subtypes.(8)

The proliferative effects of estrogen and progesterone are mostly mediated by their receptors. There are two types of estrogen receptors (ERs), ERα and ERβ.(9) Estrogen receptors play a pivotal role in mammary gland maturation, puberty and pregnancy. Expression of ERα, which is found in nearly 50-80% of breast cancers, is a predictor of a better prognosis and a lower chance of recurrence.(10) ERβ, another estrogen receptor that also has been detected in breast tumors, is suspected to contribute to hormonal sensitivity and resistance.(11-13)

In the presence of exposure to high levels of estrogens, due to its proliferative effects, replication errors occur; and the accumulation of these errors leads to genetic mutations and the development of breast cancer. (2) Parallel to the mentioned effect, some estrogen metabolites may act as carcinogens by damaging DNA. The carcinogenic effect of estrogen could be justified to some extent by these reasons (1-3).

It seems that AMH also plays a paradoxical role in breast cancer development. Some laboratory experiments revealed protective effects of AMH by stimulating apoptosis and reducing breast tumor growth. (14, 15) On the other hand, a strong positive correlation was observed between higher AMH levels and the risk of breast cancer at menopausal age. It may be because patients with higher levels of AMH usually reach menopause at a later age, and thus have a longer duration of exposure to high concentrations of steroid sex hormones (17-18).

The effect of AMH on breast cancer is conflicting. Some studies indicated that AMH decreases the level of estrogen by inhibiting CYP19A1 (aromatase), a member of the cytochrome P450 superfamily, which is responsible for the aromatization and transformation of androgens to estrogens. Inhibition of aromatase interferes with the balance of estrogen and androgen concentrations in favor of decreasing the level of estrogen.(18) In addition, AMH can influence steroid synthesis by altering hormone receptor expression. According to several studies, AMH can reduce expression of LH receptor (LHR) mRNA (19-21) or blunt an LH-mediated increase in LHR expression.(22)

A study conducted by Ge and colleagues revealed that the risk of ER+, PR+, and ER+/PR+ breast cancer increases with higher levels of AMH.(23) A similar result was reported by Jalaeeifar and co-authors regarding a positive correlation between an increased risk of estrogen and progesterone receptor positive tumors, and higher levels of AMH in comparison with women without breast cancer. The proposed mechanism for the contribution of AMH in the development of breast cancer is the effect of AMH on epithelial cell proliferation, which is implemented through respective receptors.(24)

According to the above findings, AMH affects estrogen biosynthesis pathways through different mechanisms; and estrogen are the most crucial risk factor in the development of breast cancer, especially estrogen and progesterone receptor positive subtypes. Thus, there may be a correlation between AMH and the estrogen and/or progesterone receptors of breast cancer.

Although the level of AMH was slightly higher in ER-positive breast cancer in our study, this was not statistically significant; which may be due to the overall low number of patients.

Our study had some limitations. The sample size was small, and we could not stratify patients according to their age groups. It seems that with a larger sample size, an association between AMH and the ER or PR status of breast cancer might be found. We propose a similar study with a larger sample size to evaluate this association further.

**Conclusion**

The results of the present study indicated the level of Anti Mullerian hormone was not related to the hormone receptor status of breast cancer even though the AMH level were categorized.

**Declaration section:**
Conflict of interest: The authors deny any conflict of interest in any terms or by any means during the study.

Acknowledgments

The authors appreciate the valuable cooperation of Dr Shole Ebrahimpoor.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013. This study was approved by the Research Ethics Board of Alborz University of Medical Sciences.

CONSENT FOR PUBLICATION

Informed consent was obtained from each participant.

AVAILABILITY OF DATA AND MATERIALS

All relevant data and materials are provided with in manuscript.

FUNDING

None.

Contributors’ Statement Page:

Dr. Leila Haji Maghsoudi and Dr. Sadaf Alipour: conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript.

Dr. Bita Eslami and Dr. Ramesh Omranipour: Designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.

Dr. Azin Saberi and Dr. Amirmohsen Jalaeefar: Coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

References


Table 1 Characteristics of all participants and tumors, and AMH levels.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD/ Number (percentages)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.76 ± 4.99</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.17 ± 3.90</td>
</tr>
<tr>
<td>Age of Menarche (years)</td>
<td>13.39 ± 1.28</td>
</tr>
<tr>
<td>Parity (n)</td>
<td>1.53 ± 1.30</td>
</tr>
<tr>
<td>Breastfeeding time (month)</td>
<td>17.58 ± 18.06</td>
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<tr>
<td>AMH level before treatment (ng/ml)</td>
<td>3.19 ± 2.94</td>
</tr>
<tr>
<td>ER status Positive Negative</td>
<td>77 (77%) 23 (23%)</td>
</tr>
<tr>
<td>PR status Positive Negative</td>
<td>71 (71%) 29 (29%)</td>
</tr>
</tbody>
</table>

Table 2. Comparison of the categorized AMH levels according to ER and PR status.

<table>
<thead>
<tr>
<th>AMH level Categories (ng/ml)</th>
<th>ER Positive</th>
<th>ER Negative</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>23 (29.9)</td>
<td>6 (26.1)</td>
<td>0.78</td>
</tr>
<tr>
<td>1-3</td>
<td>21 (27.3)</td>
<td>8 (34.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;3</td>
<td>33 (42.9)</td>
<td>9 (39.1)</td>
<td></td>
</tr>
<tr>
<td>&lt; 1.2 [?] 1.2</td>
<td>24 (31.2)</td>
<td>7 (30.4)</td>
<td>0.95</td>
</tr>
<tr>
<td>&lt;0.7 [?] 0.7</td>
<td>14 (18.2)</td>
<td>3 (13)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AMH level Categories (ng/ml)</th>
<th>PR Positive</th>
<th>PR Negative</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>22 (31)</td>
<td>7 (24.1)</td>
<td>0.79</td>
</tr>
<tr>
<td>1-3</td>
<td>20 (28.2)</td>
<td>9 (31)</td>
<td></td>
</tr>
<tr>
<td>&gt;3</td>
<td>29 (40.8)</td>
<td>13 (44.8)</td>
<td></td>
</tr>
<tr>
<td>&lt; 1.2 [?] 1.2</td>
<td>23 (32.4)</td>
<td>8 (27.6)</td>
<td>0.64</td>
</tr>
<tr>
<td>&lt;0.7 [?] 0.7</td>
<td>14 (19.7)</td>
<td>3 (10.3)</td>
<td>0.26</td>
</tr>
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