DETERMINATION ANTI-MÜRELLIAN HORMONE AND VITAMIN D IN BREAST CANCER PATIENTS

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ABSTRACT

Anti-mürellian hormone and Vitamin D in patients with proved breast cancer have been estimated to find the possibility of using such parameters as biomarkers in the diagnosis of breast cancer patients compared to control. Sera were collected from (32) patients to estimate the levels of [AMH] and [VIT.D] at the period (March/2017-July/2017). All patients samples were female with the range age (30-41) years. The present study showed a significance difference in statistic results at (P<0.05) AMH and VIT.D concentration of patients compared to healthy control. The results also showed a positive correlation between [AMH] and [VIT.D] of patients which the value of correlation is (-0.91749) at level 0.05 and 0.01. The relation between AMH and VIT.D is inverse. Samples were collected from (9) patients before chemotherapy treatment and (23) samples were collected from other patients during chemotherapy treatment. According to t-test there was significant difference in both [AMH] and [VIT.D] between the previous groups at level (0.05). This study recommended to use [AMH] and [VIT.D] in early detecting of the breast cancer disease in women at fertile age.

KEYWORDS: Anti-mürellian hormone, Vitamin D, breast cancer, biomarkers.

INTRODUCTION

1.1 BREAST CANCER

Breast cancer is a kind of cancer that develops from breast cells[1] Signs of breast cancer may include a lump in the breast, a change, in breast shape, dimpling; of the skin, fluid
coming, from the nipple, or a red scaly, patch of skin. In those with distant spread of the disease, there may be bone, pain, swollen lymph, nodes, shortness of, breath, or yellow skin. Breast cancer, is the, leading cause of cancer in young women, accounting for 25% of cases diagnosed, by age 40. At diagnosis, young, breast cancer patients, face a myriad of. Challenges, including decisions on, preserving, fertility prior to cancer treatment. It is well established. Anti-müllerrian Hormone, Testing of Ovarian Reserve.

AMH or Anti-müllerian hormone is a protein. hormone produced by granulose cells (cells lining the egg sacs or follicles) within the, ovary. AMH can be measured in the blood at any time in the menstrual, cycle as it is stable throughout, the cycle. It is a marker for ovarian (egg), reserve. Also known, by various other names, is a glycoprotein hormone from the transforming growth, factor beta superfamily, whose key roles are in growth differentiation and folliculogenesis.

It is first. made in primary, follicles that advance from the primordial follicle stage. At these stages follicles are microscopic and cannot be seen by ultrasound.

AMH production is highest, in prenatal and, small, natal stages (less than 4mm diameter) of, development. Production, decreases and, then pause as follicles grow. There is nearly no AMH; made in follicles over, 8mm. That chemotherapy, used to treat; breast cancer, increase; damage to the ovarian reserve, or the quantity, and quality of, remaining oocytes resulting in loss of fertility and premature ovarian failure impacts ovarian reserve, suggesting an independent effect of malignancy on reproductive potential. As more female breast cancer patients consider future fertility, it is important to, determine; if ovarian’ reserve is affected, by cancer, prior to any, therapeutic, intervention. This will allow us to better understand the interplay between cancers and, more female breast cancer patients consider future.

Ovarian, reserve is measured by hormone and, ultrasound, biomarkers, including Anti-müllerrian hormone (AMH), follicle stimulating hormone (FSH), inhibin B (inhB) and natal’ follicle count; (AFC). Among these measures, AMH; is a good; candidate biomarker because it is relatively; stable across the menstrual cycle and has been associated with, reproductive outcomes; from; time to menopause to assisted reproduction (ART), responses in normal, and; infertile; women.
Vitamin D: is the name given, to a group of; fat-soluble; prohormones (substances that usually have little; hormonal activity by; themselves but that the body can turn into hormones). Vitamin D; aids the body; use calcium and phosphorus to make strong bones and; teeth. Skin exposed to sunshine can; create vitamin D, and vitamin D can also be obtained from certain foods. Vitamin D deficiency can cause a weakening, of the bones that is called rickets in children and osteomalacia in adults.\textsuperscript{[15]}

Two main composes of vitamin D that are substantial to humans are vitamin D2, or ergocalciferol, and vitamin D3, or cholecalciferol. Vitamin D2 is made/ naturally/ by plants, and vitamin D3 is made; naturally by the body; when skin islexposed to ultraviolet radiation, in sunlight. Both forms are converted to 25-hydroxyvitamin D in the liver. 25-Hydroxyvitamin D then travels through the blood to the kidneys, where it is further modified to 1, 25-dihydroxyvitamin D, or calcitriol, the; active form of vitamin D in the body. The most accurate; method of evaluating a person’s vitamin D status is to measure the level of 25-hydroxyvitamin D in; the blood.\textsuperscript{[16]}

Most; people get,at least some of the vitamin D they need; through sunlight’ exposure. Dietary sources include a few foods that naturally contain vitamin D, such as fatty fish, fish liver oil, and eggs. However, most dietary vitamin D comes from foods fortified with vitamin D, such as milk, juices, and’ breakfast cereals. Vitamin D can also be obtained through dietary complements. Vitamin D’s most; important role, in the; human body is to keep bones; healthy and strong by helping the; body; absorb calcium.\textsuperscript{[17]}

However, recent; research has pointed; to additional; ways that; vitamin D deficiency factors, into our overall; health, including; its role in chronic diseases like diabetes, autoimmune disease, obesity, cardiovascular disease and cancer, as well as an association with a higher risk of; both overall and cancer; mortality. Instead’ of only affecting cells that live inl the bone. Vitamin D is able to affect many different, types of cells in different organs in the body. The way it does this is by, turning genes within that cell, “on and off.” In other words, vitamin D affectsl the way a cell performs its function, and it can control the growth or the death of that cell.\textsuperscript{[18]}

Many studies have latterly concentrated on the relationship of vitamin D levels to cancer. Low Vitamin D levels have been correlated with a 30 to 50 percent increased risk of colon, prostate and breast cancer.\textsuperscript{[19, 20]}
The cancers for which the most human data are available are colorectal, breast, prostate, and pancreatic cancer. Numerous epidemiologic studies have shown that higher intake or blood levels of vitamin D are associated with a reduced risk of colorectal cancer. Taking into account that knock-out experiments have shown that vitamin D receptor null mice not only experience uterine hypoplasia but also impaired folliculogenesis, it might be hypothesized that vitamin D deficiency may have a detrimental effect on female ovarian reserve. This may be further supported by previous reports demonstrating that serum 25-OH Vitamin D levels correlates with antimullerian hormone (AMH) levels in women of advanced reproductive age.

In humans, the vitamin D receptor is present in many female organs, including the ovary, uterus, and placenta. The active form of vitamin D (calcitriol) has many roles in female reproduction. Bound to its receptor, calcitriol is able to control the genes involved in making estrogen. The uterine lining produces calcitriol in response to the embryo as it enters the uterine cavity, shortly before implantation. Calcitriol controls several genes involved in embryo implantation. Once a woman becomes pregnant, the uterus and placenta continue to make calcitriol, which helps organize immune cells in the uterus, so that infections can be fought without harming the pregnancy. Poor vitamin D status has been associated with certain pregnancy complications such as gestational hypertension and diabetes.

Women about to undergo in vitro fertilization (IVF) can provide valuable insight into the role of vitamin D, since it is possible to examine each aspect of reproduction, from egg development to implantation of the embryo. A recent study found that women with higher vitamin D levels were significantly more likely to achieve pregnancy from IVF compared to women with lower levels of vitamin D. This study was repeated in another IVF center, which confirmed a four-fold difference in pregnancy rates between vitamin D replete and deficient women. In another study looking at the recipients of donor eggs, vitamin D levels in the recipients were associated with clinical pregnancy, emphasizing that the critical role of vitamin D in pregnancy may be within the uterus. Though more research is needed, it appears that vitamin D levels are associated with IVF success, and that its most important role in reproduction may be at the uterine lining. No studies have yet evaluated whether giving vitamin D improves IVF outcomes. But the data that does exist at this point suggests that a role for vitamin D supplementation may exist as a means of improving one’s natural fertility both among the fertile and the infertile.
The research that implicates vitamin D deficiency as playing a role in not only fertility but in overall health is compelling, and it is backed by sound physiologic hypothesis. [28]

**MATERIALS AND METHODS**

**2.1. Ethical Approval**
A valid consent was achieved from hospital administration and from each female (patients and controls) before their inclusion in the study. For every female or her followers, the procedure had been informed before the samples were collected, making absolutely sure that they understood the procedure that was to be carried.

**2.2 SAMPLES COLLECTION**
Thirty two patients, (female only) with diagnosed breast cancer in different stages with range of age between [31-42] years, and thirty two healthy female as control with age range [29-41] years which they were volunteers. The patients were visitors for cancer center in Marjan Teaching Hospital in Hilla city.

**2.3 COLLECTION OF BLOOD AND SERUM PREPARATION**
By aseptic technique, five milliliter of venous blood was obtained by venipuncture; from all subjects (patients and controls). After cleaning of puncture site with antisepsis (70% ethanol alcohol), blood sample was obtained with sterile disposable syringe and dispersed into sterile tube. Then put the samples from 10 -20 minutes after that the components of blood were separated by centrifuge instrument to get the serum.

**2.4 PROCEDURE OF AMH TEST**
Enzyme Immunoassay for the quantitative Determination of Anti-mürellian hormone (UNION-AMH) in BIOTECH Manufactures is the kit that used to determine S.AMH. Expected values for females in age range [31-40] years are (0.14-10.40) ng/mL.

AMH was measured by Immunology analyzer (union instrument made in China).

**2.5 PROCEDURE OF VITAMINE D TEST**
MAGLUMI 25-OH Vitamin D (CLIA) Kit has been designed for the quantitative determination of 25-OH Vitamin D IN HUMAN SERUM. Normal value of VIT.D between (30-100) ng/mL.
The test has to be performed on MAGLUMI FULLY – Chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (Including Maglumi 600, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 3000 and Maglumi 4000).

3-RESULT AND DESCUSSION

Serum Anti-Mürllain hormone and Vitamin D levels were determined as follows: From sixty four samples were collected selectively at age between [29-42] years [32] samples of patients and [32] samples of healthy control. There were 9 patient samples collected before chemotherapy treatment and other samples were collected after chemotherapy treatment. Most results were offered as mean ± standard deviation (SD). The statistical significance was accepted when probability p<0.05 and p<0.01. Analysis were achieved employing the Microsoft Excel 2010.

3.1. AMH CONCENTRATION IN SERUM

The mean of AMH concentration in serum had shown decrease in patients of breast cancer in comparison to that healthy control.

Table 1: The level of [AMH] in both patients and control.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>COUNT</th>
<th>Mean±SD ng/ml</th>
<th>SD ng/mL</th>
<th>RANGE OF AGE</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH OF PATIENT</td>
<td>32</td>
<td>0.06±0.019007</td>
<td>0.019007</td>
<td>30_42 YEARS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>AMH OF CONTROL</td>
<td>32</td>
<td>6.4813±1.5</td>
<td>1.5</td>
<td>29_41 YEARS</td>
<td></td>
</tr>
</tbody>
</table>

According to descriptive statics and t. test two samples mean and Anova Two-factor without replication (between groups); there’s significance difference between mean of patients and control.

Anti-Müllerian hormone (AMH) high levels during chemotherapy. Treatment-induced amenorrhoea is a reversible phenomenon, but few data were available on long-term AMH changes in breast cancer.[29]

3.2 VIT.D CONCENTRATION IN SERUM

The mean of VIT.D concentration in serum had shown decrease in patient of breast cancer in comparison to that healthy control.
Table 2: The level of [VIT.D] in both patients and control.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>COUNT</th>
<th>Mean±SD ng/ml</th>
<th>SD</th>
<th>RANGE OF AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIT. D OF PATIENT</td>
<td>32</td>
<td>6.35±2.85431</td>
<td>2.85431 ng/mL</td>
<td>30_42 P&lt;0.05</td>
</tr>
<tr>
<td>VIT. D OF CONTROL</td>
<td>32</td>
<td>41.219±9.07607</td>
<td>9.07607 ng/mL</td>
<td>29_41</td>
</tr>
</tbody>
</table>

According to descriptive statistics and t.test two samples mean and Anova two- factors without replication there’s significance difference between mean of Vit. D of patients and control.

Experimental evidence has also suggested a possible association between vitamin D and cancer risk. In studies of cancer cells and of tumors in mice, vitamin D has been found to have several activities that might slow or prevent the development of cancer, including promoting cellular differentiation, decreasing cancer cell growth, stimulating cell death (apoptosis), and reducing tumor blood vessel formation (angiogenesis).\[^{30,33}\]

3.3 CORRELATION BETWEEN [AMH] AND [VIT.D]
The mean of [AMH] and the mean [VIT.D] had shown correlation between the both parameters.

Table 3: The Correlation Between [Amh] And [Vit.D]

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Mean±SD ng/ml</th>
<th>SD</th>
<th>CORRELATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>[AMH] PATIENT SAMPLE</td>
<td>0.06±0.019007</td>
<td>0.01 9007 ng/mL</td>
<td>-0.91749</td>
</tr>
<tr>
<td>[VIT.D ] PATIENT SAMPLE</td>
<td>6.35±2.85431</td>
<td>2.85431 ng/mL</td>
<td></td>
</tr>
</tbody>
</table>

According to data analysis of micro soft Excel 2010 and spss 16, there’s significant correlation between AMH and VIT.D concentration at level 0.05 and 0.01. The relation between AMH and VIT.D was inverse. New research finds that low levels of vitamin D influence AMH test results. In fact, if the individual has low vitamin D, patient could end up with a false positive for low AMH.\[^{34}\]

The next figure showed the correlation between [AMH] and [VIT.D].
3.4. EFFECT OF CHEMOTHERAPY TREATMENT

There were many patient samples collected before chemotherapy and other samples were collected after chemotherapy treatment. The next table showed the effect of chemotherapy on serum [AMH] of patients.

**Table 4: Effect of Chemotherapy on Serum [Amh] of Patients.**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>No. of cases</th>
<th>Mean±SD ng/ml</th>
<th>Std.S</th>
<th>T.test probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients without chemotherapy</td>
<td>9</td>
<td>0.0755±0.0174</td>
<td>0.0174</td>
<td>0.0062203</td>
</tr>
<tr>
<td>Patients with chemotherapy</td>
<td>23</td>
<td>0.06±0.01901</td>
<td>0.01901</td>
<td></td>
</tr>
</tbody>
</table>

According to t.test the previous table showed the difference in means between the two groups of patients, there were significant difference between them.

Anti-Müllerian hormone (AMH) levels fall during chemotherapy. Treatment-induced amenorrhea is a reversible phenomenon, but few data were available on long-term AMH changes in breast cancer.\[^{37}\]

**Table (5): Effect of Chemotherapy on Serum [Vit.D] Of Patients.**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>NO. of cases</th>
<th>Mean±SD ng/ml</th>
<th>Std.S</th>
<th>T.test probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient without chemotherapy</td>
<td>9</td>
<td>8.811±1.40128</td>
<td>1.40128ng/ml</td>
<td>0.0000367</td>
</tr>
<tr>
<td>Patient with chemotherapy</td>
<td>23</td>
<td>5.25416±2.90075</td>
<td>2.90075ng/ml</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>
According to t-test there were significant difference between patients treated with chemotherapy and patients treated without treatment.

Drugs, and particularly chemotherapy drugs can deplete vitamin D levels. And good vitamin D levels are essential to prevent many different illnesses from Alzheimer’s to Ricketts and bone weakness in old age.[35]

Importantly, chemotherapy can reduce vitamin D to dangerously low levels in the bodies of cancer patients, causing severe vitamin D deficiency, and vitamin D levels are crucial to a cancer patient. In February 2009, researchers from Roswell Park Cancer Center, studying patients with colorectal cancer, noted that vitamin D deficiency was linked to a heightened risk of the disease and lowered survival. They then showed that chemotherapy was associated with ‘severe vitamin D deficiency’. Each of three different chemotherapy treatments studied reduced plasma vitamin D levels down to 21.3 ng/ml, where 30-50 is an optimal range. The researchers recommended that colorectal cancer patients should be considered for ‘aggressive vitamin D replacement strategies.[36]

Study on breast cancer showed that ‘tailored vitamin D supplementation’ could keep plasma levels in the acceptable range in women with breast cancer even during chemotherapy.[37]

4. CONCLUSIONS

According to this study, we concluded there were a relationship between the concentration of Antimülline hormone and breast cancer patients, also there were a relation between the concentration of Vitamin D and breast cancer patients. Both of [AMH] and [VIT D] were lowered in breast cancer patients and their were inverse correlation between [AMH] and [VIT D].

Chemotherapy present with high effects on [AMH] and [VIT D] which resulted in low concentrations.

5. RECOMMENDATIONS

The parameters [AMH] and [VIT.D] can be used as biomarkers for breast cancer patients. Study other markers related to breast cancer for early detection and management of the disease.
Patients should be vitamin D replacement to keep plasma levels in the acceptable range in women with breast cancer even during chemotherapy.

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