Determination fo Anti-mürellian Hormone and Vitamin D in Breast Cancer Patients.

Nadia. H. Kadhum
Dept. Of Department of Laboratory and Clinical Sciences, college of Pharmacy, University of Babylon
nadia.h.kadhum@gmail.com

Abstract

Anti-mürellian hormone and Vitamin D in patients with proven 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 of (32) patients had been taken to estimate the levels of [AMH] & [VIT.D] at the period (March/2017-Julyl/2017) .All studied patients samples were female with the range age (30-41) years. The present results showed a significance difference in statistic results at (P<0.05)fo AMH & VIT.D concentration of patients compader to healthy control.

The results also showed a good correlation between [AMH] & [VIT.D] of patients which the value of correlation is (-0.91749) at level 0.05 and o.o1. The relation between A MH&VIT.D is inverse.

There were(9) patients detcelloc the samples before chemotherapy treatment and(23) patients rieht samples when they treated with chemotherapy. According to t-test there was significant difference in both [AMH] & [VIT.D] between the previous groups at level (0.05).

So this study recommended to use [AMH] & [VIT.D] in early detecting of the breast cancer disease in women at fertile age .

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

الخلاصة

بينت الدراسة الحساب الكمي لكل من هرمون AMH وفيتامين D في مصل دم مرضى سرطان الثدي وقد تمت ألمقارنه مع مجموعة السيطرة من الأشخاص الأصحاء . وكان الهدف من الدراسة اعتماد كلا من العاملين كمؤشرات حيوية مهمة في تشخيص مرض سرطان الثدي .

لقد تم إجراء البحث في الفترة ألزمنيه الممتدة بين آذار /2017وتموز/2017 تم خلالها جمع 32 عينه من مصل الدم لمرضى سرطان الثدي من الإناث للأعمار من(30−42) عاما أما مجموعة السيطرة فكانت من الإناث الأصحاء من الأعمار(29−41) عاما بواقع 32 عينه كذلك . أظهرت النتائج الحالية وجود فروق ذات دلالة إحصائية في نتائج الإحصاء عند ألمقارنه بين قراءات المرضى والأصحاء لكل من هرمون AMH وفيتامين D عند P<0.05 .

وأظهرت النتائج علاقة مترابطة جيده بين هرمون AMH وفيتامين D في مصل دم (0.91749−) عند 0.01 و 0.05 المرضى حيث تعتبر العلاقة بينهما عكسية.

وتم اخذ 9عينات قبل حصولها على العلاج الكيماوي وبعد حصولها عليه وكان هناك فرق معنوي بين القراءات قبل وبعد الحصول على العلاج الكيماوي عند0.05.

وطبقا لهذه الدراسة فأني أوصي باستخدام هرمون AMH وفيتامين D في الكشف المبكر عن سرطان الثدي للنساء في عمر الخصوبة

الكلمات المفتاحيه: مؤشرات حيوية ،HMA،فيتامين Dسرطان الثدي.

Introduction

1.1 BREAST CANCER:

Breast cancer is a kind of cancer that develops' from breast cells. Breast cancer is cancer that 'develops from breast tissue(NCI. , 2014).

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 (NCI., 2014).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(Dev, 2012) , young breast cancer patients face a myriad of Challenges, including decisions on preserving fertility prior to cancer treatment. It is well established Anti-mürellian Hormone Testing of Ovarian Reserve (Taguchi ,2010).

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 super family, whose key roles are in growth differentiation and folliculogenesis. .(Richard , 2008)

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 .(Warne & Fairley, 1973; Su & Sammel , 2010)

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 incurs damage to the ovarian

reserve, or the quantity and quality of remaining oocytes (Warne & Fairley , 1973) resulting in loss of fertilityand premature ovarian failure impacts ovarian reserve. (Partridge , 2008).

, 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 patient Partridge AH, Gelber s consider future.

Ovarian reserve is measured by hormone and ultrasound biomarkers, including Anti-mürellian hormone (AMH), follicle stimulating hormone (FSH), inhibin B (inhB) and natal follicle count (AFC) (Gelber 2008). Among these measures, AMH is a good candidate biomarkerbecause it is relatively stable across the menstrual cycle and has been associated with reproductive outcomes from time to menopause to assisted reproduction (ART) responses innormal and infertile women. (Broekmans, 2007)

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.

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 is exposed 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.

Vitamin D's most important role in the human body is to keep bones healthy and strong by helping the body absorb calcium.

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 in 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 affects the way a cell perform sits function, and it can control the growth or the death of that cell.

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.(Otten & Hellwig, 2006; Michaelsson, 2010)

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. (Kinuta .2000; Yoshizawa, 1997). 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.v 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.

Hypothesis (Gandini, 2011; Woolcott, 2010; Jenab, 2010; Ozkan, 2010; Nikolaos 2008).

2.Matrials & 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 Sample Collection

Thirty two patients, female only with diagnosed breast cancer in deferent stages with range of age between (31-42) years and thirty two healthy female in control with na age range (29-41) years which they are volunteers. The patients were visitors for cancer center ta Marjan Teaching Hospital in Hilla city.

2.3 Collection Of Blood And Serum Preparation:

Blood samples were obtained from patients and control group. The vein on the front of elbow forearm is all most employer. The tourniquet was put around the arm and applied procedure on the area vein with iodine disinfected then drawn 5mL of blood. Then put the samples from 10 -20 minutes after that the components of blood were separated by centerfuge instrument to get on serum.

2.4 Procedure Of Amh Test:

Enzyme Immunoassay for the quantitative Determination of Anti-mürellianhormone (UNION-AMH) in BIOTEECH 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-Resulte And Descussion

Serum Anti-Mürllain hormone and Vitamin D levels were determined as follows:
From 64^{th} 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 taking chemotherapy and other samples were collected after chemotherapy treatment .Most results are offered as mean ± standard deviation (SD).The statistical significance was accepted when probability $p<0.05$ and $p<0.01$ .Analysis si 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:

GROUPS	COUNT	MEAN ± SD ng/mL	RANGE OF AGE	
AMH of patient	32	0.06 ± 0.019007	30_42 YEARS	P<0.05
AMH of Control	32	6.4813 ± 1.5	29_41 YEARS	

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

Anti-Müllerian hormone (AMH) levels fall during chemotherapy. Treatment-induced amenorrhea is a reversible phenomenon, but few data are available on long-term AMH changes in breast cancer.(Institute of Medicine .National Academy Press, 2010).

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.

GROUPS	COUNT	MEAN± SD	RANGE OF AGE	
VIT. D of Patient	32	6.35 ± 2.85431 ng/mL	30_42	P<0.05
VIT.D of Control	32	41.219± 9.07607 ng/mL	29_41	

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). (Cranney, 2007; Holick, 2006. Anne-Sophie, 2014; Thorne, 2008) .

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]

GROUPS	MEAN± SD	CORRELATION
[AMH] PATIENT SAMPLE	0.06 ± 0.01 9007 ng/mL	-0.91749
[VIT.D] PATIENT SAMPLE	6.35 ± 2.85431 ng/mL	

According to data analysis of micro soft Excel 2010 and spss16 ,there's significance correlation between AMH & VIT.D concentration at level 0.05 and o.o1. The relation between AMH &VIT.D is inverse. New research finds that low levels of vitamin D influence AMH test results. In fact, if the individual has low vitamin D, the individual could end up with a false positive for low AMH.(Moreno, 2005).

The next figure shows the correlation between [AMH] & [VIT.D].

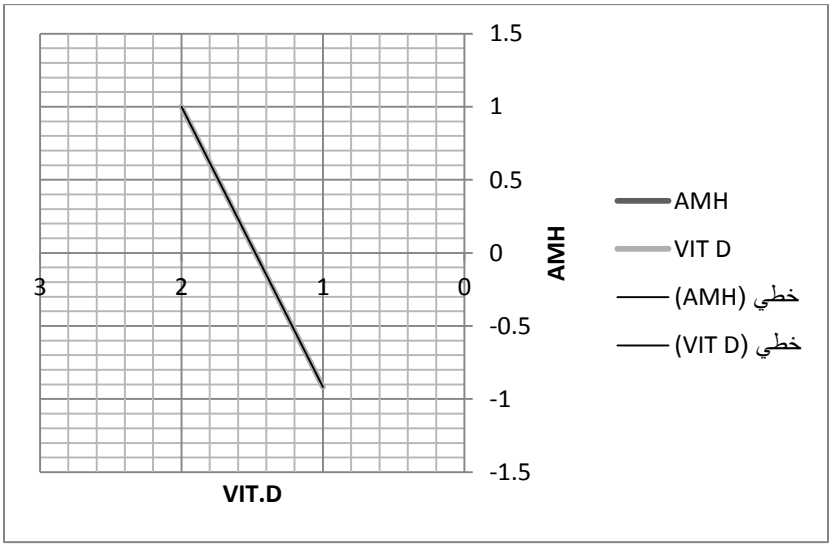

Figure No 1: Correlation Between [AMH] & [VIT.D] OF PATIENTS

3.4.Effect Of Chemotherapy Treeatment:

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

Table 4: Effect of chemotherapy on serum [AMH] of patients

GROUPS	No.of cases	MEAN± ng/ml	Std.S	T.test probability	
Patients without chemotherapy	9	0.0755 ±	0.0174	0.0062203	P<0.05
Patients with chemotherapy	23	0.06 ±	0.01901		

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

Anti-Müllerian hormone (AMH) levels fall during chemotherapy. Treatment-induced amenorrhea is a reversible phenomenon, but few data are available on long-term AMH changes in breast cancer.(Volpe, A Hum repro'd Update, 2010; 16(2): 113–130).

Table 5:effect of chemotherapy on serum [VIT.D] OF PATIENTS:

GROUPS	NO.of cases	MEAN±	Std.S	T.test probability	
Patient without chemotherapy	9	8.8111±	1.40128ng/ml	0.0000367	P<0.05
Patient with chemotherapy	23	5.25416±	2.90075ng/ml		

According to t.test there were significance difference between patients not treated with chemotherapy and patients treated with chemotherapy.

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. (Holt , 2002) .

Importantly, chemotherapy can reduce vitamin D to dangerously low levels in the bodies of cancer patients, causing severe vitamin D deficiency. And this happens at exactly the moment good 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.

A 2016 study on breast cancer by Dr. William Jacot and colleagues from Montpelier, France showed that 'tailored vitamin D supplementation' could keep plasma levels in the acceptable range in women with breast cancer even during chemotherapy. This followed 2012 research.

4. Concolusion

According to this study, there were a relation between the concentration of Antimülline hormone and breast cancer patients, also there were a relation between the concentration of Vitamin D and breast cancer patient.

Both of [AMH] and [VIT D] are lowered in breast cancer patient.There is an is inverse correlation between [AMH] and [VIT D].

Chemotherapy affected on [AMH] and [VIT D] which lowered them concentrations.

5.Recomendation

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.

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