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Determination of genetic polymorphism of breast cancer

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



صدق الله العلي العظيم

(الملك:1)

Dedication

Before we go, we offer our highest thinks,
appreciation gratitude and love ...

To those who bore the most sacred message in life,
To those who paved the way for us the path of science
and knowledge, to all our distinguished professor, and
especially to the thanks and appreciation of

Dr .Dina M. R. AL-khafaf

To my angel in life to the meaning of love and to the
meaning of tenderness and devotion....My dear father

To the one who was the secret of my success and the
tenderness of a surgical balm to... dear mother

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1.Introduction

Breast cancer is the type of high-risk cancer, which the most common invasive cancer in women,(1) and the second main cause of cancer death in women, after lung cancer. Where the happening of this cancer in the West is very much, compared to the east, where find a few recording of infected this cancer. And the reason for this is due to the consciousness, of health and culture in those areas , in addition to the nature of social relations, which one of the most important risk factors in the infect cancer. Where it recorded the highest reading of the breast cancer in the United States (U.S.) there are around 3.1 million breast cancer survivors. Where the chance of any woman dying from breast cancer is around 1 in 37, or 2.7 percent. About 10.4% of the breast cancer include of all cancer happening among women in the world. (2) During the period of 2000 to 2009 has raised infected of breast cancer in all ages specially the age group between 60-69 in Iraq , so it was affecting younger age groups than the developing countries as Iraq and Egypt. (3) Where cases were recorded in west of Iraq breast cancer among females is low but it is relatively higher in younger age group compared to other studies. (4) Where it was noted some of studies in Kirkuk that the age of breast cancer patients is ranged 20 to 80 years with an average of 47 years. (5) Including the 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, a newly inverted nipple, or a red or 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. (6)

Where including many risk factors for being infected by breast cancer female as obesity, irregularity of physical exercise, drinking alcohol, ionizing radiation, older age, prior history of breast cancer, and family history. About 5–10% of cases are due to genes inherited from a person's parents (7), breast cancer including two types BRCA1 and BRCA2 among others. Breast cancer most commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk. Cancers developing from the ducts are known as ductal carcinomas, while those developing from lobules are known as lobular carcinomas (8). In addition, there are more than 18 other sub-types of breast cancer. Some cancers, such as ductal carcinoma in situ, develop from pre-invasive lesions. (9)

Smoking tobacco appears to increase the risk of breast cancer, with the greater the amount smoked and the earlier in life that smoking began. The higher the risk in those who are long-term smokers, the risk is increased 35% to 50%. in additionally Environmental and genetics factors raise the incidence of breast cancer by activation or inactivation of certain genes that leading to neoplastic transformations, or abnormal cell growth.(10)The development of molecular biology was one of the greatest achievements in biological science in the century XX. The discovery of Polymerase Chain Reaction (PCR) brought enormous benefits and scientific developments such as genome sequencing, gene expressions in recombinant systems, the study of molecular genetic analyses, including the rapid determination of both paternity and the diagnosis of infectious disease. (11)

Recently, a technological -2- vation of PCR, known as Real-Time PCR, has become increasingly important in clinical diagnostics and research laboratories due to its capacity for detection of infection, disease and other types of cancer on molecular level .(12) In human cancer, oncogenes such as (Her-2) have been identified and used as molecular markers.(Her-2) is one of the most frequently studied BC genes due to its over expression observed in 20–30% of aggressive cases. High levels of (Her-2) have also been found in several ovarian and endometrial tumors.(13) Clinical studies have shown that (Her-2) gene up regulation and (Her-2) protein over production predicts poor prognosis in BC patients lacking estrogen and progesterone receptors, inducing a high rate of cell proliferation and tumor chemotherapy resistance .(14) The discovery of (Her-2) gene over expression in BC tissues and its association with aggressive clinical behavior has generated diagnostic interest by using (Her-2) gene expression as a predictive marker for Herceptin treatment response. Immunohistochemistry (IHC) is the first methodology selected for (Her-2) protein evaluation since it is the most economical; however, it is not the most precise. (15)

(11) This work was aimed to determining genetic polymorphism for women with breast cancer in AL-Diwanyia city.

2. Materials and Methods

2.1 Sample collection

Five ml of blood sample were obtained from 10 infected female, age ranged between 30 – 50 year and 5 of healthy female, age ranged between 36- 55 years. Sample of blood were collected in EDTA tube and stored under freezing condition (-20°C) for later DNA extraction.

2.2 DNA extraction

DNA extracted from blood sample according to instructions of geneaid kit as follow briefed procedure:

1. 200µl of blood transferred to 1.5 ml tube.
2. 20µl of proteinase K was added and pipetted 5 times then incubated for 5 mints.
3. 200µl of GSB buffer was added with shaking vigorously then incubated for 5 mints.
4. 200µl of absolute ethanol was added and mixed.
5. GD column was placed in 2 ml collection tube
6. Mixture was transferred into GD column and centrifuged for 1 mints.
7. 400µl of W1 buffer was added and centrifuged for 20 sec.
8. 600µl of wash buffer was added and centrifuged for 30 sec.

9. 100 μ l of elution buffer was added and let stand for 1 min then centrifuged for 30 secs to elute.

2.3. Preparation and running of gel electrophoresis

1. Agarose solution (1%) was prepared by adding 1 g of agarose to 100ml of 0.5 X TBE.
2. Stirred of solution on hot plate until dissolving or (use of microwave for 1min) of agarose and clearing, then cooled to 45oC
3. Ethidium bromide (2.5 μ l) was added.
4. About 2.5 cm from any end of tray the comb was vertically fixed.
5. the tray was filled (about 6mm depth) by agarose solution.
6. Solidification of gel was allowed at 25oC for 20min.
7. Fixed comb was released.
8. Gel in the tray was placed in chamber of electrophoresis.
9. Chamber of electrophoresis of was submerged by electrophoresis buffer to cover the gel to depth about 1mm.
10. Two μ l loading buffer was added to each DNA sample with mixing.
11. Sample (20 μ l) was loaded carefully by micropipette in agarose wells.

12. leads was attached red to red, black to black in voltage supply unit.

13. Running was performed at 5V/cm until separation of DNA fragments.

2.4. Master Mix ingredients 2X

iNtRON^os Maxime PCR PreMix Kit (i-Taq) 2X concentrated solution contain of:

1. Taq DNA polymerase (5 unit/ μ l). One unit (U) of Taq polymerase is defined as the amount of enzyme required for catalyze the incorporation of 10 nanomoles of deoxyribonucleotides into acid-insoluble material in 30 minutes at 74°C.

2. 2.5 mM of each dNTP (dATP, dCTP, dGTP and dTTP).

3. Reaction buffer (10x).

4. Gel loading buffer (1x).

For each 50 μ l reaction of PCR was performed as follow:

1. Thawing of PCR Master Mix (2X) then gently vortex and briefly centrifuge.

2. Above ingredients was placed in thin walled tube of PCR on ice and add the following:

DNA template	4 μ l
Forward primer	2.5 μ l
Reverse primer	2.5 μ l
Nuclease-free water	To 41 μ l
Total volume	50 μ l

3. The samples was spin down and gently vortex.

2.5 RAPD Amplification

RAPD polymerase chain reactions were done on LABNET cyclor machine using five different arbitrary primers-OP Operon(OPA-20, OPB-01, OPD-01, OPAB-14 and OPZ-05). Amplification conditions were 35 cycles of 94°C /four minutes, 94°C / 30 seconds, 36°C / one minute, and 72°C / two minutes with a final extension step of 72°C /eight minutes, PCR products were run on 1.8% agarose gel and stained with Ethidium bromide then analyzed using UV transilluminator, standard DNA ladder 100bp (Bioneer, South Korea) was used. RAPD analysis was employed for each primer separately and repeated three times using the same conditions to confirm the results, the presence of a DNA bandon the gel was represented as “1” and its absence was represented as “0”for later calculation

3. Results and discussion

Two different random primers were screened in an attempt to detect a possible genetic polymorphism between (10) females infected with breast cancer and (50) healthy females in Al-Qadisiyah region as illustrated in table (1).

Table 1. arbitrary primers used for RAPD diversity experiments

Primer name	Sequence
OPD-01	ACCGCGAAGG
OPZ-O5	TCCCATGCTG

Table 2. The numbers of DNA bands generated in samples using primer

Sample number Number of amplified bands Patient

Sample number	Number of amplified bands
Infected NO.1	6
Infected NO.2	6
Infected NO.3	6
Infected NO.4	6
Infected NO.5	6
Healthy NO.1	4
Healthy NO.2	4

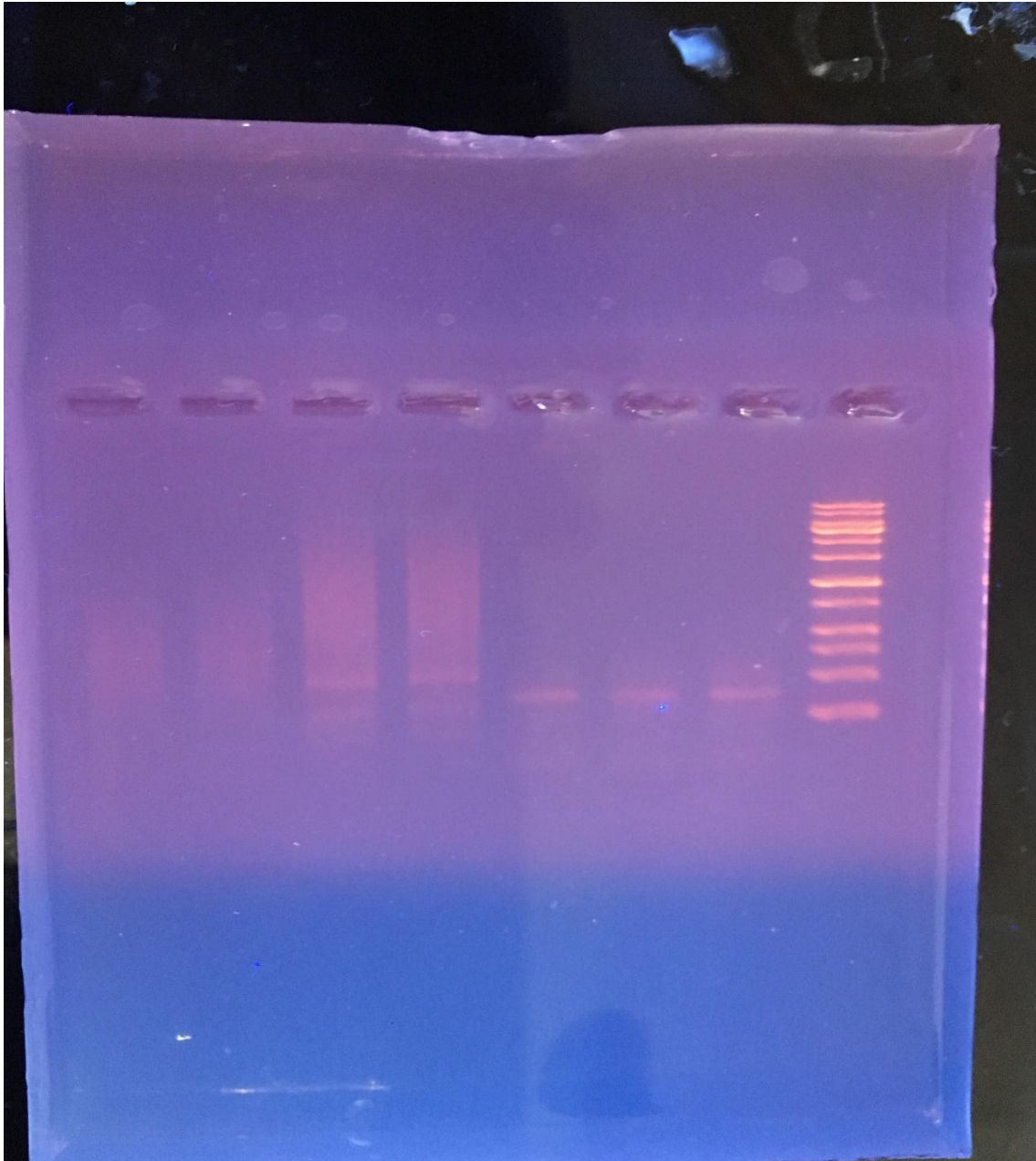


Figure1. RAPD Profile generated by primer OPZ-05 :
Lanes1-5 Infection' samples; lanes 6-7 Healthy samples and
lane A, Molecular weight (100-bp ladder) on 1.8% agarose
gel electrophoresis. Arrows indicate the polymorphic
distinguished bands

There are many traditional methods and protocols were using by researcher and laboratory specialists to detect, diagnosis breast cancer and classify it into the knowing types, but these methods are consuming more time and there results not very accurate comparing with the new molecular methods do, so the accurate and rapid diagnosis of breast cancer is a difficult problem in Iraq and several modern techniques based on molecular principles of breast cancer in Iraq and outside toward early diagnosis, classification of breast tumor as benign or malignant, prognosis and follow-up tumor-therapy response being adopted by many researchers in Iraq in special PCR-based methods^{22,23} and for data purposes^{24,25}

RAPD it's the most common and used molecular technique that using for analysis which has been applied to detect the genetic diversity among genomes or genes, due to its merits as fast, low-cost and applicable in many laboratories^{26, 27}. Applying RAPD analysis has been used in a lot of studies in the field of genetic instability in breast cancer²⁶⁻²⁸ and in lung cancer¹⁷. The ability of RAPD analysis to detect the genetic instability which represents the differences between normal and malignant cells that may include insertion, deletion and alternation in the oncogenes or suppressor genes that could cause cancer¹⁶.

4. References

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