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Genetic Polymorphism in Iraqi Females Diagnosed with Breast Cancer Using Random Amplification of Polymorphic DNA Technique

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Abstract : Objectives: This study aims at detection of a possible genetic alternation in the genomic DNA of Iraqi females identified with breast cancer and the opportunity of applying the potential amplified DNA fragment(s) as a molecular probe in future studies and applications. **Methods**: Blood samples were collected from ten female patients with breast cancer and ten healthy females, DNA was extracted from each sample then Random Amplification of Polymorphic DNA Technique has been conducted with five different arbitrary primers (OPA-20, OPB-01, OPD-01, OPAB-14 and OPZ-05). **Findings**: RAPD analysis with primer OPZ-05 could detect two polymorphic DNA bands in the genome of healthy females, but these bands were absolutely absent in the genomic DNA of patients. **Novelty /Improvement**: these two novels amplified DNA bands could be of the promising application as a molecular probe for detecting the absence of breast cancer mutation (alternation) sits in the suspected females in future.
Keywords: Breast cancer, Diversity, Genetic marker, RAPD, PCR,Qadisiyha.

Introduction

Breast cancer is a widely spread cancer in women around the world[1].In Iraq, breast cancer comes first among other types of cancer in females in Iraq and leads to death according to Iraqi Cancer Registry/Ministry of Health in the year of 2009[1,2].Breast cancer a big medical challenge in Iraq which needs an increasing public awareness and governmental programs for detection, monitoring and treatment of it[3]. Many researchers have studied the breast cancer with a lot of molecular biology techniques targeting early diagnosis, progress and treatment; such as Allele-Specific Amplification[4],Amplified Fragment Length Polymorphism (AFLP)[5], loss of Heterozygosity (LOH)[6],Restriction Fragment Length Polymorphism (RFLP)[7],determination of single nucleotide polymorphism[8-10], simple sequence repeat (SSR)[11], short tandem repeat (STR)[12], Variable Number Tandem Repeats[13] and Random Amplified Polymorphic DNA(RAPD)[14, 15].

RAPD analysis which is semi-quantitative method widely used to detect the polymorphism in the genome, taxonomy and carcinogenesis and genotoxicity researches[16].Many researchershave used RAPD analysis to study polymorphism in lung cancer[17], human lymphoblastoid cells[18], Gastrointestinal stromal tumors[19],Hepatic cancer[18]. RAPD analysis is easy and applicable in the most of the laboratories with basic equipment and instruments[16].

The aim of the current study is to employ the RAPD analysis to detectany possible genetic marker that might be used further to discriminate between the normal and tumor genomic-related breast cancer mutations in

an attempt to establish a comprehensive understanding of breast cancer detection, prognosis, and treatment in Iraqi female patients.

2.Materials and Methods

2.1Patients

This study was conducted on 10 females with malignant breast cancer and 10healthy females(control), aged between (20-69) years. Blood samples have been collected from patients and healthy individuals attending AD Diwaniyha Teaching Hospital-Oncology ward. About four milliliters of blood withdrew from each patient and healthy individual and placed into Ethylenediaminetetraacetic acid (EDTA)-tubesthen transported to the laboratory under cooling circumstances as soon as possible.

2.2 DNA Extraction

DNA was isolated from peripheral blood using Favor Prep Blood Genomic DNA Extraction Mini Kit (South Korea) according to the manufacturer's instructions at Medical Biotechnology Department/College of Biotechnology/ University of Al-Qadisiyha and stored at -20˚ C for Polymerase Chain reaction.

2.3 RAPD Amplification

RAPD polymerase chain reactions were done on LABNET cycler 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 calculations.

3. Results and Discussion

Five different random primers were screened in an attempt to detect a possible genetic polymorphism between ten females identified with breast cancer and ten normal females in Al-Qadisiyha regionas illustrated in table (1).

Table 1.arbitrary primers used for RAPD diversity experiments

Primer name	Sequence
OPA-20	GTTGCGATCC
OPB-01	GTTTCGCTCC
OPD-01	ACCGCGAAGG
OPZ-05	TCCCATGCTG
OPAB-14	AAGTGCGACC

Table 2. The numbers of DNA bands generated in samples using primer "OPZ-05"

Sample number	Number of amplified bands
Patient No.1	12
Patient No.2	12
Patient No.3	12
Patient No.4	12
Patient No.5	12
Patient No.6	12
Healthy No.1	14
Healthy No.2	14

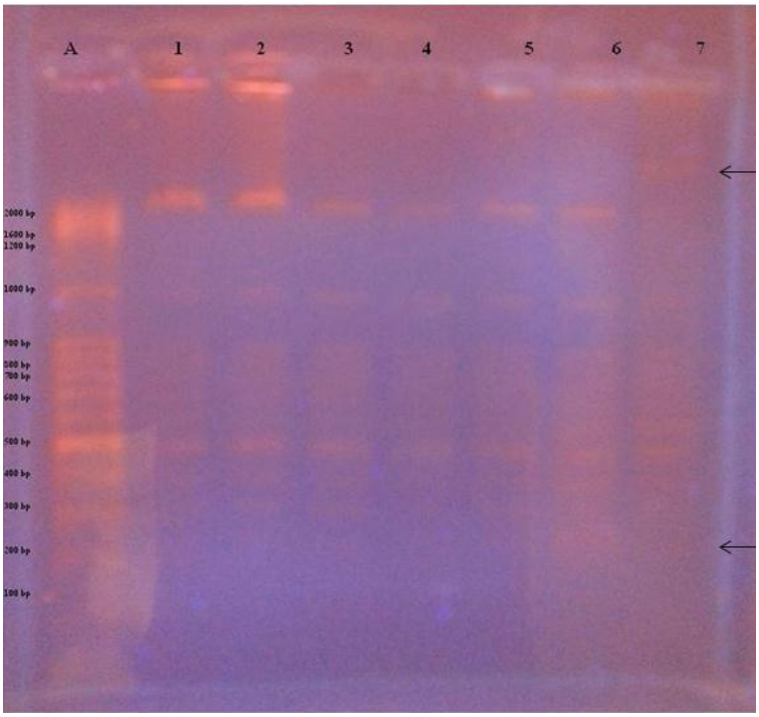

Figure1. RAPD Profile generated by primer OPZ-05: Lanes1-5 patients' samples; lanes 6-7 controls samples and lane A, Molecular weight (100-bp ladder) on 1.8% agarose gel electrophoresis. Arrows indicate the polymorphic distinguished bands

The results revealed that primer OPZ-05 could detect the polymorphic bands as a genetic 200polymorphism among patient and healthy samples figure (1), while the other four primers showed no amplification. All the samples shared 12 monomorphic bands with molecular weights ranged approximately from 2000bp to 400bp table (2). And the electrophoresis on agarose gel showed also two distinguished bands as polymorphic bands as they have sizes of > 2000bp and the other about 250 bp in the genomic DNA of healthy individuals. The primer (OPZ-05) efficiency was0.022 and its power discriminatory percentage was 2%and the calculations were done according to[21].

There are many conventional 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 considered as time-consuming and not very accurate as the new molecular methods do, so the accurate and rapid diagnosis of breast cancer is a huge problem in Iraq[1]andseveralmodern 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].

One of the most widely used molecular techniques is RAPD 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 cancer[26-28] and in lung cancer[17]. 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 cancer[16].

Our findings in the current study could verify two novel polymorphic bands as genetic markers obtained by RAPD analysis between the malignant and normal cells in both patients and healthy female samples using primer OPZ-05;one of these band has molecular weight > 2000bp and the other is about 250 bp.

These two bands could be as a result of genetic alternation in the genome of patients, which is similar to the findings[21].

Our outcomes of determining novel genetic markers using primer OPZ-05 could be or further importance if it could be purified, cloned and sequenced to pinpoint the exact the nucleotide alternation as it could be used a molecular probe for future diagnosis , prognosis and following-up treatment of breast cancer in Iraq.

4. Conclusion

These two novel DNA bands that discovered by the current study using RAPD primer OPZ-05 could be possible genetic markers in the genomic DNA of females with breast cancer while they were absent in genomic DNA of healthy females. The amplified bands could be valuable as it could be sequenced to be used as a molecular probe for diagnosis and monitoring breast cancer in the area of Iraq.

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