



Detection of DNA Hypermethylation in Blood Samples of Breast Cancer Iraqi Patients

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Abstract: In cancers, epigenetic alterations such as DNA methylation are involved in the earliest phases of tumorigenesis. Aberrant DNA methylation has been recognised as one of the most common molecular abnormalities in breast cancer and is considered a candidate biomarker of diagnosis. In this study DNA methylation was examined whether it could be correlated with breast cancer in Iraq using two classical restriction enzyme pairs *HpaII* – *MspI*. Thirty women with breast cancer (BC) Iraqi patients and 25 apparently healthy individuals were subjected to this study. Results show that 22 out of 30 (73.3%) Iraqi BC patients have DNA hypermethylation when compared with control group, which indicate that DNA hypermethylation may play an important role in breast cancer cases in Iraq. Results of this approach support the idea that DNA hypermethylation enable breast cancer prediction and using of this biomarker for prognostic and therapeutic goal. So, this research recommends for an extensive Iraqi study include larger sample size to confirm this important result.

Key words: Breast cancer, DNA methylation , early detection.

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Introduction

DNA Methylation occurs on cytosine residues of the CpG dinucleotides in DNA. About 3–6% of cytosines are methylated in mammals. Approximately 70–80% of CpG sites in the human genome are methylated. These islands extend about 0.5–3 kb, occur on average every 100 kb in the genome and are often found in the promoter area of genes. DNA Methylation had a critical role in gene expression via several routes. First, methylation of a CpG island is associated with loss of transcription of the target gene. Secondly, 5-methylcytosine (5mC) residues are susceptible to deamination to thymine, resulting in a transition mutation.

Thirdly, it is possible that inappropriate hypomethylation could be linked to deregulated gene induction (1, 2). Hypermethylation of gene promoters has been detected in the serum/plasma DNA of breast cancer cases so it is an early detection marker of breast cancer (3, 4). Breast cancer (BC) is the most common cancer among women and one of the leading causes of death all over the world (5, 6). Breast cancer is a multifactorial disease, the molecular background behind its development is not well understood, but is correlate with the sequence of genetic aberrations, leading to expanded gene-expression changes in breast tumor cells. Many studies evaluated specific molecular prognostic markers for breast cancer such as Human

Mammaglobin 1 (MGB 1) and cytokeratin-19 (CK19) gene expression which might be of value for detection of breast cancer, discrimination between benign and malignant breast tumors (7,8). Genetic mutations along with epigenetic alterations contribute to these changes. In breast cancer, epigenetic such as DNA hypermethylation has shown promise as a potential biomarker for early detection, therapy monitoring, and assessment of prognosis or prediction of therapy response (9). Over the past decade, aberrant DNA methylation has been recognised as one of the most common molecular abnormalities in BC (10, 11). Methylation of certain genes has been related to clinical and pathological characteristics of breast tumours, and is considered a biomarker of diagnosis (12). The objective of this study was to find the correlation between the DNA hypermethylation and breast cancer progression in Iraqi.

Materials and Methods Patients and clinical samples

Thirty blood samples were collected from Iraqi breast cancer women patients, who were diagnosed by physician of the Baghdad Medical city, Nuclear Medicine Hospital /Baghdad, Iraq during the period from October 2015 to August 2016. The personal information for each patient was achieved, which included: name, age, and the pathological data, including histologic tumors stage were obtained from the

clinical records of the B.C patients and authorized by their physicians and 25 blood samples of apparently healthy control were collected.

DNA extraction

For Molecular genetic studies / DNA extraction, blood samples (3ml) were collected in EDTA tubes from 30 Iraqi BC patients, and 25 apparently healthy subjects. Total genomic DNA extracted from the whole blood was applied using genomic DNA extraction kits (Geneaid, China). Then, DNA concentration and purity were measured by nanodrop . DNA bands were visualized using UV light after electrophoresis in a 1% (w/v) agarose gel (13).

Methylation Detection by Restriction Enzyme

DNA extracted from blood samples was digested and detected for DNA methylation using restriction enzyme *MspI* (nonsensitive to DNA methylation) and *HpaII* (sensitive to DNA methylation) which was mix with DNA samples as in (Table 1) and incubated for 4 hr at 37°C in the appropriate buffer. After incubation the mixture of restriction enzyme introduced to agarose gel electrophoresis and visualization products, 5 µl of each mixture with 2 µl loading dye was electrophoresed in 2 % agarose (14).

Table (1): Mixture of restriction enzyme and DNA samples .

| Component | Quantity (μl) |
|------------------|---------------|
| DNA | 10 |
| Buffer | 2 |
| Enzyme (10 U/μL) | 0.5 |
| D.W | 7.5 |
| Total volume | 20 |

Results and Discussion

Patients and clinical samples

The mean age of patients with breast cancer under study was 50.5 ± 9.85 years old ranging from 30-68 years old. Results of this study comes with finding of increasing risk of breast cancer with age in addition to using of menopausal hormone therapy (15, 16). This may be attributed to many factors, such as environmental factors, the nutrition, low exercise, poor health education. The exposure to a high dose of depleted uranium may be one of the reasons for the increased breast cancer risk in the Iraqi community. Furthermore, there are no

national screening programs for the breast cancer patients Iraq.

DNA Extraction

The result showed that, the full amount of DNA obtained (Figure 1) using genomic DNA purification kits (Geneaid, China) was very efficient method for DNA extraction .The extracted DNA concentrations from patients' blood samples and controls range were between 114-117 ng /μl . For each sample the concentration of DNA per volume was taking into consideration. DNA purification ranging between 1.8 – 2.0 since good yields of genomic DNA were obtained (Figure 1).

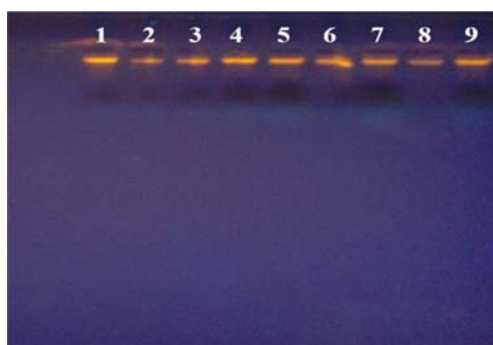


Figure (1): Genomic DNA bands visualized under UV after staining with ethidium bromide on 1% agarose gel at 1 volt/cm² for 30 min.

Methylation detection by restriction enzyme

In this study two classical restriction enzyme pairs (*HpaII* - *MspI*) which recognize specific sequence C↓CGG From 5'→3' and 3'→5' located in promoter were used . *MspI* non sensitive to DNA methylation (has an ability to identify specific sequences C↓CGG and cut DNA if methylated or unmethylated) , for this the DNA appear as smear in the gel under UV light . However, *HpaII* is unable to cut DNA when the internal cytosine is methylated (has an ability to identify the specific sequences but couldn't cut methylated DNA), so the methylated DNA appear as specific band in the gel under UV light. This property makes *HpaII-MspI* pair to a valuable tool for rapid methylation analysis. (17). Extracted DNA from the blood samples of Iraqi BC patients and control individuals were treated with two

enzymes *MSPI* and *HPaII* .The DNA samples treated with *MspI* appears as smear in the gel under UV light for patients and control samples because *MSPI* cut DNA if methylated or unmethylated . While in *HPaII* treatment, DNA samples which appears as specific band for BC patients indicate the presents of DNA methylation as *HpaII* sensitive to DNA methylation (Figure 2). Twenty two (73%) DNA samples of BC Iraqi patients revealed DNA methylation. In control group, DNA appears smear in the gel under UV light when treated with *MSPI* and could found as a less or not clear DNA band in the gel when treated with *HPaII* under UV light , this means that DNA of apparently healthy control contains low methylation compare with DNA hypermethylation of BC group, therefore appears as specific band in the gel when treated with sensitive methylation enzyme *HpaII* .



Figure (2): Enlarged image, (a) DNA from control treated with *MSPI* and *HPaII*, (b) DNA from breast cancer treated with *MSPI* and *HPaII*

These results agree with a previous study which provide evidence that well defined DNA methylation profiles

enables breast cancer subtype prediction and support the utilization of this biomarker for prognostication and

therapeutic stratification of patients with breast cancer (18). Jovanovic *et al.* (19) described in their review the various aspects of epigenetics and in particular DNA methylation in breast carcinogenesis and their potential application for diagnosis, prognosis and treatment decision. However, Dehbid *et al.* (20) evaluated DNA methylation of MAP9 gene in breast cancer as epigenetic biomarker, this study indicated MAP9 gene methylation changes in breast cancer and it can be used as molecular biomarker for breast cancer diagnosis. So, the results here suggesting that DNA methylation appear a significant role in breast cancer prognosis in Iraq, which may be useable as a dynamic biomarker for early diagnosis and monitoring.

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