Research article
Evaluation of DNA methylation of MAP9 gene in breast cancer as epigenetic biomarker

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ABSTRACT

Introduction and Aim: The DNA methylation is involved in the regulation of gene activity and abnormal DNA methylation is associated with various diseases, including cancer. MAP9 (Microtubule-Associated Protein 9) gene methylation was investigated as a potential epigenetic biomarker for cancer in this work. The results were published in Cancer Research.

Materials and Methods: The present study was on 40 breast cancer samples and 20 healthy samples to identify diagnosis biomarkers for breast cancer. DNA was extracted from the whole blood of breast cancer patients and healthy samples and were converted to bisulfite by using EpiTect Fast DNA Bisulfite Kit –Part 1 from Qiagen company. Then used Qia gene methylation kit to identify methylated site an epigenetic marker (MAP9) using HRM software in RT-PCR.

Results: The findings of the present investigation revealed that the methylation of the MAP9 gene in breast cancer patients were 28 (70%) compared to healthy patients 2 (10%) at a significant difference (P<0.01).

Conclusion: The MAP9 gene is hypermethylated in breast cancer patients, and it has the potential to be exploited as a molecular biomarker for the detection of breast cancer.

Keywords: Breast cancer; methylation; MAP9 gene.

INTRODUCTION

Women are more than twice as likely as men to get breast cancer, which ranks second overall and first among women. In 2012, 1.67 million new cases of breast cancer (25% of all incident cancer cases) were estimated worldwide. Female breast cancer is the most frequent kind of cancer among women in both richer and less developed countries, with slightly more cases estimated to have occurred in Low-Middle Income Countries (LMICs) (883000 cases) than in more economically developed regions (794000 cases) in 2012 (1).

Women who are at greater risk of breast cancer owing to family history and/or particular genetic mutations develop the disease at a younger age than women who are at average risk, and screening mammography is less sensitive in younger women (2). Early detection of breast cancer increases treatment options, including surgical resection and therapeutic interventions (3). Thus, finding markers that can help detect cancer early, particularly in younger women, that complement and/or improve existing methods will help in reducing incidence and mortality from breast cancer (4).

Epigenetic markers, such as DNA methylation, histone modifications, and microRNAs (miRNAs), play a significant role in gene expression regulation in both normal development and illness (5, 6). They also function as prognostic indicators for several diseases (7, 8), potential therapeutic targets, both of which are often seen in cancer (9, 10). When DNA methyltransferases modify CpG dinucleotides, they do so by attaching a methyl group to one of the pyrimidine rings of thymine (7). Promoter methylation is considered to suppress gene expression by attracting methyl-binding domain proteins (MBDs), which alter chromatin shape and inhibit transcription factors from binding to the promoter (12, 13). In BC, several studies have indicated that tumor suppressor genes, such as BRCA1, are silenced as a result of hypermethylation of their promoters (14), E-cadherin (15) and TMS1(16). Although the Wilms' tumor suppressor 1 (WT1) gene has been shown to be overexpressed in breast tumor tissue, this is despite the fact that its promoter has been hypermethylated (17).

MATERIALS AND METHODS

Patient groups and controls
The research was carried out between August 2019 and February 2020 on a total of 60 individuals whose
ages varied between 24 - 47 years. It included 10 breast cancer patients with early diagnosis, 30 patients under treatment and 20 healthy women. All of the samples were gathered from oncology Teaching Hospital /Medical city in Baghdad, every group except the control group was recruited from sources outside of the hospital. The factors selected and affecting in this study are, age, province, BMI and family history of breast cancer and co-morbidities such as diabetes and hypertension.

**Collecting blood samples from women**

Three ml of blood were taken from each woman (patients and control) group, by drawing blood (venipuncture) from vein by disposable medical syringe. Drawn blood were placed directly in the EDTA tubes and stored at -70°C, pending checking for molecular genetic analysis (DNA Extraction) using for quantitative qRt-PCR technique.

**DNA extraction, concentration, and purity**

All samples were used to measure DNA methylation in AMP9 gene by using RT-pcr. The genomic DNA was extraction according to Geneaid company genomic DNA according to Geneaid Company (Taiwan), Then Determination of the concentration of DNA using QuantusTM fluorometer and determination of the purity of DNA using a NanoDrop apparatus were done.

**Detection DNA Methylation of AMP9 gene by using RT-PCR**

All samples of DNA after concentration and purity were converted to Bisulfite by using EpiTect Fast DNA Bisulfite Kit –Part 1 from Qiagen company. Then used EpiTect Fast DNA Bisulfite Kit –Part2 from Qiagen company for cleanup of converted DNA.

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td>5 min</td>
<td>95°C</td>
</tr>
<tr>
<td>Incubation</td>
<td>10 min</td>
<td>60°C</td>
</tr>
<tr>
<td>Denaturation</td>
<td>5 min</td>
<td>95°C</td>
</tr>
<tr>
<td>Incubation</td>
<td>10 min</td>
<td>60°C</td>
</tr>
<tr>
<td>Hold</td>
<td>Indefinite</td>
<td>20°C</td>
</tr>
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</table>

Primers were designer by using Beacon designer version 8.21 software. The primer sequences are Forward primer: TTATAGCGATATAGGGGAGGGAAG and Reverse primer: CCGCGACAAAATAAAACCAATACC.

Application of RT-PCR program: Hold at 94°C for 5min. Denaturation at 95°C for 15 sec, annealing 61°C for 15 sec., 72°C for 15 sec, repeats for five cycles, then 95°C for 15sec., 61°C for 25sec, repeats for 40 cycles according to the Qia gene methylation kit (Table1).

**RESULTS**

According to the findings of the present investigation, the methylation of the MAP9 gene in breast cancer patients were 28 (70%) compared to healthy patients 2 (10%) at a significant difference (P<0.01), while the unmethylated pattern were 12(30%) in breast cancer patient compared to healthy patients 18 (90%). This data means hypermethylation of the study region in MAP9 gene for breast cancer patients as shown in Table 2 and Fig.1.

**Table 1: Temperatures used in PCR reaction**

<table>
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<tr>
<td>Hold</td>
<td>Indefinite</td>
<td>20°C</td>
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**Table 2: Data of MAP9 gene methylation for breast cancer patients**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of samples</th>
<th>Methylation pattern</th>
<th>( x^2 ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Methylated %</td>
<td>UnMethylated %</td>
</tr>
<tr>
<td>Group 1</td>
<td>40</td>
<td>28/ 70%</td>
<td>12/ 30%</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>2/ 10%</td>
<td>18/ 90%</td>
</tr>
<tr>
<td>( x^2 ) value</td>
<td>-</td>
<td>55**</td>
<td>30**</td>
</tr>
</tbody>
</table>

**Fig. 1:** Breast cancer samples analyzed by methylation- sensitive high-resolution melting
DISCUSSION

Breast cancer is the most often diagnosed cancer in women, and it is also the leading cause of cancer-related death in women, which is approximately 23% of the total cancers and 14% of the cancer deaths (18). Breast cancer is referred as the silent killer, since in the early stages of breast cancer, breast pain and symptoms are usually not significant and sensible (19). As a result, the adoption of suggestive biomarkers of breast cancer in the early stages of the illness might provide the individual with early warning. According to the results of the previous research (20), The MAP9 gene was shown to be significantly altered in breast cancer, with considerable alterations in DNA methylation. In addition, our research revealed that the MAP9 gene is highly hypermethylated in breast cancer at (P<0.01). The results demonstrate the importance of MAP9 gene methylation in breast cancer and suggest that it might be exploited as a molecular biomarker for the detection of breast cancer. According to several studies, hyper methylation of genes can reduce their expression (20). Therefore, the hyper methylation of MAP9 gene may lead to decreased of gene expression. It is possible that the decrease in expression of MAP 9 gene in cancer leads to instability of P53. Inhibition of apoptosis in cancer caused, following the non-stabilized P53 that is characteristic of cancer (20). In conclusion, it was discovered that the MAP9 gene is considerably hypermethylated in breast cancer, suggesting that it might be used as a diagnostic for the disease in the future.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest for the study.

REFERENCES