

# **Qualitative and Quantitative Identification of DNA Methylation Changes in Blood of the Breast Cancer patients**

**Harem Othman Smail**

**Supervised By**

**Asst. Prof. Dr. Dinya Asad Mohamad**

# Aims of the research

The aim of this study was to identify DNA methylation changes in the promoter region of the three different genes (**ESR2, OPN and BRCA1**) in breast cancer patients and to compare DNA methylation changes between **healthy** and **breast cancer patients**.

# Cancer:

Cancer is a collection of diseases discriminate by the abandoned progress and spread of abnormal cells; **it is the second leading cause of death after cardiovascular diseases .**

# Breast cancer :

In women, breast cancer is the most common class of cancer worldwide with more than one million cases diagnosed annually, **followed by cancer of the lung and colon**, making it the leading cause of cancer deaths in women with >400,000 deaths per year .

# Causes of breast cancer :

There are two main factors causes breast cancer either environmental or genetic factors.

Like other cancer, mixed between two these factors genetic, environmental also with and **epigenetic** alterations of multiple cancer genes including **oncogenes** and **tumor suppressor genes**.

# **Cancer Epigenetics :**

Epigenetics refers to the study of heritable changes that cannot be explained by **changes in the DNA sequence** .

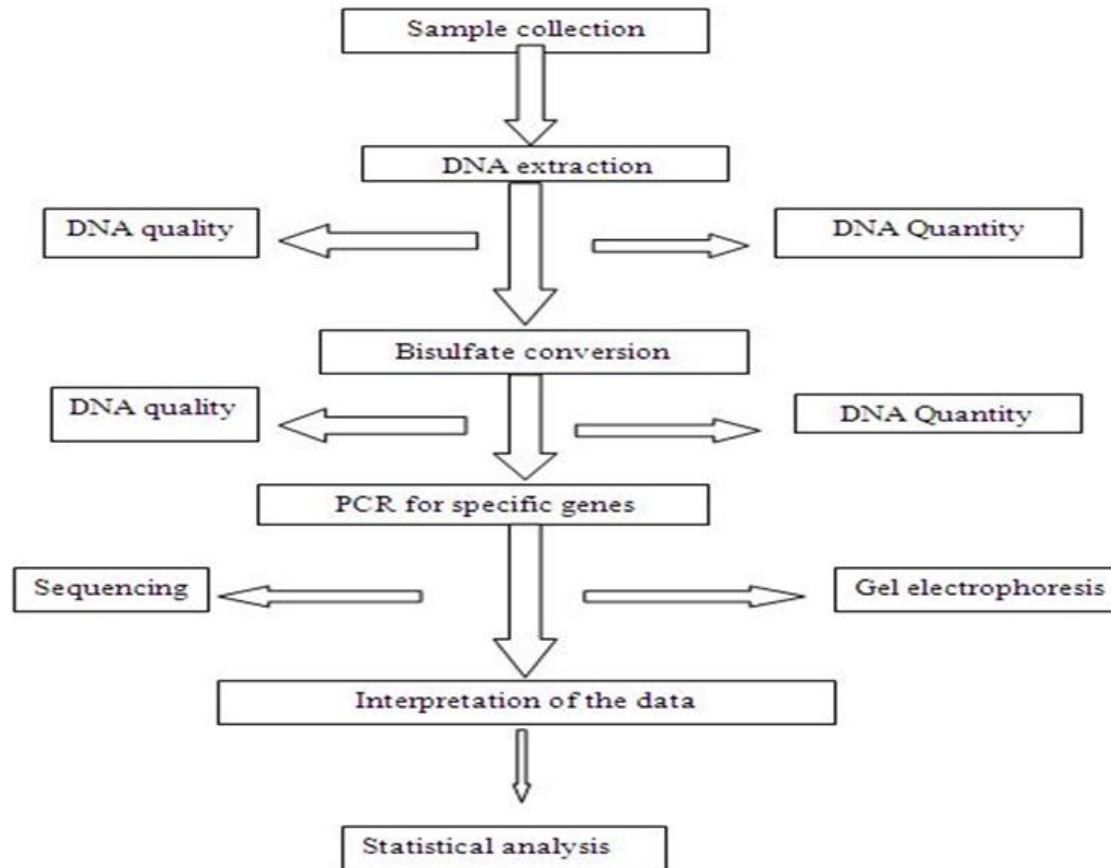
# **DNA methylation :**

DNA methylation is a covalent chemical modification, resulting in the addition of a methyl (**CH<sub>3</sub>**) group at the carbon five position of the **cytosine** ring .

# **Materials and Methods**



# Research design



## **Methods:**

Sample collection: A total of 38 blood samples were collected from women with different grades of breast cancer in Hiwa hospital.

Blood samples divided into three groups, 13, 14 and 11 blood samples for ESR2, OPN and BRCA1 genes respectively .

# Bisulfate primer seeker

The free program and it recommended multiple options for both forward and reversed primer and choice on of them for each one.

[www.zymoresearch.com/tools/bisulfite-primer-seeker](http://www.zymoresearch.com/tools/bisulfite-primer-seeker)

# RESULTS

**DNA quantity before bisulfite conversion**

The mean absorbance of DNA for 13 samples selected for ESR2 gene was **1.6** and mean concentration was **20.5 ng /ul.**

The mean absorbance of 14 samples selected for OPN gene was **1.9** and DNA concentration was **25.2 ng/ul**

The mean DNA absorbance of the 11 samples selected for BRCA1 gene was **1.6**, and DNA concentration was **22.06 ng/ul**



# DNA Quality

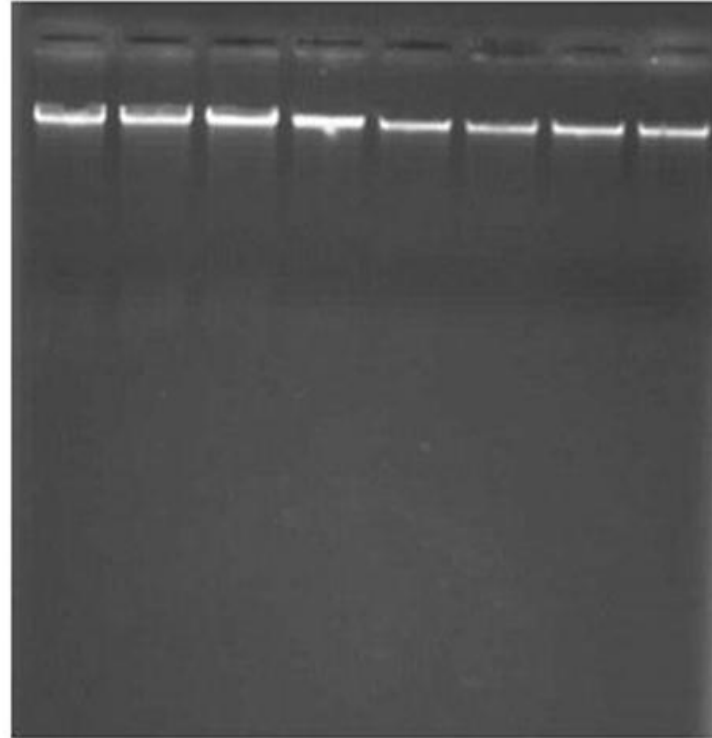


Figure 4.1 quality of DNA extraction before bisulfite conversion.

**DNA quantity of bisulfite converted samples**

The mean absorbance of DNA for 13 samples selected for ESR2 gene was ( **1.2** ) and mean concentration was ( **5.6 ng /ul**).

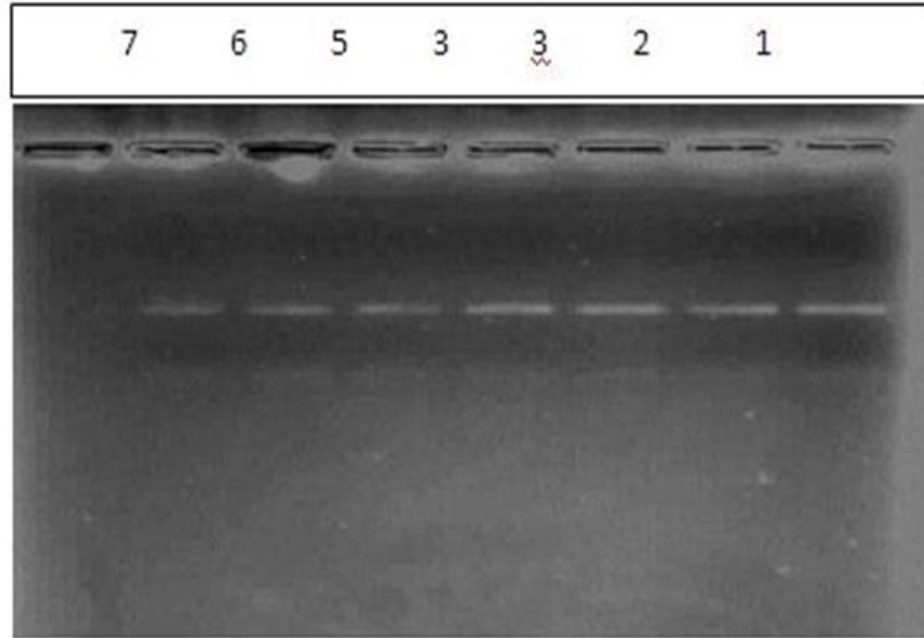
The mean absorbance of DNA for 14 samples selected for OPN gene was **(1.3)** and mean concentration was **(6.1ng /ul )**.

The mean absorbance of DNA for 11 samples selected for BRCA1 gene was (**0.9**) and mean concentration was (**4.3 ng /ul**).

## **DNA Quantity of Converted Methylated Human Control :**

To determine and ensure that the DNA was bisulfite converted, methylated human control was run in the agarose gel in parallel with some samples before PCR. The absorbancy of the methylated human control was **0.974**, and DNA concentration was **13.4 ng/ul**.

# DNA quality after bisulfite conversion

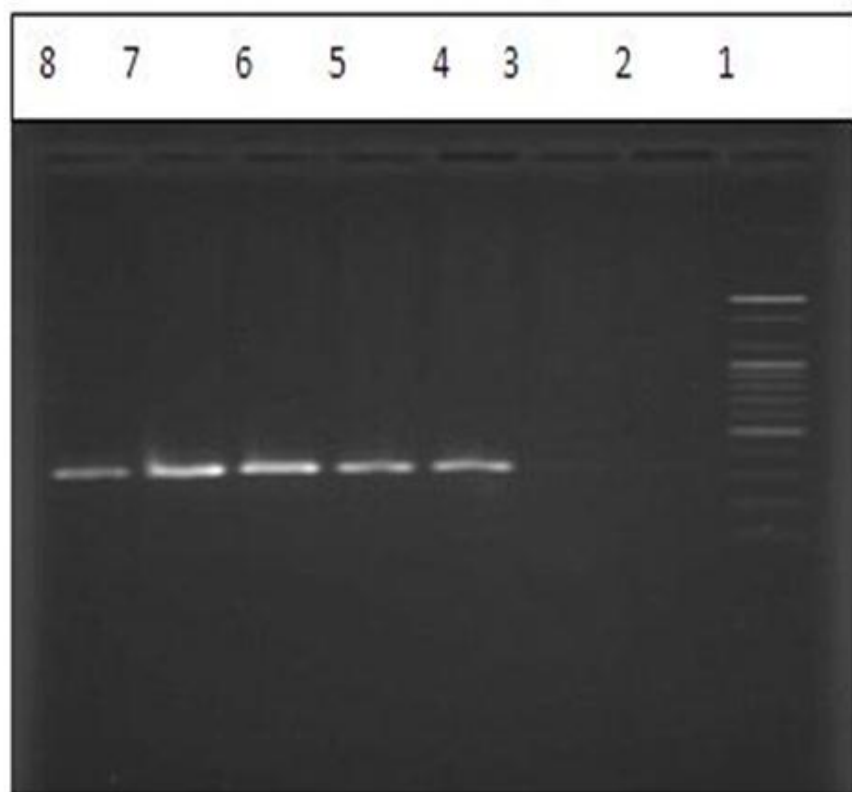


**Figure 4.2: Bisulfite converted DNA**

**\*line 1: Human control, line 2- 7 Bisulfite converted DNA**

**Amplification of ESR2 gene promoter  
region:**





**Figure 4.3: PCR-amplified promoter region of ESR2 gene.**

**\*Line 1: 100bp DNA ladder, line (2-8): 343 bp methylated ESR2 promoter of samples 1, 2, 3, 4, 5, 6 and 7.**

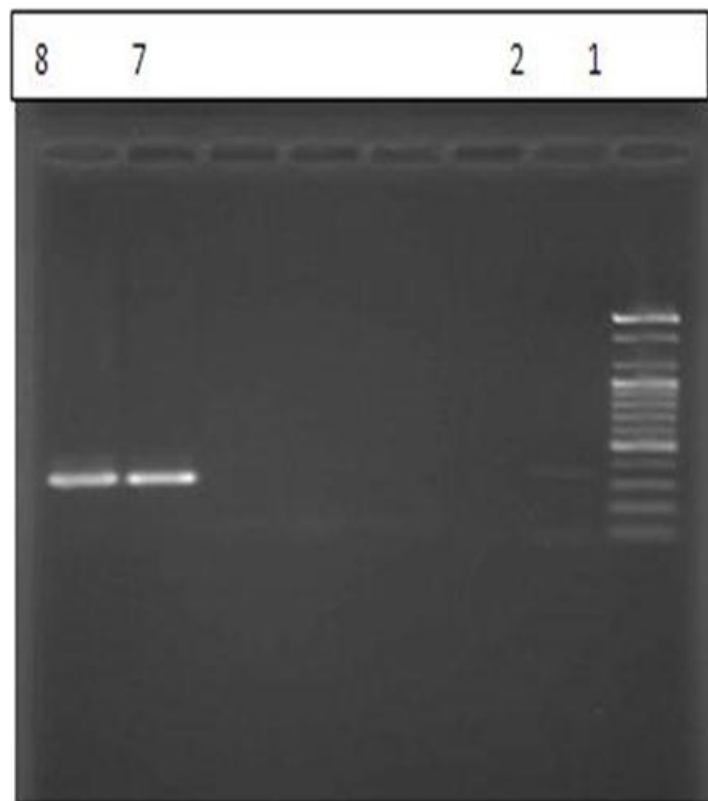
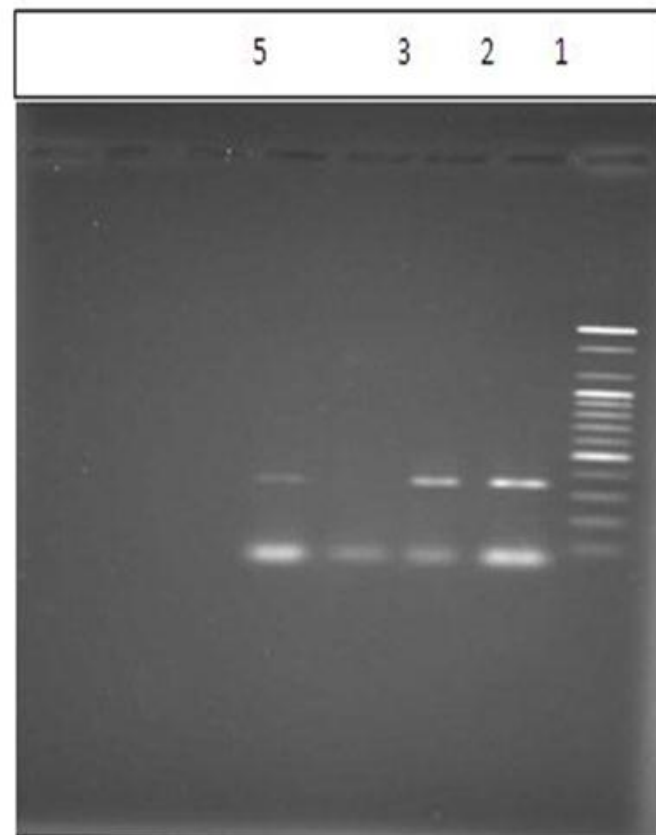


Figure 4.4: PCR-amplified promoter region of ESR2 gene.

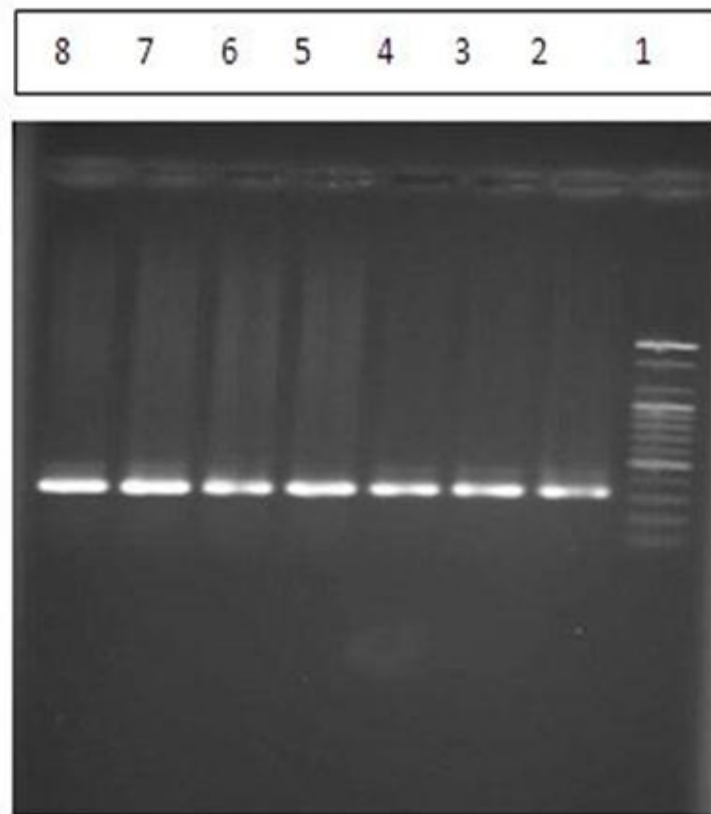
\*Line 1: 100bp DNA ladder, line (2, 7 and 8): 343 bp methylated ESR2 promoter of samples 8, 9 and 10.



**Figure 4.5: PCR-amplified promoter region of ESR2 gene.**

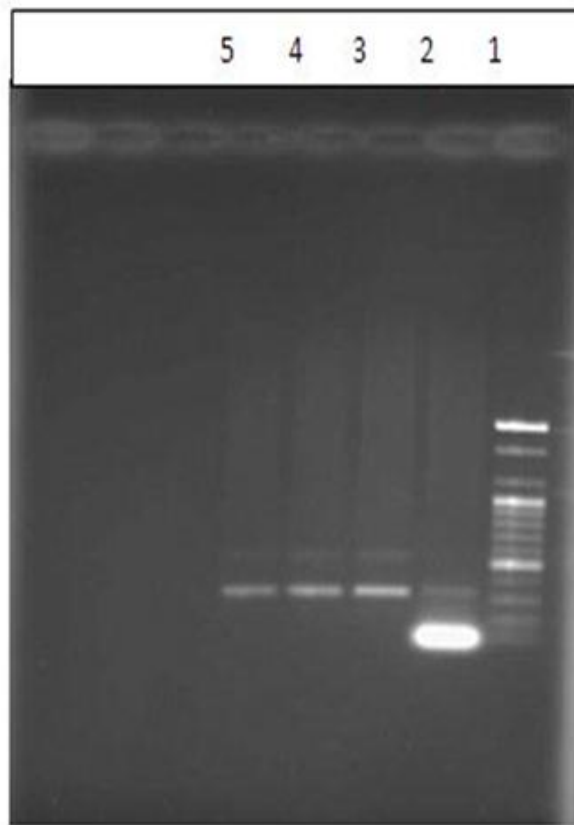
**\*Line 1:100bp DNA ladder, line (2, 3 and 5): 343 bp methylated ESR2 promoter of samples 11, 12 and 13.**

# **Amplification of OPN gene promoter region**



**Figure 4.6: PCR-amplified promoter region of OPN gene.**

**\*Line 1: 100bp DNA ladder, line (2-8): 324 bp methylated OPN promoter of samples 14, 15, 16, 17, 18, 19 and 20.**



**Figure 4.7: PCR-amplified promoter region of OPN gene.**

**\*Line 1: 100bp DNA ladder, line (2-5): 324 bp methylated OPN promoter of samples 21, 22, 23, and 24.**

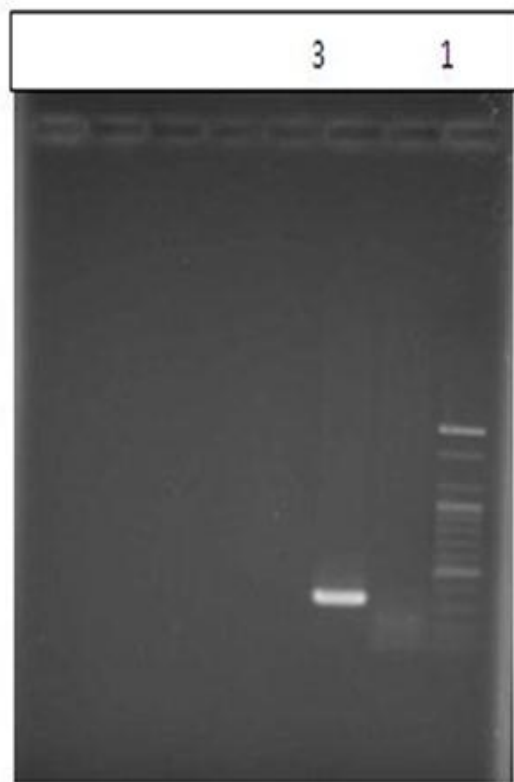
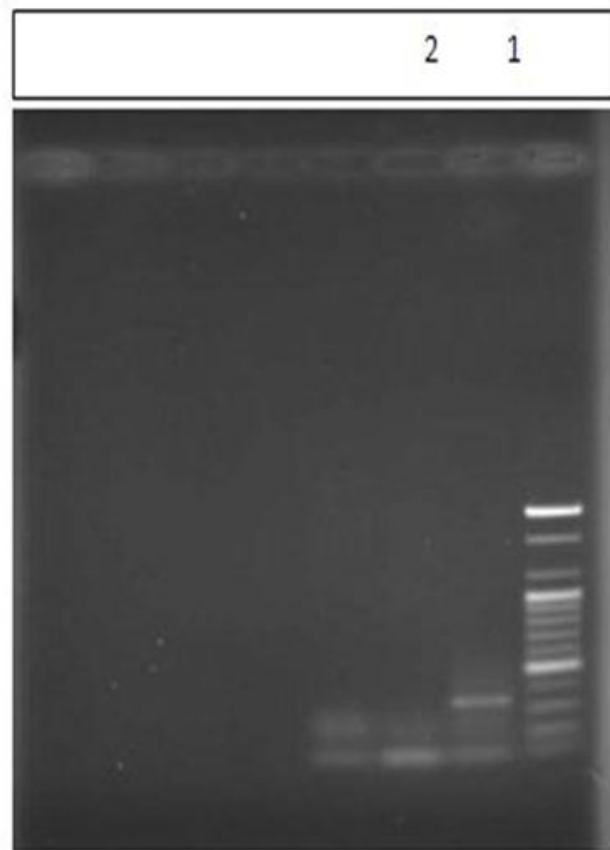


Figure 4.8: PCR-amplified promoter region of OPN gene.

\*Line 1: 100bp DNA ladder, line (3): 324 bp methylated OPN promoter of samples 25.



**Figure 4.9: PCR-amplified promoter region of OPN gene.**

**\*Line 1: 100bp DNA ladder, line (2): 324 bp methylated OPN promoter of samples 26.**



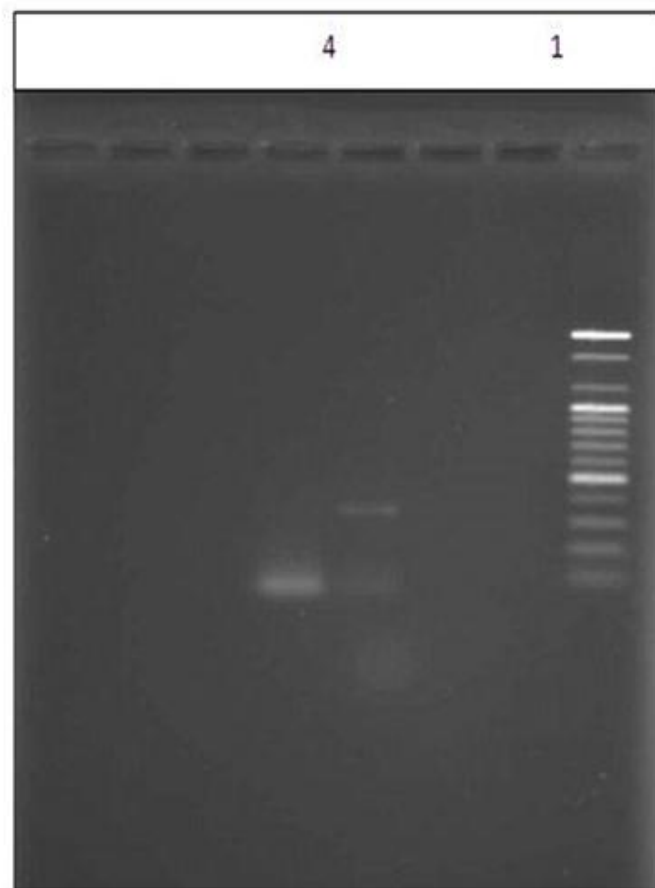
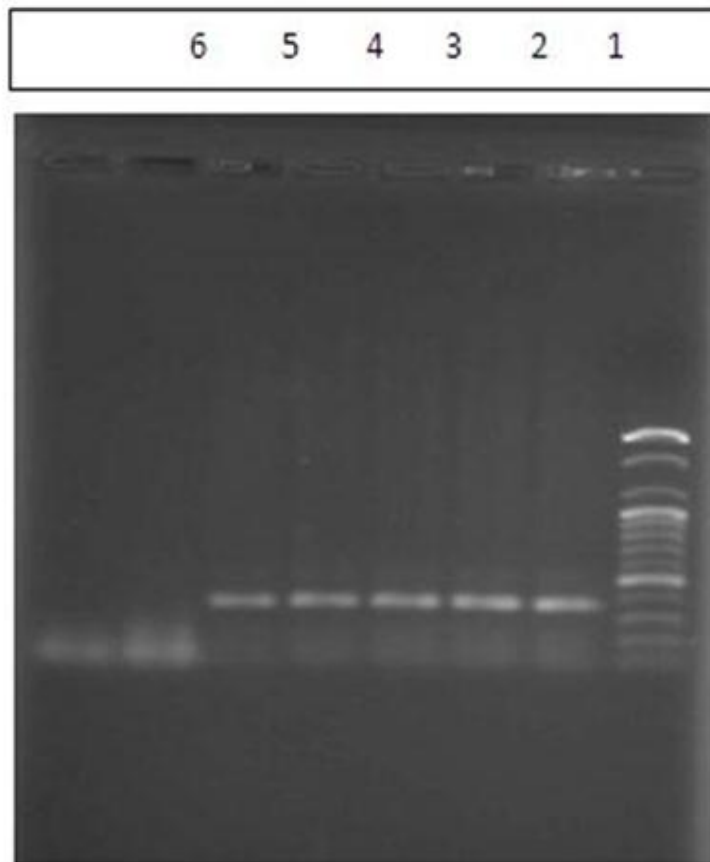


Figure 4.10: PCR-amplified promoter region of OPN gene.

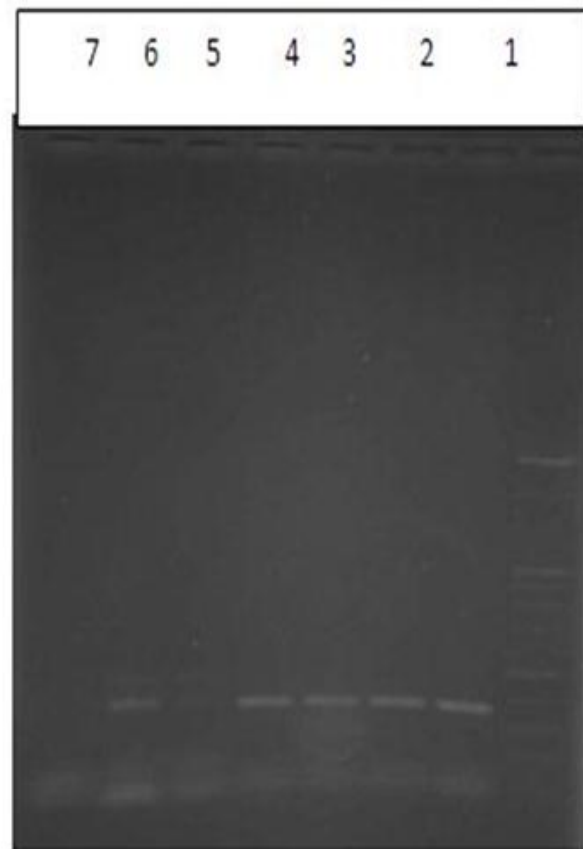
\*Line 1: 100bp DNA ladder, line (4): 324 bp methylated OPN promoter of

# **Amplification of BRCA1 gene promoter region**



**Figure 4.11: PCR-amplified promoter region of BRCA1 gene.**

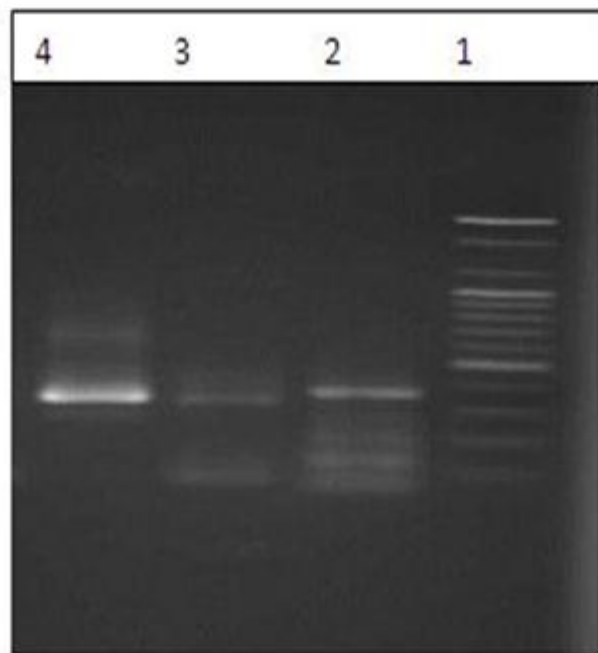
**\*Line 1: 100bp DNA ladder, line (2- 6): 350 bp methylated BRCA1 gene of samples 28, 29, 30, 31, and 32.**



**Figure 4.12: PCR amplified products of BRCA1 gene promoter region.**

**\*Line 1: 100bp DNA ladder, line (2- 7): 350 bp methylated BRCA1 gene of samples 33, 34, 35, 36, 37, and 38.**

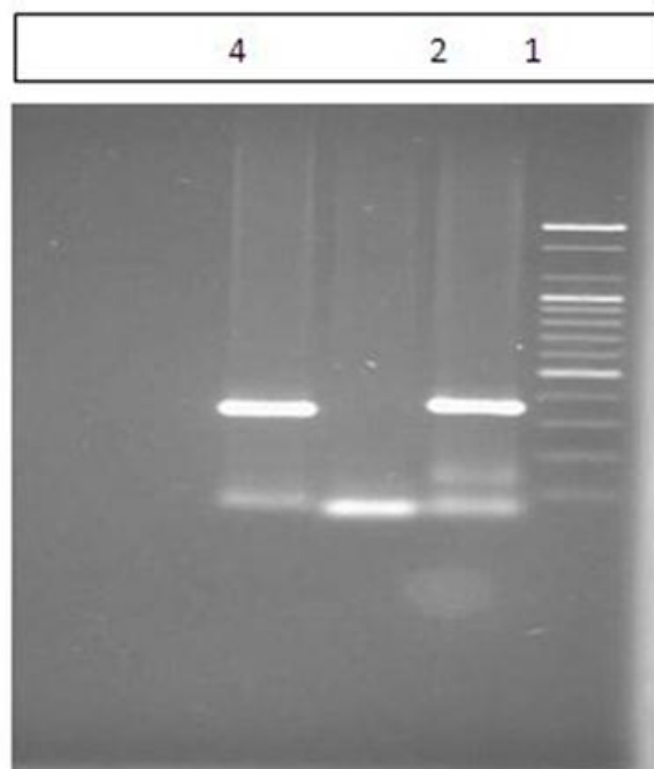
**Qualitative identification of bisulfite  
converted control of BRCA1, OPN, and  
ESR2**



**Figure 4.13 PCR amplified products of bisulfite converted control of BRCA1, OPN, and ESR2 genes promoter regions.**

**\*Line 1: 100bp DNA ladder, line 2: BRCA1 (350)bp, line 3: OPN (324) bp and line 4: ESR2 (343) bp.**

**Qualitative identification of methylated  
control of ESR2, BRCA1 and OPN gene:**



**Figure 4.14: PCR amplified products of methylated control of BRCA1, OPN genes promoter region.**

**\*Line 1: 100bp DNA ladder, line 2: BRCA1 (350) bp, line 4: OPN (324) bp.**



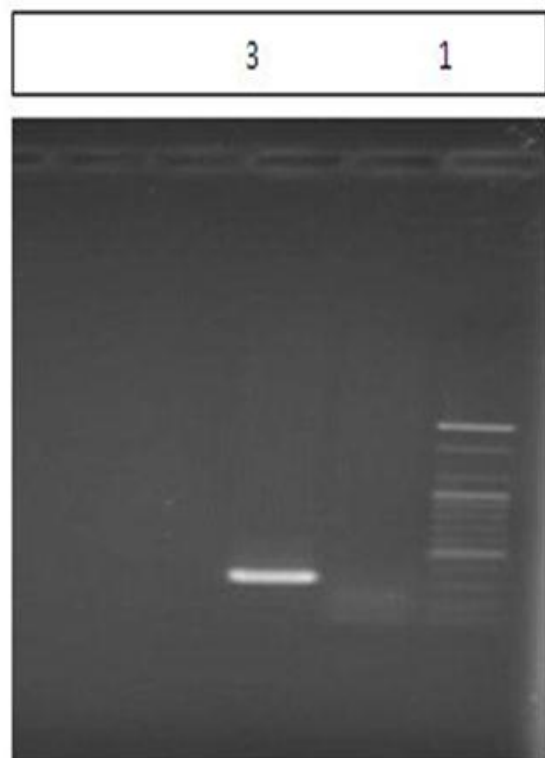


Figure 4.15: PCR amplified product of methylated control of ESR2 gene promoter region.

\*Line 1: 100bp DNA ladder, line 3: ESR2 (343) bp.

# **Quantitative Identification of CPG methylation of ESR2 gene**

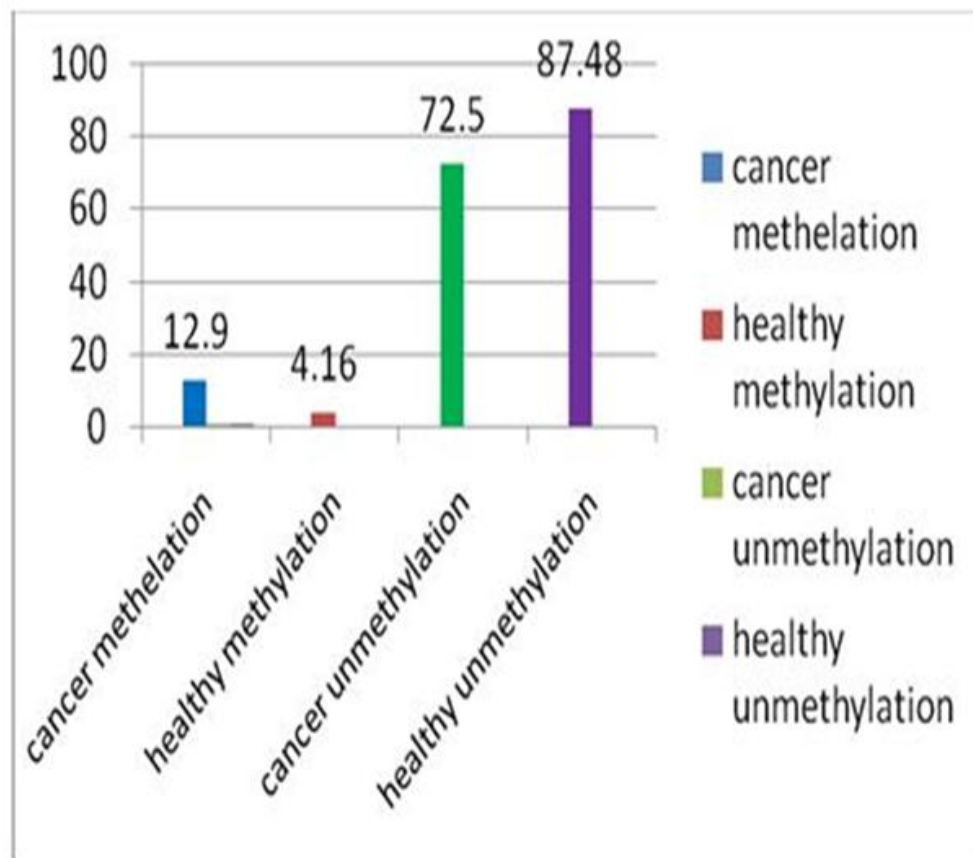
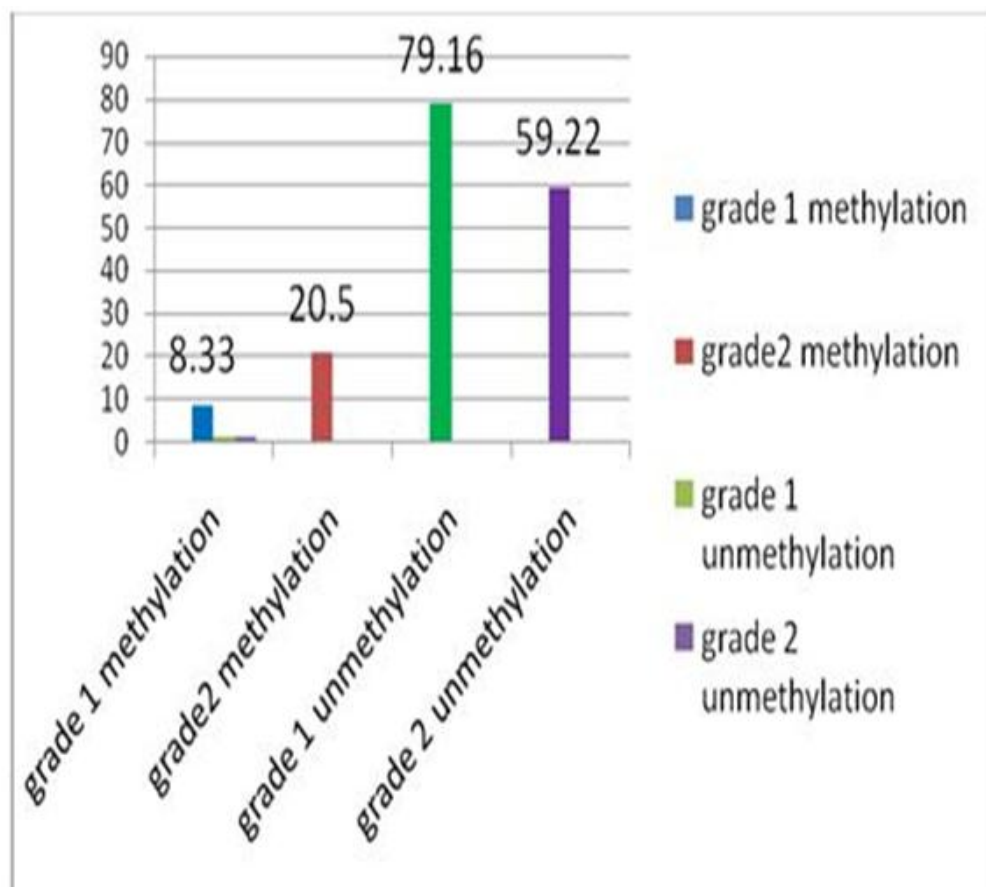
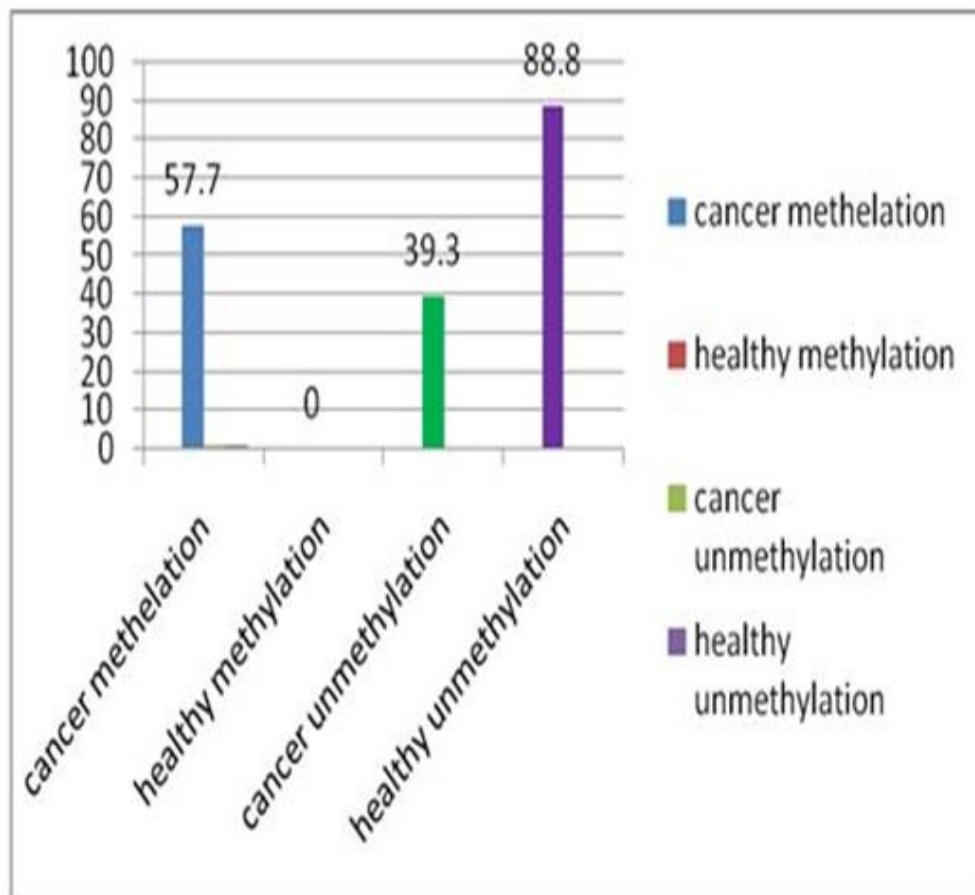


Figure 4.16: Total methylation and unmethylation between cancer and healthy samples of ESR2 gene.

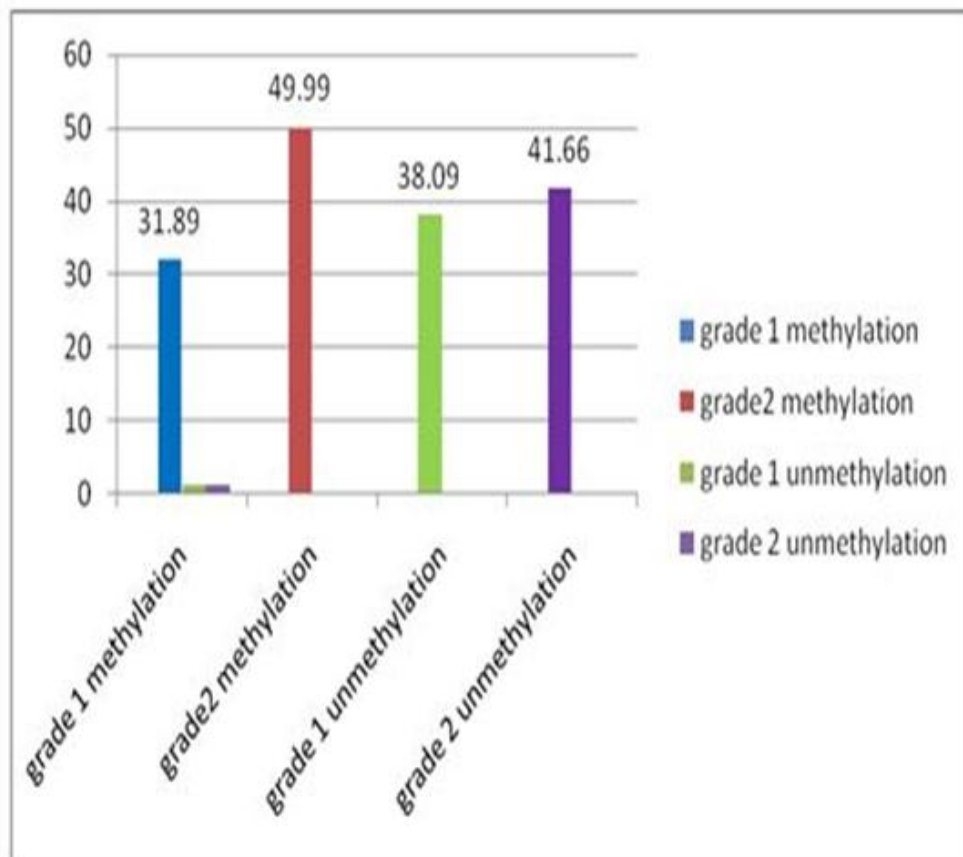


**Figure 4.17: Total methylation and unmethylation between grade 1 and grade 2 of breast cancer samples of ESR2 gene.**

# **Quantitative Identification of CPG methylation of OPN gene**



**Figure 4.18: Total methylation and unmethylation between cancer and healthy samples of OPN gene**



**Figure 4.19: Total methylation and unmethylation between grad1 and grade 2 breast cancer samples of OPN gene.**

# **Quantitative Identification of CPG methylation of BRCA1 gene**



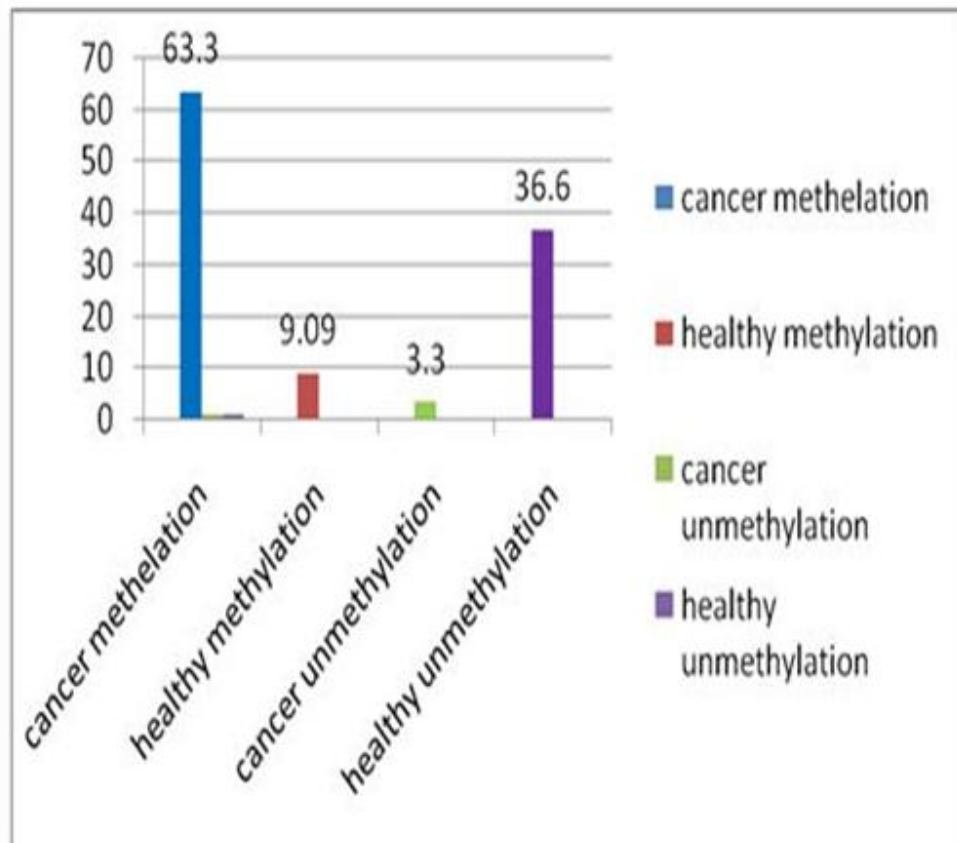
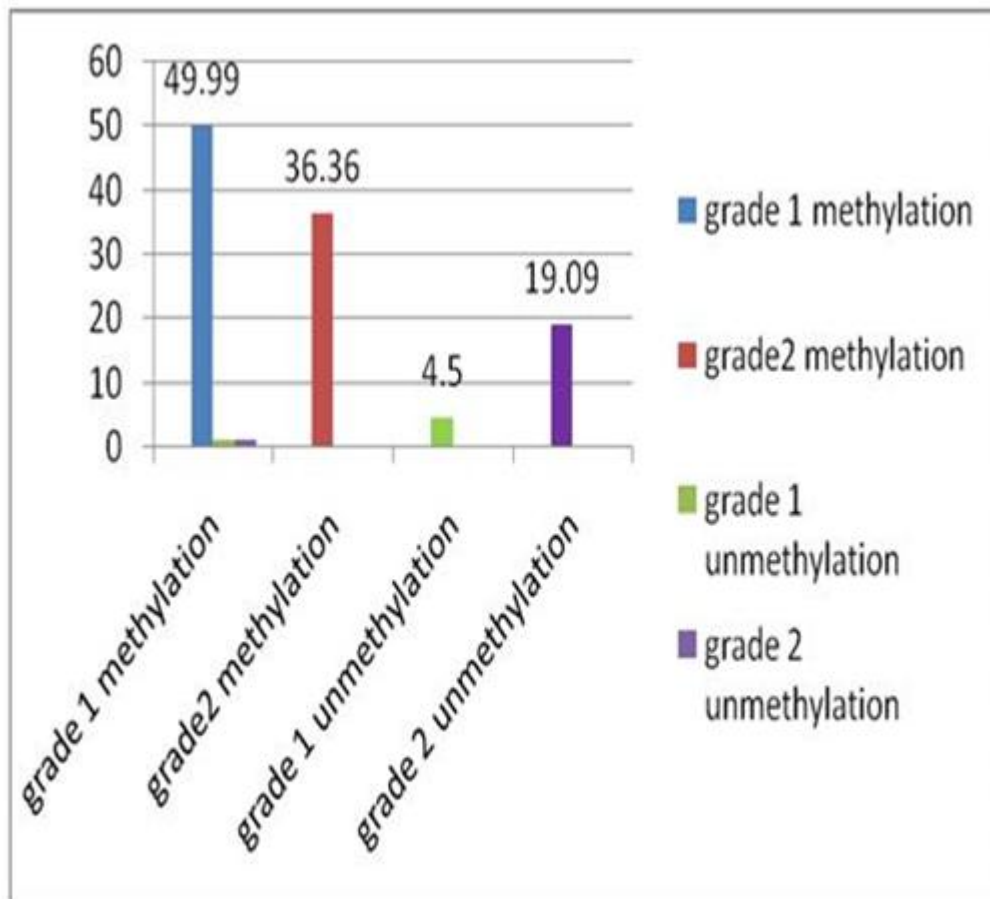


Figure 4.20: Total methylation and unmethylation between cancer and healthy samples of BRCA1 gene.



**Figure 4.21: Total methylation and unmethylation between grade 1 and grade 2 breast cancer samples of BRCA1 gene.**

# **Statistical Analysis**

Fisher exact test used to compare methylation and unmethylation between healthy and cancer samples and also between grade 1 and grade 2 of each gene. For ESR2 genes, the value was (0.034) between healthy and breast samples that indicate significant result ( $p < 0.05$ ). The result of statistical analysis between grade 1 and grade 2 of ESR2 samples was (0.007) which also showed significantly ( $p < 0.05$ ).

For OPN gene, neither statistical analysis between healthy and breast cancer samples (0.0) nor between grad1 and grade2 (0.265) at ( $p < 0.05$ ) was significant.

Regarding BRCA1 gene, the Fisher exact test statistic value was (0.0) between healthy and breast cancer samples ( $p < 0.05$ ) and the value was (0.028) between grade1 and grade2 of breast cancer. The two values were significant.

# Conclusions

1-DNA quantity decrease after bisulfite conversion reaction.

2-Size of ESR2 343 bp, OPN 324 bp and BRCA1 350 bP.

3-Bisulfate conversion rates are different from the samples of among genes (ESR2, OPN and BRCA1).

4-ESR2 are more converted than OPN and OPN more converted than BRCA1.

5-Total CPG DNA methylation is different between all three genes.

# Recommendations



1-Using of other techniques to determine DNA methylation statuses such as CoBRA (combined bisulphite restriction analysis), Methylation-specific PCR (MSP), Pyrosequencing and MethyLight™.

2- Evaluation the relation between DNA methylation on the gene expression.

3- DNA methylation profiling of different breast cancer genes.

4- Comparison of DNA methylation between different stages of breast cancer

Thanks for your time