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Full Length Research Paper

Evaluation of methylation status in glutation S-transferase P1(GSTP1) gene promoter in human breast cancer and its relation to tumor grade and stage

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Glutation S-transferase P1 (GSTP1) gene methylation in promoter CpG islands has been described as a specific biomarker for many types of cancer including breast cancer as a tumor suppressor gene. At the present study we found that the GSTP1 gene promoter to be methylated in breast cancer tissues. For studying the difference of sequence in hypermethylated GSTP1 promoter in cancer tissues and non methylated status in normal tissues, we analyzed the cytosine methylation status as epigenetic changes in 50 tumors from patients with breast cancer and 50 normal breast tissues that were obtained from the adjacent regions of the breast tumors of the same patients. In order to study the promoter methylation status for GSTP1 gene in breast cancer, 40 CpG sites [nucleotide(nt) 197, 190, 187, 185, 183, 182, 176, 162, 155, 152, 148, 145, 141, 132, 127, 124, 112, 109, 101, 99, 81, 77, 74, 71, 54, 53, 48, 47, 43, 42, 40, 38, 23, 22, 15, 14, 13, 11, 8, 4] were screened. The GSTP1 methylation was detected in 41.3% of the breast tumors which was associated with higher tumor grade (p=0.467) and tumor stage (p = 0.048).

Key words: GSTP1, promoter, methylation, pattern, breast cancer.

INTRODUCTION

Breast cancer is the second leading cause of death among women after lung cancer. Detection of premenopausal breast cancer without X-ray exposure is of particular importance in early detection. Thus alternative approaches to breast cancer detection are clearly needed. Detection of changes in DNA methylation which is the most common molecular alteration in human neoplasia (Wu et al., 2010), including breast cancer (Bedgia et al., 2006), may offer an alternative method to screening (Hoque et al., 2006). Cytosine methylation has critical role in control of gene activity that mostly occurs in cytosines that precede guanines, called 'CpG islands' (Esteller, 2008). Theses regions span the 5' end of the regulatory region of many genes, which usually not methylated in normal cells (Weber et al., 2007). Hypermethylation of CpG islands in gene promoter blocks the ability of transcription factors to interact with the promoter and inhibits gene expression (Li et al., 2008). DNA methylation is one of the known mechanisms for control of certain tissue-specific genes that have important role as tumor suppressor genes such as GSTP1, E-cadherin, BRCA1 in many types of cancer including breast cancer.

It is reported that CpG islands are hypermethylated in >80% of hepatocellular carcinomas, ~ 30% of breast cancers and >90% of prostate cancers (Maxwell et al., 2009).

GSTP1 is the only gene of the human glutathione S-transferase (GST) P subfamily (Suzuki et al., 2005).GSTs provide protection to mammalian cells against electrophilic metabolites of carcinogens and reactive

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oxygen species (Chow et al., 2007).GSTp1 plays a role in regulating the Map kinase pathway via protein-protein interactions as it is an inhibitor of c-Jun NH2-terminal kinase I, a kinase involved in stress response, apoptosis and cellular proliferation (Holly et al., 2007).

Recently, GSTP1 CpG island hypermethylation was found to be significantly associated with tumor size, lymph node metastasis and relapse free-survival in breast cancers (Gao et al., 2009). The involvement of GSTP1 in drug metabolism, potential effect on cancer therapy and other tumor features, indicates the importance of evaluation of mechanisms for it's regulation in normal and cancer tissues (Ronneberg et al., 2008).

In this research, we studied tumors from breast cancer patients and also normal breast tissues from the adjacent regions of the breast tumors of the same patients as control tissues. The promoter region of the gene, is selected at 40 CpG sites with specific structures like as: ccg, ctg and cgcg (the order of sequence for cytosine and guanine nucleotides which are in DNA) in the prompter analyzed by semi-quantitative measures, and the relationship between methylation degree with tumor's grade and stage was evaluated.

MATERIALS AND METHODS

Patients and tissue specimens

Samples of breast cancers were obtained from 50 women (mean age of 48.2±10.55 year) who had undergone surgery at the Tabriz University Hospital (Imam Reza) of Medical Sciences (Retrospective observational study with Cluster random sampling in time intervals for 1 year). The samples were obtained according to institutional guidelines, during the surgery as a fresh and immediately taken to -80° deep freeze. Normal breast tissues were also obtained from the surgery region of the same patients, about 3 cm away from the tumor site (n = 50). As all specimens were the part of routine pathology and surgery mastectomy procedures, therefore, all the samples were coded by number and patients' information were kept in secure.

Determining of stage was performed according to AJCC-02 TNM stage system and determining of grade was performed according to WHO international protocol by surgeon as GI, GII and GIII.

Gene sequence alterations typically were identified by direct sequencing of PCR-amplified bisulfite treated genomic templates, BSP (Bisulfite treated specific PCR), that included the promoter region.

DNA isolation

Genomic DNA was isolated from tumor and normal tissues using SDS-protease K and phenol-choloroform method as previously described (Pourabbas et al., 2009). Samples were first digested with protease K; DNA was extracted from 200 µl protease K digested samples, following the manufacturer's protocol (Fermentase DNA isolation kit,Letonia). DNA concentration was measured using a spectrophotometer.

Bisulfite conversion

Extracted DNA was treated with sodium bisulfite as described before with some modifications. Briefly 5 µg of DNA was denatured using NaOH and treated with sodium bisulfite for 8 h in 55°C. Bisulfite treated DNA was amplified with BSP specific primer set (forward: 5'-TTT GGGAAAGAGGGAAAGGTTT -3',nt -4954 to -4979) and (reverse: 5'-CCCCATACTAAAAACTCTAAACCCC-3',nt -5281 to -5306). 2 µg of treated DNA were entered into a 25 µl polymerase chain reaction (PCR) mixture containing 0.5 µM forward and reverse primers each; 1X Taq buffer,0.1 mM four deoxynucleotide triphosphate,1.125 µM $MgCl_2$, 1.25 µl DMSO and 1.2 U Taq ploymerase (Fermentase,Letonia),15.75 µl ddH_2O. PCR was carried out with the program as followed: a 1 min cycle in 95°C followed by 30 cycles of 30 s in 94°C,40 s in 58°C,45 s in 72°C and a final extension cycle of 5 min in 72°C.

The untreated breast tissue genomic DNA was used as negative control and universal methylated DNA (Invitogen) sample as ready to use as positive control. PCR products then, were purified using PCR product purification kit (Fermentase,Letonia) and checked by electrophoresies of 5% polyacrylamide gel. The resulting products were sequenced on an ABI automated sequencer with big dye terminator (Perkin-Elmer,CA).

Classification and quantification of methylation levels

Determination of methylated CpG sites was performed by comparing the C versus T nucleotide peaks in bisulfite treated tumor samples in each 40 specified CpG sites that remained as C and was converted to T in bisulfite treated normal samples. Methylation was considered 100% if all 40 CpG sites were methylated. Methylation levels less than 40 CpG sites were classified as partial methylation and absence of methylation was named as unmethylated.

Statistical analysis

The percentage of methylation was caculated by chi-square test and significant difference between cancer and normal samples. Cancer grade and stage with methylated CpG sites,was analyzed by using spearman regression test.

RESULTS

Methylation status in tumor and samples

Out of 46 breast cancer samples, 28.3% (13 samples) were completely methylated, 13% (6 samples) were partially methylated and 58.7% (27 samples) were unmethylated. The overall hypermethylation rate in breast cancer tissues was 41.3% (19 out of 46). On the other hand, the majority of the normal samples (87.2%) or 41 out of 47 were unmethylated. There were significant differences between tumor and normal samples in the methylated CpG sites (methylation pattern) (Figure 1).

Methylation of the GSTP1 promoter and its relation to grade of tumor

Out of 50 tumor samples, 5 samples had unknown grade and were excluded from analysis. Out of 45 samples, 10 were classified as Grade I (well differentiated), 30 Grade

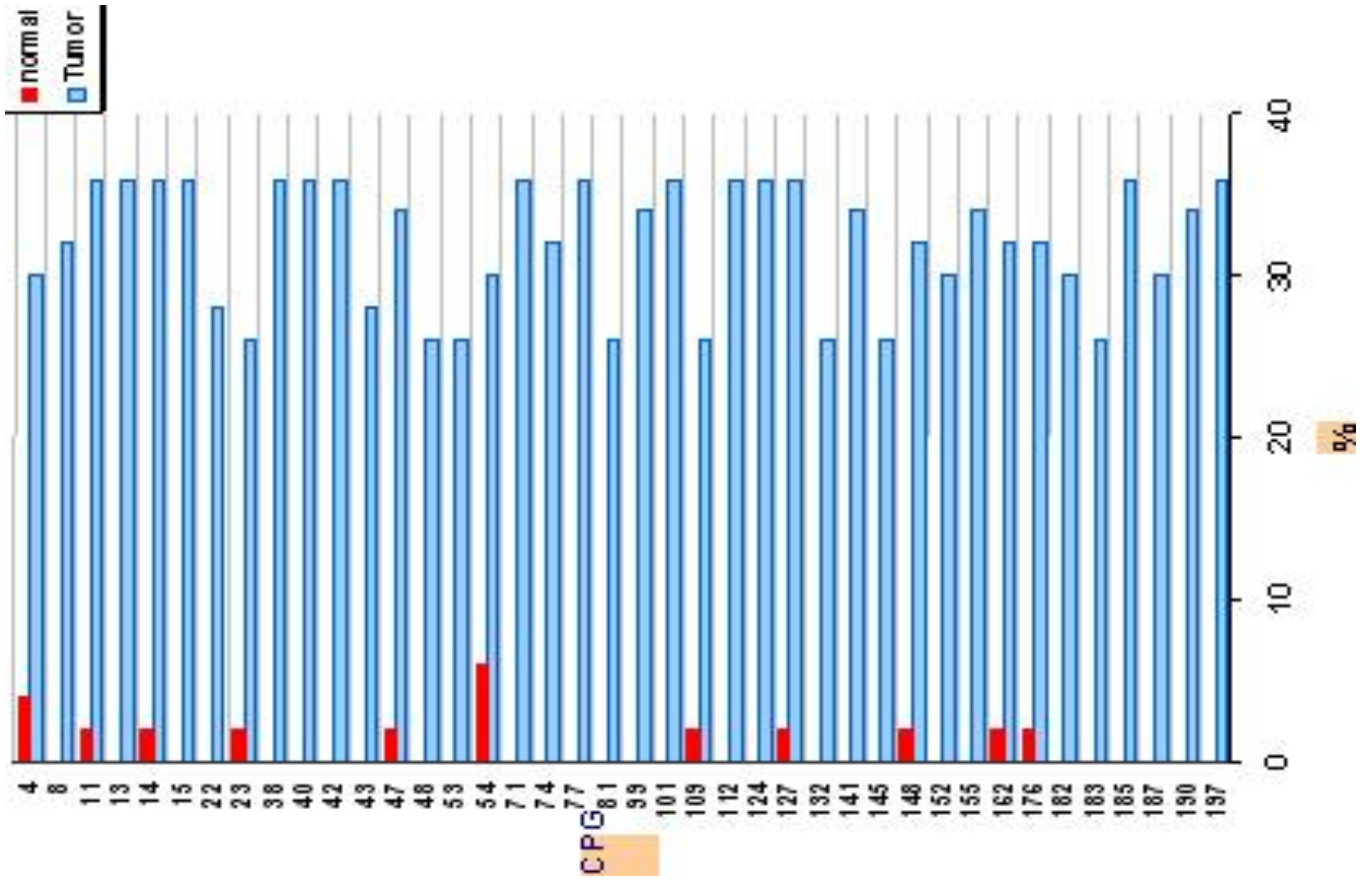

Figure 1. Comparison of methylation pattern on GSTP1 promoter region in breast cancer and it's adjacent normal tissues (p = 0.000).

II (moderately differentiated) and 5 Grade III (poorly differentiated). Also 4 samples of Grade II, had missed in methylation sequence analysis and were excluded from the calculations (total samples were 41). Analysis for methylation showed that among 10 Grade I tumor samples, 2 (20%) were fully methylated and 9 samples (34.6%) were partially methylated and 6 samples (60%) were nonmethylated. Among 26 grade 2 tumors**,** 9 samples (34.6%) were fully methylated, 3 samples (11.5%) partially methylated and 14 samples (53.8%) were nonmethylated. Finally, out of 5 grade 3 tumor samples, 1 sample (20%) was full methylated and 1 sample (20%) partially methylated, 3 samples (60%) were nonmethylated. Statistical analysis did not show any significant direct relationship between methylation and grade of tumor samples (Figure 2).

Methylation pattern of the GSTP1 promoter CpG sites and its relation to grade of tumor

The spearman regression test analysis showed no significant relationship between methylated sites and tumor grade (p = 0.467).

Methylation of GSTP1 gene and it's relation to tumor's stage

Among the 7 different stages that have been reported in different studies, in this study, only 3 stages were found in tumor samples including stage 1, 2 and 3a. There were 3 samples in Stage 1, 29 in Stage 2 and 14 in Stage 3a. (Total 46 known stage samples). Out of the 3 stage 1 tumor samples, 3(100%) were full methylated, out of 29 stage 2 tumors, 8(27.6%) were full methylated, and 4 (13.8%) were partial methylated and 17(58.6%) were nonmethylated.

Among 14 Stage 3a, 2(14.29%) were full methylated and the same number for partial methylation and 10 (71.43%) were nonmethylated in tumor breast samples (Figure 3).

Methylation pattern of the GSTP1 promoter CpG sites and its relation to stage of tumor

The spearman regression test analysis showed the highest significant relationship between stage of tumor and methylation at 183, 176, 162, 152, 148, 145, 132,

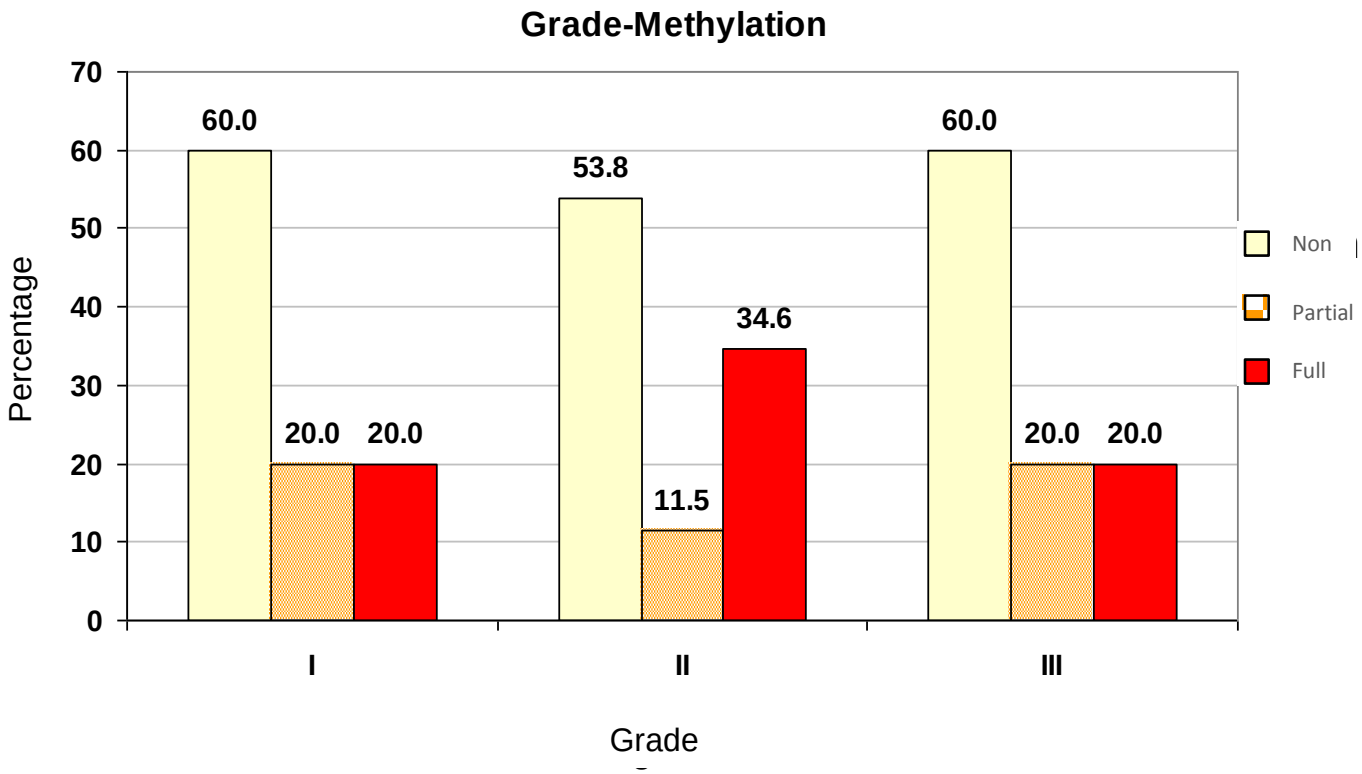

Figure 2. Comparison of tumor grade and methylation status in tumor samples.(correlation is significant at the 0.05 level, 2 tailed) (p = 0.8).

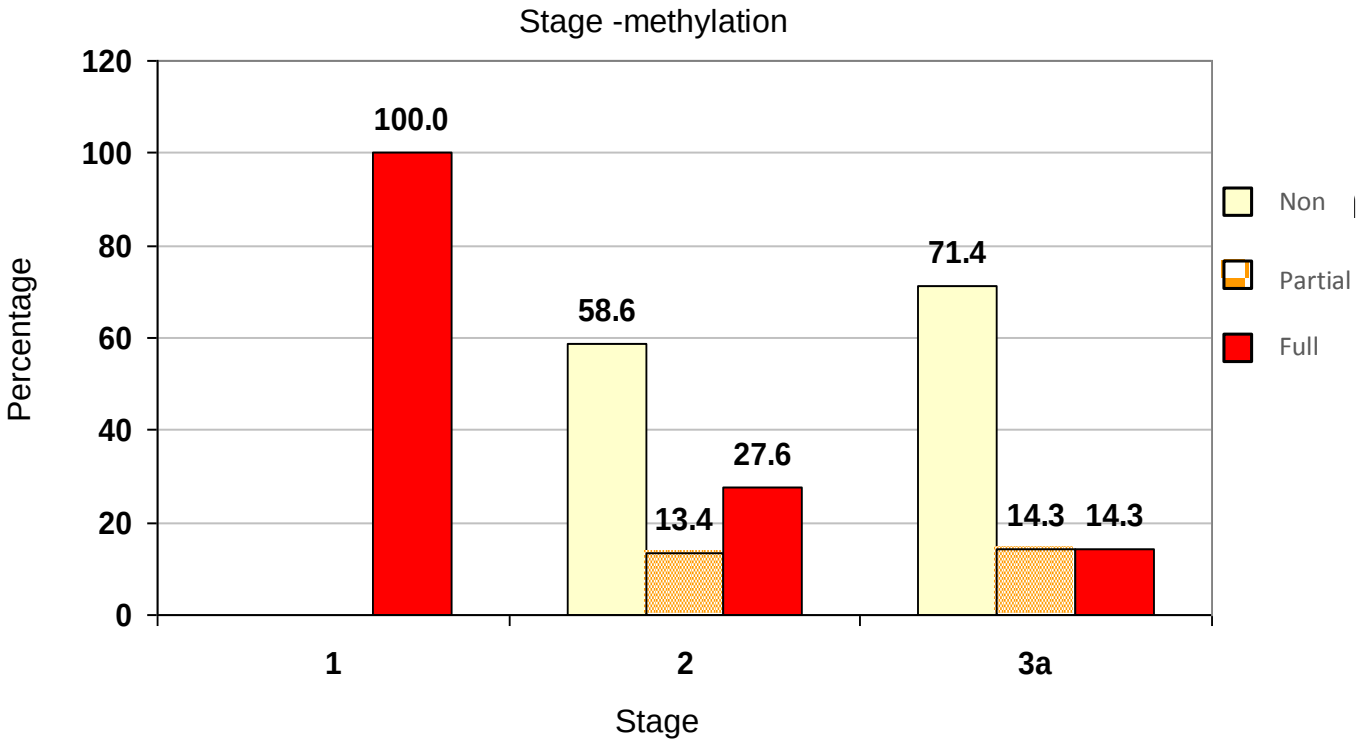

Figure 3. Comparison of tumor stage and methylation status in tumor samples. (correlation is significant at the 0.05 level,2 tailed) (p = 0.046).

Table 1. Methylation pattern and tumor stage in breast tumor samples.

		Correlation Coefficient	Sig. (2-Tailed)
	CPG_197	0.23	0.111
	CPG_190	0.21	0.135
	CPG_187	0.19	0.196
	CPG_185	0.23	0.111
	CPG_183	0.33(*)	0.021
	CPG_182	0.27	0.061
	CPG_176	0.28(*)	0.049
	CPG_162	0.28(*)	0.049
	CPG_155	0.21	0.135
	CPG_152	0.35(*)	0.013
	CPG_148	0.28(*)	0.049
	CPG_145	0.33(*)	0.021
	CPG_141	0.21	0.135
	CPG_132	0.33(*)	0.021
	CPG_127	0.23	0.111
	CPG_124	0.23	0.111
	CPG_112	0.23	0.111
	CPG_109	0.33(*)	0.021
	CPG_101	0.23	0.111
STAGE	CPG_99	0.29(*)	0.039
	CPG_81	0.33(*)	0.021
	CPG_77	0.23	0.111
	CPG_74	0.28(*)	0.049
	CPG_71	0.23	0.111
	CPG_54	0.27	0.061
	CPG_53	0.33(*)	0.021
	CPG_48	0.33(*)	0.021
	CPG_47	0.29(*)	0.039
	CPG_43	0.34(*)	0.017
	CPG_42	0.23	0.111
	CPG_40	0.23	0.111
	CPG_38	0.23	0.111
	CPG_23	0.33(*)	0.021
	CPG_22	0.25	0.075
	CPG_15	0.23	0.111
	CPG_14	0.23	0.111
	CPG_13	0.23	0.111
	CPG_11	0.23	0.111
	CPG_8	0.36(*)	0.010
	CPG_4	0.27	0.061

109, 99, 81, 74, 53, 48, 47, 43, 23 and 8 nts (p = 0.048) (Table 1).

DISCUSSION

Cancer is a disease associated with both genetic and epigenetic changes. Epigenetic gene regulation has been recognized to play a role in the etiology of cancer. Abnormal DNA methylation is a hallmark of cancer and often mediates silencing of gene (Xiang et al., 2008).

In this study, we provided a status of methylation extant in the promoter of the detoxification enzyme GSTP1. Previously, the hypermethylation of GSTP1 promoter has mainly been studied by methylation specific PCR (MSP) and was found to be methylated in 24 to 30% of breast tumors (Muggerud et al., 2010; Esteller et al., 2001). Bae et al. (2005) reported that GSTP1 is one of the

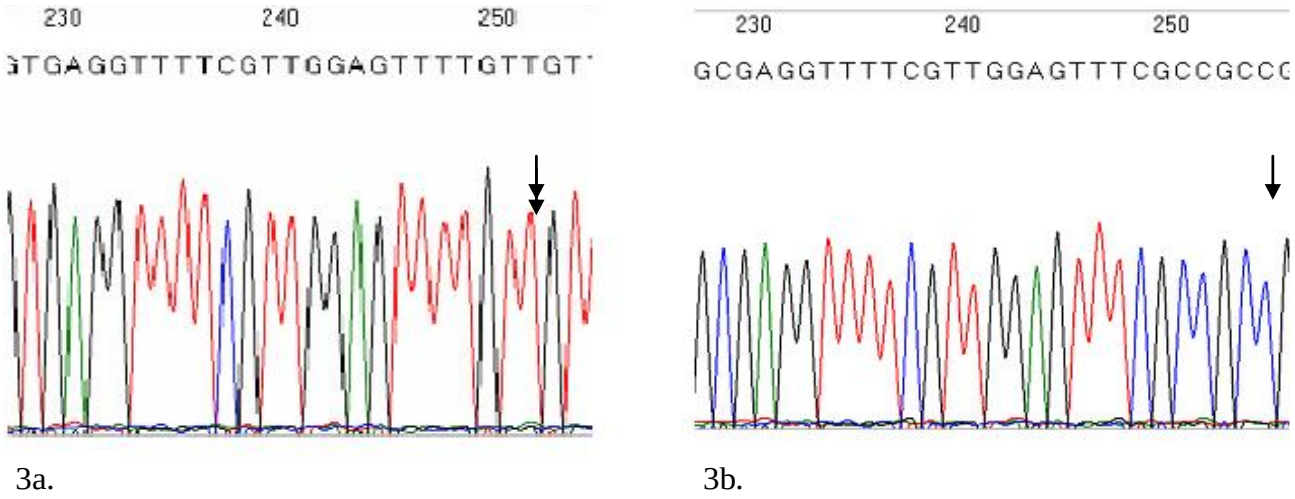

Figure 4. Examples of direct sequencing chromatograms. Bisulfite treated DNA was amplified and sequenced on ABI automated sequencer. The sequences represents CpG sites one of which marked by arrows. 3a) Complete conversion of cytosine to thymine (non-methylated) normal breast tissue. 3b) Complete non-conversion of cytosine to thymine (complete methylated) breast tumor tissue.

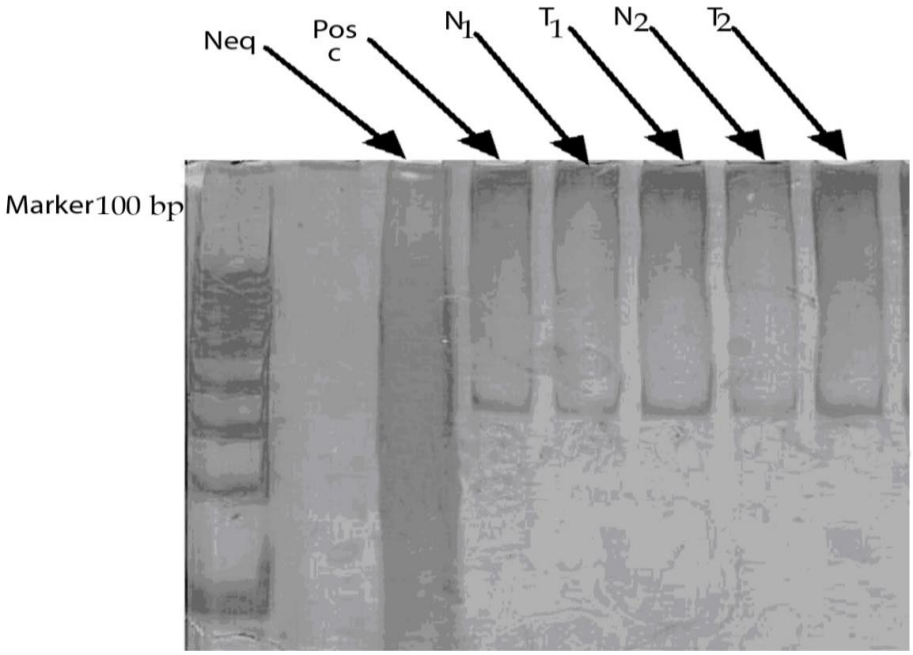

Figure 5. Electerophoresis of BSP products in acrylamide gel, produces visible bands both in methylated and nonmethylated samples with BSP specific designed primers that amplifies both methylated and non methylated DNA samples (positive control is ready to use positive universal methylated DNA sample and negative control is non bisulfite treated genomic DNA).

hypermethylated genes during breast cancer. Quantitative DNA methylation profiling is a powerful tool to identify molecular changes associated with tumors for GSTP1, it might be predictive factor for the response to and efficacy of doxorubicin treatment (Dejeux et al., 2010).

We found that the GSTP1 promoter was methylated in 41.3% of the breast tumors analyzed by bisulfate specific PCR (BSP) method and comparison was made between tumor tissues and normal neighbor breast tissues by direct bisulfate sequencing (Figure 4 and Figure 5). Most of them were seen in Stage 2 that also had the highest

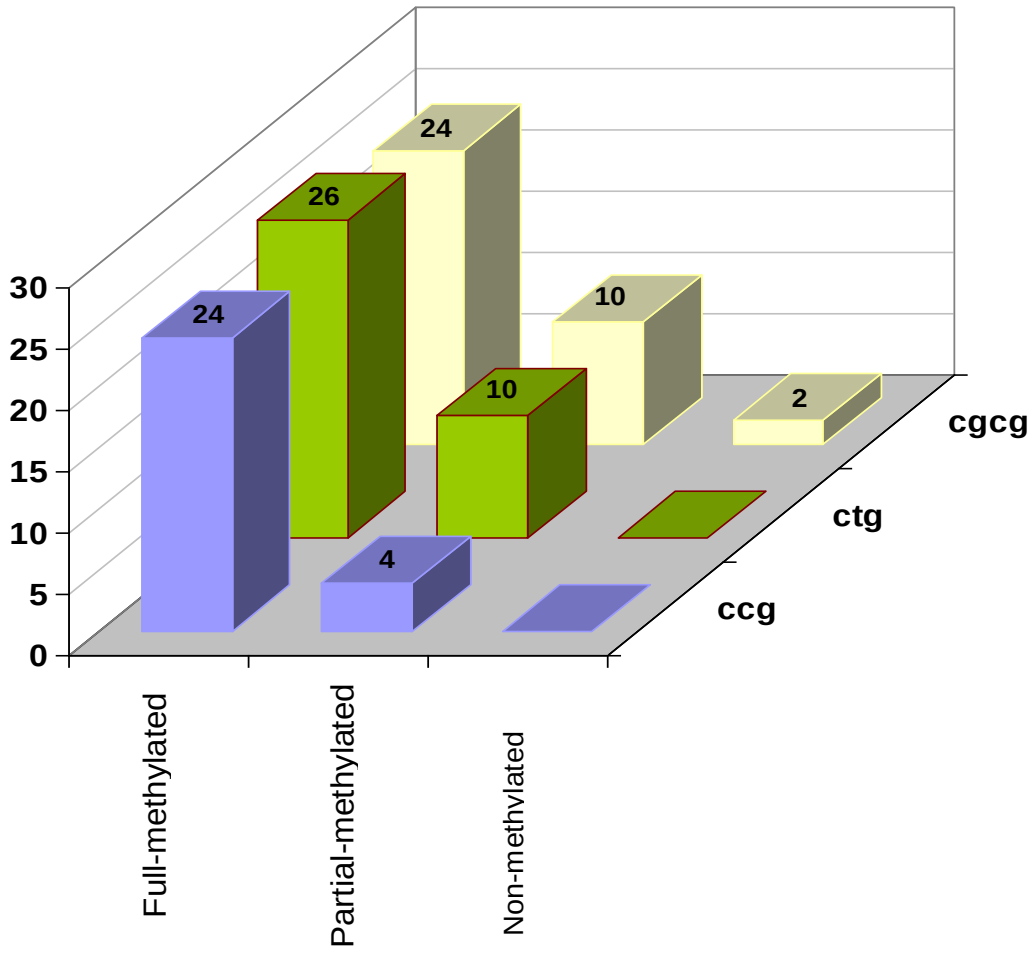

Figure 6. Comparison of ccg,ctg and cgcg sites methylation status in GSTP1 promoter region in full,partial and non methylated levels(chi-square test; p=0.000).

number in all tumor samples. The comparison of normal neighbor tissues with tumor tissues, showed the significant difference between methylation status among them (p = 0.000). There was also statistically significant difference in methylation pattern between normal and tumor samples (p = 0.000).

According to several studies, GSTP1 gene is methylated in various types of tumors. Fang et al. (2007) had found effectiveness of DNA methyltransferase inhibitors in demethylation of the CpG islands in the promoters and reactivation of methylation silenced genes such as $P16^{ink4}$, retinoic acid receptor β, human mutL homolog 1 and glutathione-S-transferase π. They showed that these activities in human esophageal, colon, prostate and mammary cancer cell line and the activity can be enhanced by longer term treatment with polyphenolic compounds, the present study was performed on breast tumor and normal tissues from the same area.

It has been reported that the pattern of aberrant methylation of individual or multiple genes can be associated with clinically useful information, such as cancer risk assessment, cancer progress, early detection and responses to therapy, therefore, these features make DNA hypermethylation as an excellent biomarker candidate (Vaziri Gohar, 2010). As the special structure of CpG sites, There were also some CG contained sites as ccg, ctg and cgcg that had methylated in significant values in breast tumor samples (Figure 6).

Poplawski et al. (2008) reported hypermethylation of GSTP1 in gastric cancer which was also associated with age, gender, smoking and family history. Promoter methylation of GSTP1 was best analyzed in prostate cancer. It has also been demonstrated that, GSTP1 methylation is an early event in prostatic carcinogenesis, because in high-grade prostatic intraepithelial neoplasia loss of GSTP1 expression is caused by DNA methylation. Many other types including breast cancer and cholangiocarcinoma showed a GSTP1 hypermethylated promoter. In hepatocellular carcinoma, methylation of

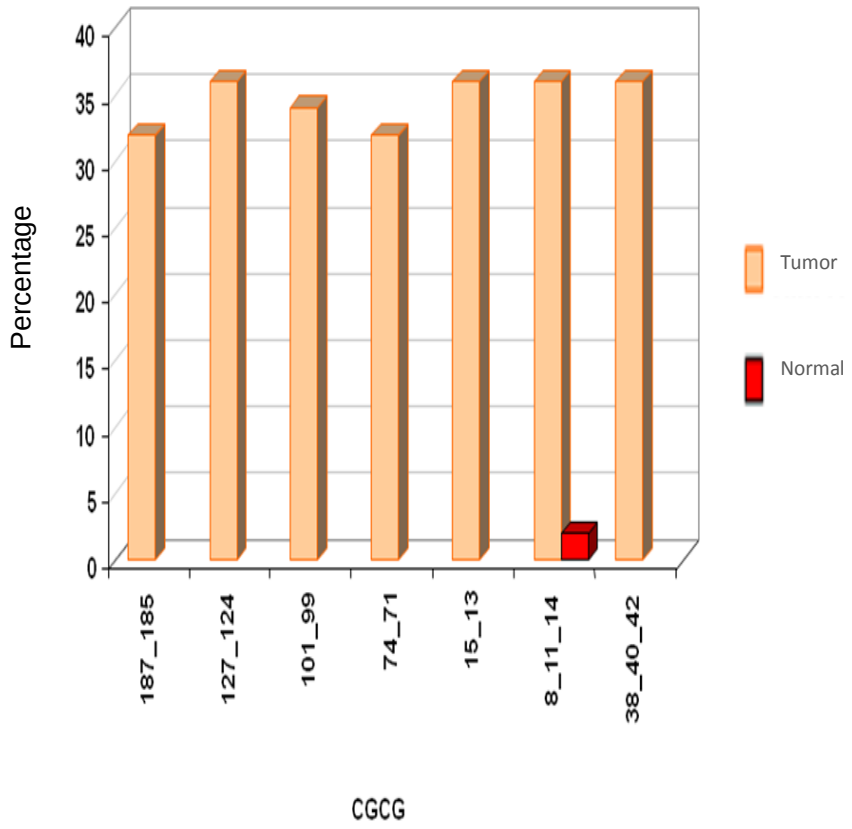

Figure 7. Prevalence of methylated cgcg sites in the promoter region of GSTP1 gene in breast cancer samples (chi-square test; p=0.000).

GSTP1 gene occurred in 41 to 85% (Tischoff and Tannapfel, 2008). The consequence of silencing or loss of GSTP1 gene as a biotransformator of electrophilic substances, is higher incidence of mutations that has been established in prostate cancer and also was found in breast cancer tissue, hypermethylation could be detected in the early stage of breast cancer, but not observed in normal or bengin breast tissue (Paluszczak and Baer-Dubowska, 2006).

Due to small number of *in situ* and lobular form of tumors in this work, evaluation of a possible increasing trend of GSTP1 methylation in malignant progression was not possible. The GSTP1 methylation status did not correlate with tumor number or chemotherapy history. There was no significant correlation between tumor grade and stage indicating lack of common methylated site related to both aspects of the tumor. Evaluating of CpG sites methylation pattern in prometr region of GSTP1 was performed in new design and methodology.

BSP product sequencing of tumor samples showed considerably higher promoter CpG sites methylation when compared with normal samples, with higher prevalence in 197, 185, 127, 124, 112, 101, 77, 71, 42, 40, 38, 15, 14, 13, 11 nt (36%) that also has correlation with those that are most prevalence in methylation according to tumors stage and grade(data not shown). This might be related to higher predisposition of some CpG sites to methylation which has a key role in cancer progression. Notably all of these sites belong to cgcg,ctg and ccg series of CpG sites that are the special composition of CpG sites, so this may have a specific mean and importance through promoter region and already have specificity to tumor samples (Figure 7).

According to King-Baton et al. (2008), treatment with genistein or lycopene at non-toxic concentrations partially demethylated the GSTP1 promoter and reactivated GSTP1 expression in human breast cancer cells. Both of these substances are known to have chemopreventive activity against prostate cancer (Klein, 2006), probable mechanism is due to inhibiting the expression of DNMT and resultant re expression of their mRNA (Xiang et al., 2008), indicating effectiveness of methylation mechanism in gene silencing (Dietrich et al., 2009).

Hypermethylation of the GSTP1 promoter with reduced expression levels is detected in precursor high-grade intraepithelial neoplasia and absence of GSTP1 expression with promoter hypermethylation is evident in prostate cancer. Inactivation of GSTP1 may leave cells

vulnerable to oxidative damage and/or tolerant to accumulation of oxidized DNA base adducts (Donkena et al., 2010). In the present study methylation is present even in early stages and grades of tumors showing the methylation progress from starting the carcinogenesis event although this changes are present also in some degrees in higher tumor stages and grades (Figures 2 and 3). GSTP1 methylation is correlated with Gleason grade and prostate volume, suggesting that quantitative GSTP1 methylation maybe of prognostic significance (Zho et al., 2004). In our study GSTP1 methylation seem to be occurring in early steps of malignancy but if treatment applying is followed, it may have significance value to prognosis of therapy effectiveness. On the other hand the relationship between methylation classes and several covariants like patient age, alcohol consumption, dietary folate, estrogen receptor status and tumor size are not included according to AJCC staging system (Christensen et al., 2010).This finding maybe explain the discrepancy of staging of tumor with methylation status.

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REFERENCES

Bae YK, Shim YR, Choi JH, Kim MJ, Gabrielson E, Lee SJ, Hwang TY, Shin SO (2005). Gene promoter hypermethylation in tumors and plasma of breast cancer patients.Cancer. Res. Treat., 4: 233-240.

Bedgia NG, Sagredo AA, Guerra I, Viguri A, Albania C, Diaz I R, Rezola R, Alberdi MJ, Dopazo J, Montaner D, Renobales M, Fernandez AF, Field JK, Fraga MF, Liloglu T, Pancorbo MM (2010). DNA methylation epigenotypes in breast cancer molecular subtypes.Breast. Cancer. Res., 12: R77-89.

Chow HHS, Hakim IA, Vining DR, Crowell JA, Tome ME, Moore JR, Cordova CA, Mikael DM, Briehl MM, Alberts DS (2007).Modulation of human glutathione S-transferases by polyphenon E intervention.Cancer. Epidemiol. Biomarkers. Prevent., 16: 1662-1666.

Christensen BC, Kelsey KT, Zheng S, Houseman EA, Marsit CJ, Wrensch MR, Wiemels JL, Nelson HH, Karagas MR, Kushi LH, Kwan ML, Wiencke JK (2010). Breast cancer DNA methylation profiles are associated with tumor size and alcohol and folate intake.PloS. Gen., 6: e1001043.

Dejeux E, Ronneberg JA, Solvang H, Bukholm I, Geisler S, Aas T, Gut IG, Dale ALB, Lonning PE, Kristensen VN, Tost J (2010).DNA methylation profiling in doxorubicin treated primary locally advanced breast tumors identifies novel genes associated with survival and treatment response. Mol. Cancer., 9: 68-81.

Dietrich D, Lesche R, Tetzner R, Krispin M, Dietrich J, Haedicke W, Schuster M, Kristensen G (2009). Analysis of DNA methylation of multiple genes in microdissected cells from formalin-fixed and paraffin-embedded tissues. J. Histol. Cytochem., 57: 477-489.

Esteller M, Corn PG, Baylin SB, Herman JGA (2001). Gene hypermethylation profile of human cancer. Cancer Res., 61: 3225-3229.

Esteller M (2008) .Epigenetics in cancer.N. Engl. J. Med., 358: 1148-1159.

Fang M, Chen D, Yang C (2007) .Dietary polyphenols may affect DNA methylation.J. Nutr., S223-228.

Gao P, Yang X, Xue YW, Zhang XF, Wang Y, Liu WJ, Wu XJ (2009).Promoter methylation of glutathione S-transferase π1 and multidrug resistance gene1 in broncholoalveolar carcinoma and it's correlation with DNA methyltransferase 1 expression. Cancer, 115: 3222-3232.

Holly SL, Fryer AA, Haycock JW, Grubb SEW, Strange RC, Hoban PR (2007). Differential effects of glutathione S-transferase Pi(GSTP1) haplotype on cell peoliferation and apoptosis.Carcinogenesis, 28: 2268-2273.

Hoque MO, Feng Q, Toure P, Dem A, Critchlow CW, Hawes SE, Wood T, Jeronimo C, Rosenbaum E, stern J, Yu M, Trink B, Kiviat NB, Sidransky D (2006). Detection of aberrant methylation of four genes in plasma DNA for the detection of breast cancer.J. Clin. Oncol., 24: 4262-4269.

King-Baton A, Leszczynska JM, Klein CB (2008).Modulation of gene methylation by genistein or lycopene in breast cancer cells.Environ .Mol. Mutagene., 49: 36-45.

Klein EA (2006). Chemoprevention of prostate cancer.Annu. Rev. Med., 57: 49-63.

Li M, Paik HH, Balch C, Kim Y, Li L, Huang THM, Nephew KP, Kim S (2008). Enriched transcription factor binding sites in hypermethylated gene promoters in drug resistant cancer cells. Bioinformatics, 24: 1745-1748.

Maxwell A, McCudden CR, Wians F, Willis MS (2009). Recent evidences in the detection of prostate cancer using epigenetic marker in commonly collected laboratory samples.Labmedicine, 40: 171-178.

Muggerud AA, Ronneberg JA, Warnberg F, Botling J, Busato F, Jovanovic J, Solvang H, Bukholm I, Dale ALB, Kristensen VN, Sorlie T, Tost J (2010).Frequent aberrant DNA methylation of ABCB1,FOXC1,PPP2R2B and PTEN in ductal carcinoma in situ and early invasive breast cancer.Breast. Cancer Res., 12: R3(1-10).

Paluszczak J, Baer-Dubowska W (2006). Epigenetic diagnostics of cancer-the application of DNA methylation markers. J. Appl. Genet., 47: 365-375.

Poplawski T, Tomaszewska K, Galicki M, Morawiec Z, Blasiak J, Morawiec Z, Blasiak J (2008). Promoter methylation of cancer-related genes in gastric carcinoma. Exp. Oncol., 30: 112-116.

Ronneberg JA, Tost J, Solvang HK, Alnaes GIG, Johansen FE, Brendeford EM, Yakhini Z, Gut IG, Lonning PE, Dale ALB, Gabrielsen OS, Kristensen VN (2008). GSTP1 promoter haplotypes affect DNA methylation levels and promoter activity in breast carcinomas.Cancer Res., 68: 5562-5571.

Suzuki T, Takagi Y, Osanai H, Li L, Takeuchi M, Katoh Y, Kobayashi M, Yamamoto MM (2005). Pi class glutathione S-transferase genes are regulated by Nrf2 through an evolutionarily conserved regulatory element in zebrafish.Biochem. J., 388: 65-73.

Tischoff I, Tannapfel A (2008).DNA methylation in hepatocellular carcinoma.World. J. Gasteroenterol., 14: 174-1748.

Vaziri Gohar A (2010).Clinical application of epigenetic markers in diagnosis and treatment of cancer.J. Biol. Sci., 10: 373-385.

Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, Rebhan M, Schubeler D (2007). Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat. Genet., 39: 457-466.

Wu Y, Alvarez M, Slamon D, Koeffler P, Vadgama JV (2010). Caspase 8 and maspin are downregulated in breast cancer cells due to CpG site promoter methylation.BMC. Cancer, 10: 32-44.

Xiang N, Zhao R, Song G, Zhong W (2008). Selenite reactivates silenced genes by modifying DNA methylation and histones in prostate cancer cells. Carcinogenesis, 29: 2175-2181.