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Association between matrix metalloproteinase-2 gene variants and pathogenesis of breast cancer in sera of Iraqi women

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Abstract

Objective: The current study aims to investigate the role of matrix metalloproteinase-2 (MMP-2) in breast cancer pathogenesis in Iraqi women.

Methods: Forty-one (41) women with breast cancer and 45 control women were included in this case–control study. Body mass index, age, smoking; married status, tumor size, degree, subtype, lymph node status, pre- and post-menopause were included the phenotypic results. The polymerase chain reaction (PCR)-allele specific restriction was used to observe the rs243865 polymorphism. Genomic DNA was extracted from whole blood and genotyping with specific prefixes for amplification of the MMP-2 gene was accomplished as enzyme-restricted PCR products were digested, followed by electrophoresis on 1.5% agarose gel. In order to interpret the researchers' results, numerous statistical analyses were applied.

Results: The amplicon size of MMP-2 gene was 304 bp, and following its amplification reactions by allelic specific PCR. The amplification product for MMP-2 gene amplification SNP rs243865 gene polymorphism results exhibited one band of 304 bp, two bands of 304 bp, and one band 304 bp for individuals have genotype as wild type (CC), homozygous (TT), and heterozygous (CT), respectively. Genotype frequencies of rs243865 polymorphism were found to be consistent with Hardy–Weinberg equilibrium. Allele frequencies of C allele was 0.57, and the T allele was only 0.43 in cases of breast cancer women patient, while the frequencies of CC, CT, and TT genotypes of the rs243865 SNP were statistically significant as 31.7%, 51.2%, 17.1%, respectively. Allele frequencies of C and T were 0.78 and 0.22 for the control group, respectively, the heterozygous genotype (CT) was significantly increased the risk of breast cancer women (OR=0.3, 95% CI; 0.12–0.8, P≤0.05) with respect to those of the CC wild type. The TT genotype significantly raised the risk of breast cancer women by (OR = 0.2, 95% CI; 0.04–0.9, P≤0.05).

Conclusion: In women with breast cancer, MMP-2 expression's high association were observed with positive lymph node, histological classification of breast cancer (II) was higher than other classes and advanced clinical process (II).

Keywords: MMP-2, breast cancer, allele, polymerase chain reaction, genotype, SNP

Introduction

Breast cancer is a genetic disease caused by a multiple series of mutations in oncogenes, tumor suppressor genes, and stability genes. About 5–10% of all breast carcinomas are caused by germ-line mutations.[1] Breast cancer occurs in both men and women, although male breast cancer is rare.[3] Most masses detected on a mammogram and most lumps of the breast grow out to be benign.[4] Most carcinoma types of cells have an allowing control, while others function actively and are likely metastatic.[5] The sign and symptoms of breast cancer differ, sometimes it can be scientifically difficult to differentiate between malignant and benign tumors. A triple test must be carried out: psychiatric assessment, breast screening, and a biopsy examination.[13] The concordance of all three modalities is a standard control to prevent false detects. The risk of developing breast cancer is impacted by multiple genetic, behavioral, and environmental factors.[4] The involvement of breast cancer in every first-degree female parent almost doubles the risk for a genotype, and the risk grows continuously with the number of relatives affected.[18] Study shows that breast cancer risk correlates with the acquired dose of radiation. Risks became higher in patients under 50 years old than where the individual was diagnosed before 50.[6] Lung cancer is the most common disease diagnosed, and the major cause of death from cancer. It is closely accompanied by female breast cancer (11.6%), prostate cancer (7.1%), and colorectal cancer (6.1%).[7]

Breast cancer is a malignant tumor dart which begins in breast cells. There are several variables that will increase the chance of developing breast cancer, among most cancers. The immune system typically watches out for and kills cancer cells and cells with weakened DNA.[8] The failure of such a successful immune response and surveillance could cause breast cancer. Some people inherit DNA mutations and genes such as BRCA1, BRCA2, and P_{53}. Anyone with a family history of ovarian or breast cancer was at an elevated risk of breast cancer.[9] The pathologist classifies cancer by degree. An often-fatal feature of malignant tumors is the ability of cancer cells to infiltrate other tissues and propagate to distant organs. Proteolytic enzymes play a vital role in the development of cancer. In the human genome, over 500 genes encoding proteases or protease-like proteins are present.[10] The matrix metalloproteinase-2 (MMP-2) make up a large family of zinc-dependent endopeptidases multidomain spread in all kingdoms except protozoa. Each MMP has a signal peptide to guide trafficking.[11] MMP-2 is a well-known cancer metastasis mediator but is also believed to be active in many cancer development factors including cell growth and inflammation. That works with the degradation of type IV collagen. The MMP-2 gene is 16q13 in the chromosome, it is 17 kb long with 13 (thirteen) 110–901 bp exons and 175–4350 bp 12 introns.[16] Genetic variation that modulates the expression of MMP-2 may lead to the individual cancer susceptibility differences. In the MMP-2 promoter, two

single nucleotide polymorphisms (SNPs) have been shown to affect *in vitro* expression; C to T transformations at −1306 (rs243865).[2] As it is a part of an extracellular matrix (ECM), this results in the lack of cellular structural support, and thus in the destabilization of the basal membrane, an important step for cancer spread. Altered MMP-2 activity arising from the involvement of unique MMP-2, this included variants in the degrade of ECM and disturbance of the membrane barrier in the basement.[14] MMP-2 protein has been regulated by immune histochemical approaches in breast carcinoma cells. In processing, growth factors, including EGF-like growth factor (HB-EGF) and TGFβ are thought to play.[12] Several studies have shown that genetic polymorphism regulates the expression of MMP-2 in its promoter region and that sustained elevated levels of MMP-2 could make carries more vulnerable and aggressive to tumorogenesis. In this context, we explored the relationship between susceptibility to breast cancer and the existence of variants of MMP2 (promoter): rs243865 (1306 C/T), as possible biomarkers of breast cancer risk.

Materials and Methods

Forty-one (41) women with breast cancer with age ranged between 16 and 82 years, and another 45 apparently normal women as a control group with age ranged between 16 and 55 years were included in a case–control study during Nov. 2019 to Sep. 2020. Cases and controls were recruited from the Imam Hussein Oncology Center, Al-Hussein Teaching Hospital, Al-Hussein Medical City/Kerbala Health Directorate – Iraq. Women in control were selected from the patient's relatives in a hospital from the general population women-only individuals who did not show signs and symptoms of any chronic diseases such as high blood pressure, kidney disease, heart disease, or others were selected to take part in this study, were free of breast cancer personal or family background, and were comparable in self-declared ethnic origin to patients. The breast cancer diagnosis was consistent with the American Cancer Society's recommendations (www.cancer.org). This included mammography and breast biopsy tests to confirm breast cancer; four of these were performed in all patients. Unrelated comorbidities are observed in one subject (cases and controls). For patients from medical reports and interviews, the demographic profile and clinical bio-data were gathered using a standardized questionnaire by doctors or senior residents. This included age at research entrance, age at first breast cancer diagnosis, body mass index (BMI), menopause status pre-and post-menopause, tumor size, level of disease at the appearance, marital status, and smoking history. The histological review covered disease stage and nuclear grade status, estrogen receptor (ER), and progesterone receptor (PR) status prior to treatment (chemotherapy, surgery, and radiation), and matrix metalloprotease genotypes-2. The study was done and approval was obtained from the Research and Ethics Committee at the College of the Medicine / University of Kerbala all patients and control subjects provided written informed consent.

Regarding the MMP-2 gene, one SNP, with a minor frequency of alleles. The National Center for Biotechnology Information (NCBI) Gene SNP Gene view was used to identify Iraqi women and clinical significance (www.ncbi.nlm.nih.gov / projects / SNP/). These included rs243865 (context sequence GAGACCTGAAGCTAAAGGGTG (C / T) AAGACATAATCG TGACCTCCAAATG). MMP-2 genotyping was performed by the allelic discrimination method, using DNA extraction samples Whole blood samples from the patient and healthy control group were collected in EDTA heat tube cycles (T Professional, Biometra, Germany). DNA template supplement, Thermus aquatics DNA polymerase (Taq polymerase), deoxynucleotide triphosphates (dNTPs) and buffer solution, applied polymerase chain reaction (PCR) performed with a volume of 5 mL primer volumes, 5 mL DNA volumes, PCR temperature: 58) 25 mL total volume by gel electrophoresis separates DNA fragments by size in an agarose gel. The reproducibility of genotyping was verified by the average rate of successful genotyping per sample and SNP.

Statistical analysis was done on program PAST version 3.09. Mean±SD and percent total were used in presenting continuous and categorical data, respectively. Mean differences and intergroup significances were evaluated using Student's *t*-test and Pearson x2 test, respectively. Genetic Power Calculator was employed for calculating the study power, considering the number of study subjects (41 patients and 45 controls), BMI of the included variants, breast cancer prevalence in Kerbala/Iraq (estimated), and relative risk for heterozygous (1/2) and minor allele homozygous (2/2) genotypes.

Hardy–Weinberg equilibrium (HWE) law was used for genetic calculation which states that allele and genotype frequencies in a population will remain constant from one generation to the next generation in the absence of disturbing factors. HWE can be used to calculate the expected common homozygotes, heterozygotes, expected rare homozygotes, and the frequency range of the 2 (p and q) alleles from the observed genotypes. Calculation of odds ratios (ORs) and 95% confidence intervals (95% CIs) associated with the risk of breast cancer was determined using logistic regression analysis; statistical significance was set at *P* value≤0.05.

Results

Results showed significant differences between breast cancer patients and control women were noted in (age ; P≤0.01) BMI (P≤0.01), number of smokers (P=0.3), and number of married (P≤0.01) were noted in patients than in control women. These covariates were selected as the main covariates that were controlled for later analysis as showed demographic of study participants are listed in Table 1.

Table 1. **The demographic characteristics of BC and non-BC.**

Demographical Characteristic		Patients N = 41	Control N = 45	Statistical analysis
Age (y)		47.36 ±14.29	32.6 ± 7.02	≤ 0.01
BMI		29.6 ± 5.5	24.6 ± 3.2	≤ 0.01
Smoking	Yes	6	11	*P* value = 0.3 $\chi^2 = 1.3$
	No	35	34	
Marriage	Yes	36	23	*P* value ≤ 0.01 =13.4 χ^2
	No	5	22	

BMI: body mass index, Age (y) Student's t-test (continuous variables) and Pearson's x2 (categorical variables). Mean ± SD. , Number of subjects (percent total).

Table 2. **Genotype of MMP-2 gene (-1306 C/T) polymorphism.**

MMP-2 (- 1306 C> T) SNP (rs243865) Genotype	Patient N = 41	Control N = 45	Odds ratio	CI 95%	*P* value
CC (Ref)	13 (31.7%)	28 (62.2%)	-	-	-
CT	21 (51.2%)	14 (31.1%)	0.3	0.12 – 0.8	≤ 0.05
TT	7 (17.1%)	3 (6.7%)	0.2	0.04 – 0.9	≤ 0.05

MMP-2: matrix metalloproteinase-2; SNP: single-nucleotide polymorphism; OR: odds ratio. SNP genotyping rs243865was done by allelic exclusion method primers. (Major allele.minor allele,Minor allele C> T number (frequency). Crude (unadjusted) OR.

Table 3. **Hardy-Weinberg equilibrium for MMP2 (- 1306 C> T) (rs243865).**

MMP-2 (-1306 C> T) (rs243865) Genotype	Groups							
	Patients N= 41				Control N= 45			
	H-W observed frequency	%	H-W expected frequency	%	H-W observed frequency	%	H-W expected frequency	%
CC	13	31.7	13.47	32.85	28	62.2	27.2	60.4
CT	21	51.2	20.06	48.93	14	31.1	15.6	34.7
TT	7	17.1	7.47	18.22	3	6.7	2.2	4.9
Total	41	100	41	100	45	100	45	100
x^2	0.09				0.45			
P value HWE	≥ 0.05				≥ 0.05			
Allele frequency								
C	0.57				0.78			
T	0.43				0.22			

MMP-2: matrix metalloproteinase-2; SNP: single-nucleotide polymorphism; HWE: Hardy–Weinberg equilibrium; OR: odds ratio. SNP genotyping was done by allelic exclusion method, Major allele.minor allele.

In Table 1, patients were stratified to <50 years by age versus those aged <50 years The mean ± SD value of the age of patients breast cancer (47.36±14.29 years) was significantly higher than control (32.6±7.02 years). When analysis was performed on individual stratification age, incidences of breast cancer were significantly different P<0.01. The diagnosis records of female patients with breast cancer were enrolled in the study. Most of the patients (56.09%) were diagnosed with breast cancer before the age of 55. However, only 43% of the cases were diagnosed above the age of 55. Patients were reported to include a higher prevalence of women with prolonged menstruation (P≤0.01), past oral contraceptive users (P≤0.01), and a lower frequency of women breastfeeding (P≤0.01). A statistical disparity in marital status, educational level, smoking number (P=0.3, χ^2=1.3), and married status was observed (P≤0.01=13.4 χ^2). The prevalence of married patients among younger women under 50 years of age was substantially higher; while the characteristics of illiteracy and nulliparity were statistically lower (P≤0.05). Including palpable lumps, bloody nipple discharge, skin changes, tumor size, lymph node status, and stage of the disease, observational variations were observed with respect to the clinical appearance of the evaluated patients. A statistically significant increase in the frequency of polymorphic genotypes (CT) and (CC) was observed in the breast cancer group compared to the control group with P≤0.05. As regards allelic frequency of MMP-2 gene, C allele was statistically significantly higher in the breast cancer group the C allele significantly higher in the control group with (P value ≤0.05). By calculating the odds ratios for CT and TT, the results is 0.3 and 0.2, respectively (Table 2).

Genotyping frequencies of MMP-2 gene were analyzed with HWE. The results were showed in Table 3 for the MMP2 (-1306 C> T) (rs243865) genotype SNPs the breast cancer patients group (χ^2 = 0.09, C allele frequencies =0.57 and the T allele frequency =0.43, P≤0.05), and control (χ^2=0.45, C allele frequencies=0.78 and for allele T is 0.22, P≤0.05). The analysis of results showed that the (rs243865) of MMP-2 gene (C→T) genotype frequencies of wild genotype (CC), heterozygous genotype (CT), and homozygous genotype (TT) were 31.7%, 51.2 %, and 17.1%, respectively in breast cancer patients and 62.2%, 31.1%, and 6.7% in the control group.

The results of the Table 3 showed chi-square of examined SNPs in the smoking patients and control CC allele is low compared with no smoking in-patient and control, while the chi-square (x^2) = 5.07 is significant at P = 0.7. In the CT allele, we show the chi-square examine of SNPs in smoking patients and

control compared with no smoking number in-patient and control, while the chi-square (x^2) value 0.3 at $P = 0.6$. And the last allele TT, the result of smoking patient and control compared with no smoking patient and control the chi-square (x^2) value 2.7 at $P = 0.098$. The results showed that the chi-square (chi-square) of the SNPs that were examined in the married patient and control group with a high CC allele compared with the unmarried, the chi-square value (x^2) 5.07 at $P \leq 0.05$. For a CT allele in a married patient, the chi-square of the SNPs examined, control compared with his unmarried patient, married, and control, the chi (x^2) value is 4.4 at $P \leq 0.05$. The result is the last allele TT is the chi-square of the SNPs examined in a married patient, control compared with unmarried, the chi (x^2) value of 0.47 at $P = 0.49$. Most were married with an average of 2–4 children (95% pre- and 97% post-menopausal), 2% have a close relative of cancer of breast cancer. Relationship between BMI and the occurrence of genotypes of MMP-2, we divided the group of patients with breast cancer into two simple categories based on mean ± SD: the first includes patients with a BMI of the CC allele (31.2 ± 6.4) and the second group with a mass index is 24.3 ± 2.3 with a $P \leq 0.01$. The second allele is CT included in the patient (28.6 ± 5.5) and control is (24.7 ± 3.98) with $P = 0.05$. Finally, the TT allele is 30 ± 3.7 and the control (26.3 ± 6.4) with a $P = 0.3$. When comparing the TT and CT genotype versus the wild (CC) genotype, a significant difference was observed between the control group and the patient group with a $P \leq 0.01$.

Discussion

Breast cancer is the primary cause of cancer-related deaths worldwide. Several studies have shown enhanced expression of MMP-2 in breast cancer, and genetic functional variants in the MMP-2 gene were correlated with altered breast cancer susceptibility.[19,22] Our analysis found that the correlation of rs243865 and MMP-2 SNP with the occurrence and aggressiveness of breast cancer was correlated with breast cancer in multiple populations. The MMP-2 gene contains many polymorphisms on chromosome 16, of which the promoter variant rs243865 is related to lower promoter activity.[22] The decreased expression is predicted to be associated with a decreased risk of cancer in the development of diseases, including breast cancer. Second, several genetically engineered animal studies have linked a low degree of constitutive expression of MMP-2 with a decreased risk of tumor development. It was observed that mice missing the MMP-2 gene developed fewer tumors than wild-type mice when caused by a carcinogenic stimulus.[31] It was observed that cancer cells inserted into a vein were less likely to colonize the lungs of MMP-2 knockout mice than those of wild-type mice.[32] Although some familial risk may be due to shared environmental factors, there may be other common, low penetrance genetic variants affecting susceptibility to breast cancer. Because the MMP-2 system has a significant impact on the development and progression of cancer, including breast cancer, and because genetic polymorphisms in the

Table 4. **Demographic with MMP-2.**

Demographic with MMP-2 (- 1306 C> T) (rs243865) genotype			Patients N =41	Control N = 45	Chi square test
Smoking	CC	Yes	2	6	P value = 0.7 $x^2 = 0.2$
		No	11	22	
	CT	Yes	3	3	P value = 0.6 $x^2 = 0.3$
		No	18	11	
	TT	Yes	1	2	P value = 0.098 $x^2 = 2.7$
		No	6	1	
Demographic with MMP2 (- 1306 C> T) (rs243865) Genotype			**Patients N = 41**	**Control N = 45**	**Chi square test**
Marriage	CC	Yes	12	16	P value ≤ 0.05 $x^2 = 5.07$
		No	1	12	
	CT	Yes	15	5	P value ≤ 0.05 $x^2 = 4.4$
		No	6	9	
	TT	Yes	6	2	P value = 0.49 $x^2 = 0.47$
		No	1	1	
BMI	CC		31.2 ± 6.4	24.3 ± 2.3	≤ 0.01
	CT		28.6 ± 5.5	24.7 ± 3.98	≤ 0.05
	TT		30 ± 3.7	26.3 ± 6.4	0.3

promoters of MMP-2 are correlated with decreased enzyme activity, we sought to determine whether these polymorphisms may be associated with varying risk of breast cancer. Based on an analysis of 41 patients with breast cancer and 45 controls, we found that the MMP-2 polymorphisms influenced the risk of developing the TNM stage and metastasis of breast cancer. Subjects carrying the variant genotypes of MMP-2 were at a moderately reduced risk of cancer. In addition, it appeared that the polymorphisms in the two genes had some additive effect regarding reducing breast cancer risk and the protective effects were more pronounced in younger subjects (S55 years old), which is in line with the conception that genetic susceptibility is often associated with an early age of disease onset. Our findings were consistent with other research,[21,23] which also reported a negative correlation between rs243865 and breast cancer risk. Our results were in strong disagreement with other studies,[24,25] which showed no link between rs243865 and breast cancer risk. The rs243865 minor (T) allele was functionally associated with disruption of the binding of SP1 binding elements, resulting in decreased activity of the promoter. As in a report, data were kept for the diagnosis of women with breast cancer. By the age of 55, most patients (78.0%) were diagnosed with breast cancer. However, only cases above the age of 55 were diagnosed (21.95%).[28] Demographic and therapeutic profiles of research subjects. Highly substantial BMI ($P \leq 0.01$) and percentage of smoke ($P \leq 0.01$) between breast cancer patients and control women were found to have relevant differences. A greater proportion of people with extended menstruation ($P \leq 0.01$), former oral contraceptive users ($P \leq 0.01$), and a lower incidence of women breastfeeding ($P \leq 0.01$) compared to control persons have been identified in patients. Such covariates were selected, as these covariates were chosen as the primary control covariates.[14] As calculated by the BMI, core obesity tends to have a greater effect on the prevalence of breast cancer in African–American females than general adiposity. The pre-menopausal women have 21% an elevated value of BMI and 29% of post-menopausal women with an abdomen diameter of 90 cm are also more likely to develop breast cancer.[29] The HWP deviation in the controls is determined by measuring the difference between the observed genotype frequencies and the expected frequencies. It is used in genotyping to identify errors. Using only controls for HWP assessment is reasonable when assuming an unusual condition in the study. In the present research, it is clear that the C allele for MMP-2 (-1306 C>T) was linked with breast cancer and may be considered a risk factor for disease progression.[27] Results from these studies may have implications for possible strategies for cancer prevention, especially for communication and therapy of person-level risk. In this respect, it is reassuring that even for people with the highest decile of risk for those with a low BMI, who did not smoke or drink and who did not use MHT, the risks for non-modifiable variables were equal to those of the average woman in the general population.[26] Despite contradictory effects, local overexpression of MMP-2 is commonly considered facilitating and prevents cancer invasion and metastasis by TIMP-2. A number of studies have shown that genetic polymorphisms in MMP-1 (1G/2G) or MMP-3 (5A/6A) promoters that modify gene transcription activity may affect the invasiveness or metastasis of certain types of cancer, such as melanoma,[34] colorectal cancer,[35] and breast cancer.[33] However, neither genotype of MMP-2 was substantially associated with the tumor stage or metastatic status of breast cancer at the time of diagnosis in the present study. These observations show that the polymorphisms studied in MMP-2 do not play a major role in suppressing or inducing local MMP-2 expression as a relevant genetic factor, and may therefore not serve as a sole risk marker for metastatic disease. However, there are certain drawbacks to our results on the metastasis of breast cancer since they were collected at the time of diagnosis. It may be appropriate to further analyze wider case series with prospectively followed-up clinical results, especially the survival rate.

Conclusion

The observed data indicated that in women with breast cancer, MMP-2 expression is highly associated with positive lymph node, histological classification of breast cancer (ll) which was higher than other classes, and advanced clinical process (ll).

Conflict of Interest

None

References

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