Original Article

Investigation of Microsatellite Instability BAT25 and BAT26 in Breast Cancer Patients by Conventional Polymerase Chain Reaction

Jabbar Salman Hassan, Basim Mohammed Hanon[1], Ahmed Hasan Mohammed[2], Thana Rasheed Abd Al-Rahman

Department of Microbiology, Faculty of Medicine, Al-Nahrain University, Baghdad, [1]Department of Physiology, College of Veterinary Medicine, Wasit University, Kut, [2]Department of Pathological Analysis, College of Science, Thi-Qar University, Nasiriyah, Iraq

Abstract

Context: Breast cancer is the most common cancer among women worldwide, comprising 23% of the 1.1 million female cancers that newly diagnosed each year. **Aims:** The aim is to investigate the existence of microsatellite instability (MSI) in breast cancer of patients. **Settings and Design:** Fifty female patients with invasive ductal breast carcinoma collected. Inclusion criteria of patients include female patients with diagnostic feature of breast cancer and age range 26–42-year-old untreated with chemotherapy or hormonal therapy. **Subjects and Methods:** DNA had be extracted from frozen tissue samples of breast cancer. This protocol done according to the kit manufacture's manual of QIAamp DNA Mini Kit from Qiagen – USA. All samples tested for MSI by singleplex polymerase chain reactions (PCRs) using two microsatellite markers BAT25 and BAT26. PCR achieved in a final volume of 50 µl and after thermal cycles, gel visualization performed. **Statistical Analysis Used:** The significance of differences in proportions was analyzed using the Fisher's exact test with SPSS version 20 and values of $P \leq 0.001$ considered statistically significant. **Results:** PCR demonstrating MSI in 13 (26%) of the 50 breast cancer sample. Eight (16%) of 50 breast cancer sample were BAT25 positive with a PCR product size of 124 bp, whereas 5 (10%) of 50 breast cancer sample were BAT26 positive with a PCR product size 121 bp. **Conclusions:** The result suggests strong evidence that MSI at the BAT25 and BAT26 and have involved in the pathogenesis of the great majority of breast cancers.

Keywords: *Breast cancer, microsatellite instability, polymerase chain reaction*

Introduction

The development of breast and other cancers is a multistep process.[1,2] At least, six genetic changes may be required to convert normal breast epithelium to malignant breast cancer,[1,3] with each alteration presumably increasing proliferative or survival capacity. In contrast, many of the types of changes commonly observed in cancer cell genomes develop at immeasurably low frequencies in normal cells. This is consistent with previous proposals that one or more changes occurring during cancer evolution increase the endogenous mutation rate beyond the normal repair capacity or decrease the ability to detect and/or repair mutations, resulting in a mutator phenotype in affected cells.[4,5]

Microsatellite instability (MSI) is defined as the type of genomic instability related with defective DNA mismatch repair in tumors. MSI provides an indication of the presence of genetic instability in a given tumor by comparing the size of a subset of simple repeated sequences occurring throughout the genome (mono-, di-, tri-, and less frequently, tetranucleotide repeats) between normal and tumor DNA from the same individual.[6]

The most frequent errors associated with microsatellites are base–base mismatches that escape the intrinsic proofreading activity of DNA polymerases, and insertion–deletion loops, which are extrahelical nucleotides that form DNA hairpins. Insertions or deletions in microsatellites located in DNA coding regions generate frame shift mutations, which can lead to protein truncations.[6,7]

MSI is a situation in which a germline microsatellite allele has gained or lost repeated units and has thus undergone a somatic change in length. Because this type of alteration can be detected only if many cells are affected by the same change, it is an indicator of the clonal expansion that is typical of a neoplasm.[8]

Initially, some authors used the term replication errors, although in 1998

How to cite this article: Hassan JS, Hanon BM, Mohammed AH, Abd Al-Rahman TR. Investigation of microsatellite instability BAT25 and BAT26 in breast cancer patients by conventional polymerase chain reaction. Clin Cancer Investig J 2017;6:68-72.

Address for correspondence:
Dr. Ahmed Hasan Mohammed, College of Science, Thi-Qar University, Nasiriyah, Iraq. E-mail: ahmedhasan@sci.utq.edu.iq

Access this article online

Website: www.ccij-online.org

DOI: 10.4103/ccij.ccij_160_16

Quick Response Code:

the National Cancer Institute Workshop on hereditary nonpolyposis colorectal cancer recommended the use of the term MSI and established "MSI golden standards" that are currently employed in research and diagnostic laboratories worldwide.[9]

The mononucleotide MSI loci, BAT25 and BAT26, have the highest accuracy in predicting MSI-H tumors, with sensitivity and specificity approaching 94%–98% for both markers.[10] The quasimonomorphic[11] feature of these markers, defined as little or no polymorphism in these loci across all ethnic populations, allows the testing of tumor tissue without the need for a corresponding normal control. However, some unstable tumors may have stable BAT26 loci due to a large intragenic MSH2 deletion, causing complete absence of the BAT26 loci in the tumor tissue.[12] Other MSI loci are generally added to correctly detect these cases.

The aim of this study was to investigate the frequency and presence of MSI in invasive ductal breast carcinoma (IDC) and its correlation with clinical and pathological parameters.

The objective of this study was the detection of MSI in the BAT25 and BAT26 loci of DNA samples extracted from IDC tissue sample and amplification the product by singleplex polymerase chain reaction (PCR).

Subjects and Methods

Specimens

This study includes fifty female patients with IDC and twenty female with benign breast tumor as negative control were chosen from Al-Imamin Al-Kadhimin Teaching Hospital and from Dijlah Private Hospital during February–July 2013. Inclusion criteria of patients include female patients with a diagnostic feature of breast cancer and age range 26–42-year-old untreated with chemotherapy or hormonal therapy. The samples introduced in this study included fifty tumors tissue sample from IDC. The tissue samples were preserved in normal saline until delivered to the working laboratory. Each tumor mass preserved in normal saline and freezed at −20°C for DNA extraction and PCR.

DNA extraction

DNA had been extracted from frozen tumor of breast tissues. This protocol done according to the kit manufacture's manual of QIAamp DNA Mini Kit from Qiagen – USA.

Procedure

1. The tissue sample was removed from storage then the amount of tissue was determined by weighing 25 mg of tissue
2. A 25 mg of tissue was cut up into small pieces. Placed in a 1.5 ml microcentrifuge tube and 180 µl of buffer ATL was added
3. A 20 µl proteinase K was added and mixed by vortex then incubated at 56°C until the tissue is completely lysed. Vortex occasionally during incubation was necessary to disperse the sample
4. The 1.5 ml microcentrifuge tube was centrifuged to remove drops from the inside of the lid
5. Two hundred microliter of buffer AL was added to the sample, mixed by pulse vortexing for 15 s, and incubated at 70°C for 10 min The 1.5 ml microcentrifuge tube was centrifuged to remove drops from inside the lid
6. Two hundred microliter of ethanol (96%–100%) was added to the sample and mixed by pulse vortexing for 15 s. After mixing, briefly centrifuged the 1.5 ml microcentrifuge tube to remove drops from inside of the lid
7. Carefully, the mixture from step 6 (including the precipitate) was applied to the QIAamp Mini spin column without wetting the rim. Close the cap, and centrifuged at 6000 ×*g* (8000 rpm) for 1 min The QIAamp Mini spin Column was placed in a clean 2 ml collection tube and discarded the tube containing the filtrate
8. The QIAamp Mini spin column was opened carefully and added 500 µl buffer AW 1 without wetting the rim. Close the cap, and centrifuged at 6000 ×*g* (8000 rpm) for 1 min The QIAamp Mini spin column was placed in a clean 2 ml collection tube, and discard the collection tube containing the filtrate
9. The QIAamp Mini spin column was carefully opened and added 500 µl buffer AW2 without wetting the rim. Close the cap and centrifuged at full speed (20,000 ×*g*; 14,000 rpm) for 3 min
10. The QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the filtrate. The QIAamp Mini spin column was carefully opened and a 50 µl of buffer AE was added then incubated at room temperature for 1 min, and centrifuged at 6000 ×*g* (8000 rpm) for 1 min The step number 10 was repeated for one time to collect finally 100 µl of eluted DNA.

DNA evaluation by NanoDrop for an A260/A280 value of 1.5, the percentage of protein in the DNA preparation, for good PCR-sequence specific primer results, DNA is required with an A260/A280 quotient of 1.6 or greater. The sections of tumor tissue should contain more than 50% of neoplastic cells[13] in order to avoid false negatives. Microsatellite marker amplifications are performed as singleplex PCR reactions using DNA from tumor tissue in accordance to the recommendations by the National Cancer Institute.

Internal control

Since DNA extracted from freezing tissue sample can be variably degraded and may contain PCR inhibitors, we suggest performing a preliminary quality control to test if sample. DNA is suitable for MSI and to determine the optimal quantity for amplification.

For this purpose, a 167 bp fragment of the b-globin gene is amplified. Since the b-globin gene is present in all the cells (it never undergoes deletions) and is not polymorphic, it is a suitable target for the control PCR.[14] Positive control for b-globin: DNA from normal human lymphocytes, 50 ng/ml.[15]

Polymerase chain reaction PreMix AccuPower® Bioneer, South Korea

The powerful technology for convenient and easy to perform DNA amplification. It contains DNA polymerase, deoxynucleotide triphosphates, a tracking dye and reaction buffer in a premixed format, freeze-dried into a pellet. 25/100 bp mixed DNA ladder is especially designed for determining the size of double strand DNA from 25 to 2000 base pairs. The DNA Ladder includes 17 double strand DNA fragments ranging in size from 25 to 200 bp in 25 bp increments. Single PCR reactions were conducted to amplify the two loci BAT25 and BAT26 for screening breast cancer patients.

The PCR reaction was performed in a final volume of 50 ml, containing: 0.5 µl b-globin both forward primer, reverse primer (primer designed in Alpha DNA, Canada) [Table 1] 30 pmol/ml 0.3 pmol/µl final, 1 µl of diluted sample DNA, 20 µl mister mix, H_2O to volume, overlay the reaction mixture with 20 ml of mineral oil.

Thermal cycling: 94°C 10" + 5 × (94°C 60", 52°C 60", 72°C 60") + 35 × (94°C 30" 52°C 30" 72°C 30") + 72°C 5'.

Prepare a different master mix for each microsatellite marker (singleplex). PCR was performed in a final volume of 50 µl, containing: 20 µl of master mix, 0.5 µl forward primer and reverse primer, 30 pmol/ml 0.3 pmol/ml final 0.5 µl, (50 ng/ml) 1 µl of diluted sample DNA or 1 µl of undiluted negative control or 1 µl of positive control for amplification and H_2O to volume, overlay reaction mixture with 20 µl of mineral oil.

Thermal cycling: 94°C 10 min' + 5 × (94°C 60S", 55°C 60S", 72°C 60S") + 35 × (94°C 30S" 55°C 30S" 72°C 30S") + 72°C 5 min'.

Gel visualization

Mix 10 ml of PCR product with 2 µl of 6× loading buffer; load on a 2% agarose gel prepared with 1× tris-borate ethylenediaminetetraacetic acid buffer containing 0.5 mg/ml ethidium bromide. Run at 80 V constant until bromophenol blue reaches 1/2 of the gel. Inspect under a ultraviolet source. A single band should be visible in the sample.

Statistical analysis

The significance of differences in proportions was analyzed using Fisher's exact test. Entry of data into the computer and the Fisher exact tests performed using SPSS version 20 and $P \leq 0.001$ considered statistically significant.

Results

Fifty tissue samples from female patients with IDC enrolled in this study, the age ranged from 26 to 42-year-old. In addition to twenty female with benign breast tumor used in this study as a negative control.

All samples tested for MSI by singleplex PCR reactions using two microsatellite markers BAT25 and BAT26. The internal control B-globin appeared in the region 167 bp as shown in Figure 1 while BAT26 in 121 bp and BAT25 in 124 bp.

PCR demonstrating MSI in 13 (26%) of the 50 breast cancer sample. Eight (16%) of 50 breast cancer sample were BAT25 positive with a PCR product size of 124 bp as shown in Figure 2, while 5 (10%) of 50 breast cancer sample were BAT26 positive with a PCR product size 121 bp as shown in Figure 3. Two out of 13 samples were positive for both BAT25 and BAT26, the remainder 37 (74%) out of 50 samples showed a microsatellite stability. All benign breast tumor samples showed a negative result for MSI and give 100% for microsatellite stability.

Statistical analysis showed the presence of significant differences ($P < 0.001$) toward the role of MSI in IDC when compared with negative control. However, the results

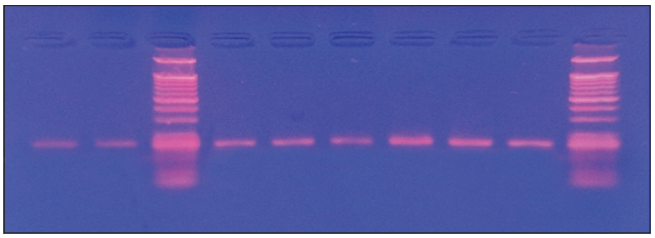

Figure 1: Gel electrophoresis (2% agarose, 7 v/cm², 1 h) of polymerase chain reaction positive products for b-globin 167 bp, L1: 25/100 bp DNA ladder was used, L2 positive control

Table 1: Primers sequences for microsatellites instability

Marker name	Genomic position	Sequences (5′-3′)	T °m	Product (bp)
b-globin	11p15.5	F: ACACAACTGTGTTCACTAGC	58	167
		R: GAAAATAGACCAATAGGCAG	56	
BAT25	4q12-4q12	F: TCGCCTCCAAGAATGTAAGT	59.7	124
		R: TCTGCATTTTAACTATGGCTC	57.0	
BAT26	2p16.3-2p16.3	F: TGACTACTTTTGACTTCAGCC	57.	121
		R: AACCATTCAACATTTTTAACCC	59.0	

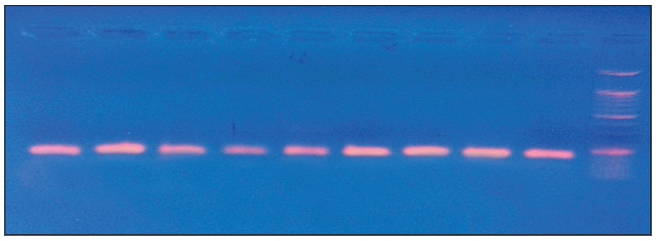

Figure 2: Gel electrophoresis (2% agarose, 7 v/cm², 1 h) of polymerase chain reaction positive products for BAT25 was shown in 124 bp, L1: 25/100 bp DNA ladder was used, L2 positive control

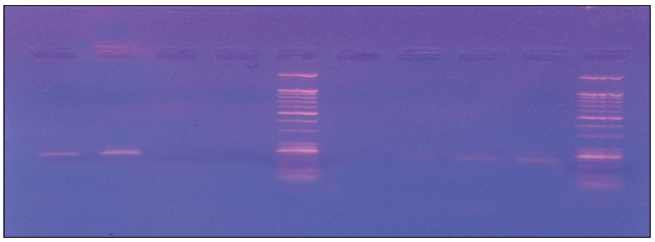

Figure 3: Gel electrophoresis (2% agarose, 7 v/cm², 1 h) of polymerase chain reaction positive products for BAT26 was shown in 121 bp, L1: 25/100 bp DNA ladder was used, L2 positive control

showed there is no correlation of the presence of MSI in IDC with the age of the patient. This study also trying to find an association between the stage of IDC and the existence of MSI, but the statistical analysis showed no correlation between these variants.

Regarding the differentiation of the cell in tissue sample of IDC, it was well in all samples and when compared between positive sample of MSI with negative one in this parameter, the results showed no significant differences between the two groups.

Discussion

DNA mismatch repair is a system for recognizing and repairing erroneous insertion, deletion, and mis-incorporation of bases that can arise during DNA replication and recombination Mismatch repair is strand-specific. During DNA synthesis the newly synthesized (daughter) strand will commonly include errors. To begin repair, the mismatch repair machinery distinguishes the newly synthesized strand from the template (parental). Any defects in this system cause errors in the replication of simple nucleotide repeat segments. This condition is commonly known as MSI because of the frequent mutations of microsatellite sequences.[16]

MSI is associated with many tumor such as endometrial cancers, colon, gastric, and pancreatic cancer. There are many studies investigating MSI in breast carcinoma at different loci using different microsatellite markers but still inconsistent.[17,18]

In this study, we have detected MSI at the BAT25 and BAT26 in 13 patients out of 50 patients These data provide firm evidence that the instability seen was specific to the breast cancer this result was similar to many other investigations working in this field as studies by Walsh *et al*.,[19] Adem *et al*.[20] Yee *et al*.[21]

Two tumors showed instability at multiple marker BAT25 and BAT26, this result may suggest that the instability seen in breast tumors represents a random background instability, this result comes in accordance with those obtained in others studies of Soo-Chin Lee *et al*.;[22] Paulson *et al*.[23]

Conclusions

We have detected somatic MSI in 26% of 50 breast cancer these data may suggest strong evidence that MSI at the BAT25 and BAT26 are involved in the pathogenesis of the great majority of breast cancers. However, these marker has be seen just as a part of this process.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Devilee P, Cornelisse CJ. Somatic genetic changes in human breast cancer. Biochim Biophys Acta 1994;1198:113-30.
2. Nowell PC. The clonal evolution of tumor cell populations. Science 1976;194:23-8.
3. Weinberg RA. Tumor suppressor genes. Science 1991;254:1138-46.
4. Loeb LA. Microsatellite instability: Marker of a mutator phenotype in cancer. Cancer Res 1994;54:5059-63.
5. Loeb LA. Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 1991;51:3075-9.
6. Karran P. Microsatellite instability and DNA mismatch repair in human cancer. Semin Cancer Biol 1996;7:15-24.
7. Jiricny J. The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol 2006;7:335-46.
8. Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, *et al.* Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science 1997;275:967-9.
9. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, *et al.* A National Cancer Institute Workshop on microsatellite instability for cancer detection and familial predisposition: Development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248-57.
10. Cicek MS, Lindor NM, Gallinger S, Bapat B, Hopper JL, Jenkins MA, *et al.* Quality assessment and correlation of microsatellite instability and immunohistochemical markers among population- and clinic-based colorectal tumors results from the Colon Cancer Family Registry. J Mol Diagn 2011;13:271-81.
11. Deschoolmeester V, Baay M, Wuyts W, Van Marck E, Van Damme N, Vermeulen P, *et al.* Detection of microsatellite instability in colorectal cancer using an alternative multiplex assay of quasi-monomorphic mononucleotide markers. J Mol

Diagn 2008;10:154-9.
12. Pastrello C, Baglioni S, Tibiletti MG, Papi L, Fornasarig M, Morabito A, *et al.* Stability of BAT26 in tumours of hereditary nonpolyposis colorectal cancer patients with MSH2 intragenic deletion. Eur J Hum Genet 2006;14:63-8.
13. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomäki P, *et al.* Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998;338:1481-7.
14. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, *et al.* Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988;239:487-91.
15. Lothe RA, Peltomäki P, Meling GI, Aaltonen LA, Nyström-Lahti M, Pylkkänen L, *et al.* Genomic instability in colorectal cancer: Relationship to clinicopathological variables and family history. Cancer Res 1993;53:5849-52.
16. Lawes DA, SenGupta S, Boulos PB. The clinical importance and prognostic implications of microsatellite instability in sporadic cancer. Eur J Surg Oncol 2003;29:201-12.
17. Vaurs-Barrière C, Penault-Llorca F, Laplace-Marieze V, Presneau N, Maugard CM, Fiche M, *et al.* Low frequency of microsatellite instability in BRCA1 mutated breast tumours. J Med Genet 2000;37:E32.
18. Ali S, Müller CR, Epplen JT. DNA finger printing by oligonucleotide probes specific for simple repeats. Hum Genet 1986;74:239-43.
19. Walsh MD, Buchanan DD, Cummings MC, Pearson SA, Arnold ST, Clendenning M, *et al.* Lynch syndrome-associated breast cancers: Clinicopathologic characteristics of a case series from the colon cancer family registry. Clin Cancer Res 2010;16:2214-24.
20. Adem C, Soderberg CL, Cunningham JM, Reynolds C, Sebo TJ, Thibodeau SN, *et al.* Microsatellite instability in hereditary and sporadic breast cancers. Int J Cancer 2003;107:580-2.
21. Yee CJ, Roodi N, Verrier CS, Pari FF. Microsatellite instability and loss of heterozygosity in breast cancer. Can Res 1994;54:1641-4.
22. Lee SJ, Berg KD, Sherman ME. Microsatellite instability is infrequent in medullary breast cancer. Am J Clin Pathol 2001;115:823-7.
23. Paulson TG, Wright FA, Parker BA, Russack V, Wahl GM. Microsatellite instability correlates with reduced survival and poor disease prognosis in breast cancer. Cancer Res 1996;56:4021-6.