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Molecular Highlighting Analysis of Mutational BRCA1- and BRCA2- Gene Products in Association with Human Mammary Tumor Virus Infection in Tissues from Iraqi Women with Breast Carcinomas

[1]Saad Hasan Mohammed Ali, [2]Shakir H. Mohammed Al-Alwany, [1]Huda Q. Muhammad Abu AL-ess, [2]Anwar Kadhim Hussein Al_Saffar, [3]Isra'a Mahdi Al-sudani

[1]College of Medicine / Baghdad UniversityIraq
[2]College of Science / University of Babylon/ Iraq
[3]College of Medicine / Al-Mustansyria University/Iraq

Abstract : Background: The most common cancer worldwide among women is breast cancer. Both internal and external factors have resulted in initiation, promotion, and progression, among them race and, to an even greater extent, viruses are influencing development of this complex multifactorial genetic-breast disease. Unsurprisingly, mutations in brca1 and 2 genes and complete loss of the function of their either proteins leads to a dramatic increase in genomic instability and a significant increase in the lifetime risk of developing breast cancer. The higher detection of hormone response elements in the HMTV Long-Terminal-Repeat (LTR) in gestational breast cancers suggests a mechanism for association of HMTV with this hormonally- responding tissues.

Objective: To analyze the impact of concordant BRCA 1& BRCA 2 expression as well as HMTV infection on breast tissues from a group of Iraqi patients diagnosed with different breast lesions.

Patients and Methods: A total number of 60 tissue specimens were examined for HMTV-envelope and BRCA 1&2 genes expression. Those samples belonged to 30 patients diagnosed with breast cancer (BC) . The remaining specimens were obtained from healthy breast tissues of 30 females. Detection of HMTV was done by ultrasensitive version of in situ hybridization method whereas immunohistochemistry detection system was used to demonstrate the expression of BRCA 1&2 genes.

Results: Detection of HMTV envelope gene-ISH reaction in tissues with BC was observed in 13 out of 30 (43.3%). No HMTV envelope gene -positive ISH reaction was detected among all the examined healthy breast tissues in the control group. The difference between the percentages of HMTV detection in tissues BC and control groups was statistically highly significant (P value = <0.0001).

Positive BRCA- 1& BRCA-2-immunohistochemical (IHC) reactions were observed in 56.7 % and 46.7% in BC tissues, respectively.

Conclusions: The significant detection of HMTV along with BRCA- 1& BRCA-2genes expression production breast cancer patients are supporting the hypothesis of an etiologic roles for that virus along with mutated and / or defected BRCA 1&2 genes in breast cancer development.

Key words : Breast Cancer, HMTV,in situ hybridization, BRCA 1&2 ,immunohistochemistry.

Introduction:

Globally, the most frequent cancer that associated with highest number of deaths among women is breast cancer. Although it is one of the most prevalent malignancy and the leading cause of death among women with cancers worldwide, the etiology and molecular mechanisms related to breast carcinogenesis remain unclear and poorly understood because of the biological heterogeneity of the breast cancer[1,2].

The International Agency for Research on Cancer (IARC) has reported that The development of breast cancer has been associated with several internal and external factors which play major roles during initiation, development, and progression of cancer, among them genetic predisposition (e.g., mutations in BRCA1/2 and other genes) as well as a family history of breast cancer, ethnicity, dense breast tissue, lifestyle, hormonal contraception and treatment after menopause, and obesity[3].Many other risk factors that promoting breast tumorigenesis, such as age, parity, diet, environmental and geographic location, and race have also been recognized[4-6].

BRCA1 is a human tumor suppressor gene found in all humans and its protein is normally expressed in the cells of breast and other tissues to help in repairing damaged DNA , chromosomal damage and DNA double-strand breaks as well as ubiquitination, transcriptional regulation as well as other functions[7].Although the terms "breast cancer susceptibility gene" (BRCA, italic for the gene and non-italic for the protein) sound as if they describe increases an oncogene, BRCA1and BRCA2 are normal and that their mutation is the abnormal where the risk for breast cancer[8].

To date, five different conditional Brca1 alleles as well as four conditional Brca2 alleles have been recognized[9].

Human BRCA-associated tumors often harbor TP53 mutations[10,11].In addition, Mammary specific deletion of *Brca* genes are found to be under the control of the whey acidic promoter (WAP), the mouse mammary tumor virus long terminal repeat (MMTV) promoter, or the cytokeratin-14 (K14) promoter where each the examined mouse models have developed mammary tumors with a long latency suggesting further genetic alterations were required[9].

The cancer risk caused by BRCA1 and BRCA2 mutations are inherited from parent, so they are classified as hereditary or germline mutations rather than acquired or somatic mutations[12].

Because humans have a diploid genome typically only one BRCA1 and BRCA2 copy BRCA1 has also been shown to be required for the activation of both S- and G2/M-phase cell-cycle arrest after DNA damage[13].BRCA1 has also been shown to interact with multiple DNA repair/recombination proteins[12]. Furthermore, the roles for BRCA1 in transcriptional regulation and proliferation are mediated through associations with CTIP, ZBRK, p300, estrogen receptor (ER), HDAC, Rb, p53, RNA polymerase II holoenzyme, cyclin D1, and c-myc[12].

Hundreds of different types of mutations in these genes have been identified, some of which have been determined to be harmful, while others have no proven impact. Only 5-10% of breast cancer cases in women are attributed to *BRCA1* and *BRCA2* mutations[14].

Women with harmful mutations in either *BRCA1* or *BRCA2* have a risk of breast cancer that is about five times the normal risk, and a risk of ovarian cancer that is about ten to thirty times normal[15].In addition, many studies have indicated that viral infections may play a role in one or more of the steps in its pathogenesis[16, 17].Many previous studies have pointed for possible viral etiology in breast carcinogenesis including MMTV ,EBV, HPV and HCMV[18].

The hypothesis of viral breast- carcinogenesis was based on the proven causal role of mouse mammary tumor virus (MMTV) in mice mammary tumors[19,20]. Yet, several researchers have shown that MMTV can directly infect human mammary tissues[17, 21-24],and subsequently have pointed for a similar virus (MMTV-like virus) known as human mammary tumor virus (HMTV),that constituted a risk factor for BC in the humans[16,17,25].

In addition, the researchers have revealed that the incidence of HMTV sequences was higher in inflammatory[26]and gestational breast cancers than in sporadic specimens[27] and then concluding that HMTV might associated with a particular malignant phenotype[28].Gestational breast cancers are those mostly associated with hormonal changes and hormone responsive elements present in the LTR of this virus[27],and in turn together point for molecular- viral associations with these hormonally responding tissues.

The presence of sequences homologous to MMTV in human breast cancer, designated as MMTV-like and then appeared to be the same virus as HMTV, has been confirmed by several groups[20,21,29-31].

The sequence- homology between them has been found to be 90% to 98%[25, 32]. It has been shown that this B-type retrovirus was integrating and becoming expressed in the infected mammary tissues with a greater frequency[33]. Existence of HMTV was suspected for decades, but this unique virus was not confirmed in human breast cancer and tumors until 2001. Evidenced role of HTMV/MMTV in human breast cancer has only recently been revealed[34].

However, the association of an HMTV agent with human breast cancer remains controversial. The aim of this work was to evaluate the rate of occurrence of envelope- gene sequences of this retrovirus (HMTV) in BC tissues from Iraqi women in Mid –Euphrates.

This study is aiming to analyze the rate of concordance of BRCA 1&2-gene translational expression and HMTV in breast tissues from a group of patients with malignant and benign breast tumors.

Materials and methods:

The study was designed as a retrospective one. It has recruited 60 selected formalin fixed, paraffin embedded breast tissue blocks; among them[30], tissue biopsies from breast carcinoma with different grades as well as[30] tissues with apparently normal bladder tissue autopsies which were collected from the archives of Forensic Medicine Institute / Baghdad and used as breast healthy control groups. Specimens were selected for analysis based on two criteria: (i) the presence of sufficient material for analysis and (ii) histological evaluation by two pathologists that demonstrates that the samples contained at least 30%tumor cells (in the case of the cancer samples). Representative sections from each case were paraffin-embedded for staining with hematoxylin and eosin to assess the histopathology diagnosis using the American Cancer Committee criteria.

The diagnosis of these tissue blocks were based on their accompanied records. A consultant pathologist reexamined all these cases to further confirm the diagnosis following trimming process of these tissue blocks.

In one hand, the detection of HMTV by ISH kit (Zyto Vision GmbH. Fischkai, Bremerhaven. Germany) was performed on 4µm paraffin embedded tissue sections using digoxigenin-labeled oligo-nucleotides probe which targets Human mammary tumor virus RNA. One section was mounted on ordinary glass slide and stained with hematoxyline and eosin, while another slide was mounted on charged slide to be used for ISH for detection of HMTV.

For the In Situ Hybridization procedure, the slides were placed in 60c hot-air oven overnight then the tissue sections were de-paraffinized and then treated by graded alcohols according to the standard methods and the details of processes for performing ISH reaction with this probe were applied according the instructions of the manufacturing company(Zyto Vision GmbH. Fischkai, Bremerhaven. Germany). The main steps for ISH procedure are:

Incubation of slides for 18 hrat 70°C on hot plate. Rehydration process was done at room temperature which include : Slides were immersed in two changes of absolute ethanol for one minute each, then Immersion in ethanol (95%) for one minute each, after that immersed in ethanol (70%) for one minute each, finally immersion in distilled water for 5 minutes to remove residual alcohol. After that, slides were allowed to dry completely by incubating them at 37°C for 5 minutes,(alternatively, dewaxing protocols routinely used in immunohistochemistry procedures, e.g. 2-5 min xylene, 2-5 min 100% ethanol, 2-5 min 96% ethanol, 1-5 min 70% ethanol, can be used, air drying of sections.

Then application (dropwise) Pepsin Solution(ES1) to the tissue/cell section and incubate for 20-30 min at 37°C in a humidity chamber. After that we immersed slides in distilled water and drain off the water, air

dried sections. Then add the probe to the center of a cover slip and place cover slip upside down on target area). Denaturation of the slides at 75°C for 5 minon hot plate, then we add the 20 µl of cDNA probe added to each section and slides were covered by cover slips be careful to avoid trapping any air bubbles. After that probe and target DNA were denaturized by placing in pre-warmed oven at 75°C for 8-10 minutes, slides were transferred to a pre-warmed humid hybridization chamber and incubated at 37°C for overnight. Then the slides were allowed not dry out at any time during the hybridization and staining. All reagents used during hybridization and detection were warmed to room temperature. At the next day, slides were soaked in pre-warmed protein block at 37°C until the cover slips fell off and should be careful not to tear the tissue, and then the slides were allowed to remain in the buffer for 3 minutes, at 37°C after cover slips were removed. Then application of AP-Streptavidin(AB9) drop wise (3-4 drops per slide) to the slides and incubate for 30 min at 37°C in a humidity chamber. Then washed in wash buffer TBS (prepared by usingWB5) and then twice times for 1 min in distilled water and application of5-bromo3-chloro3-indoly/phosphate/nitro blue tertrazolium substrate-chromogen solution NBT/BCIP(SB4) drop wise (4 drops per slide) to the slides and incubated for 40 min at 37°C in humidity chamber. Slides were incubated at 37°C for 30 minutes or until color development was developed completed. Color development was monitored by viewing the slides under the microscope. A dark blue colored precipitate forms at the complementary site of the probe in positive cells. Then the slides were rinsed in distilled water for 5 minutes, then counter staining process by immersion of the slides in Nuclear Fast Red stain for 30 seconds, then washing process was followed by immersion the slides for 1 minute in distilled water. After that Sections were dehydrated by ethyl alcohol, (95%, once for one minute then, 100% twice times for 2 minutes each); cleared by Xylene, then mounted with permanent mounting medium (DPX). Then final evaluation by light microscope.

Immunohistochemistry / Detection system (Abcam. England) was used to demonstrate the BRCA1 and BRCA2 tumor suppressor genes. This technique is based on the detection of the product of gene expression (protein) in malignant and normal cells using a specific monoclonal antibodies, i.e. Primary antibody for specific epitope (usually mouse antihuman monoclonal antibody), which binds to nuclear targeted protein.

The bound primary antibody is then detected by secondary antibody (usually rabbit or goat anti mouse) , which contains specific label (in this context we used peroxidase labeled polymer conjugated to goat anti mouse immunoglobulin) . The substrate is DAB in chromogen solution , positive reaction will result in a browning color precipitate at the antigen site in tested tissues.

Chi –square test was used to detect the significance between variables of our study. All the statistical analyses was done by SPSS program(Version– 17)&P value was considered significant when $p < 0.05$.

Results

I. Age Distribution Among Study Groups

The patient's ages ranged from 20-72 years with a mean of 46 year. Mean age of malignant tumor was 50±13 years, while in the control group it was 43.5± 5 years. There were no-significant statistical differences ($p<0.05$) between groups according to age (Table 1).

Table (3-1): Distribution of breast tumor patients according to their age

Maximum	Minimum	S.E	S.D	Mean Age	N	The Patients
72	28.00	2.34999	13.08418	50	30	**Malignant Breast Tumors**
59.6	27.6	1.08284	5.19311	43.5	30	**Healthy Br. Tissues Control**
Chi square no-Significant (P > 0.05)						**Statistical Analysis/ ANOVA**

II.Histological Grade

This study revealed that well differentiated carcinomas were seen in 19 cases of malignant group (63.3%) while 9 cases (30%) were moderately differentiated. Poorly differentiated carcinoma was seen in 2 cases (6.7%) as shown in figure (1).

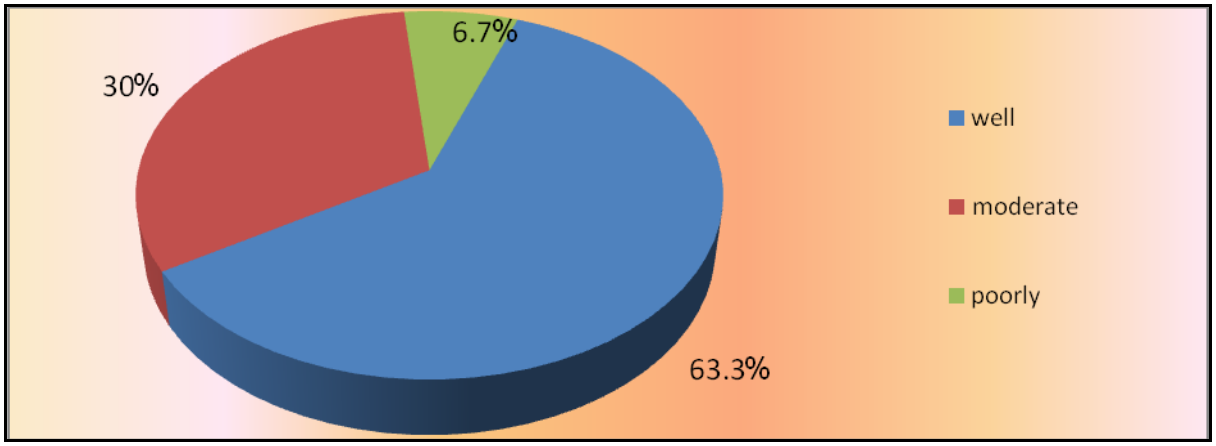


Figure (1): Frequency of tumor grade of malignant breast tumors

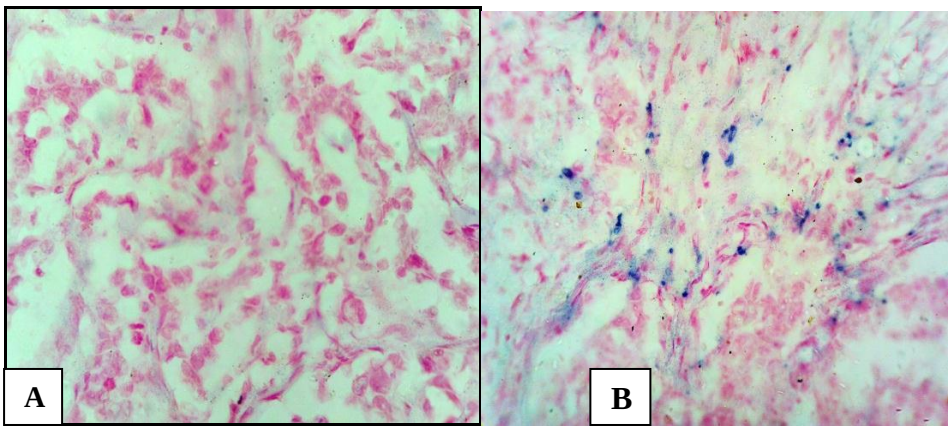
III. Positive HMTVenvelope gene - ISH Signal Scoring:

Regarding malignant breast tumor group, the total percentage of positive HMTV envelope gene – ISH detection was 43.3% (13 out of 30 cases) . No HMTV envelope gene -positive ISH reaction was detected among all the examined healthy breast tissues in the control group. Statistically, significant difference ($p<0.05$) was found on comparing the percentages of HMTV envelope gene among the study groups (Table2).

Table (2): Signal Scoring- Frequency of Positive HMTVenvelope gene - ISH Reactions.

Chi-Square - Tests	**Negative HMTVenvelope gene signaling**	**Signaling Scoring**			**Positive HMTVenvelope gene signaling**	**Studied Groups**
		Score III	**Score II**	**Score I**		
P value = 0.006 significant (p <0.05)	17/30 (56.7%)	3/13 (23.1%)	6/13 (46.2%)	4/13 (30.7%)	13/30 (43.3%)	**Malignant Br. Tumors (n=30)**
	30/30 (100%)	0/30 (0.00%)	0/30 (0.00%)	0/30 (0.00%)	0/30	**Healthy Br. Tissues (n=30)**

The highest percentage of HMTV envelope gene- positive malignant breast tumors have showed moderate scores (score II) (46.2% :6 out of 13 cases) .



Figure(2) :In Situ Hybridization(ISH) for HMTV Deduction Infiltrative Breast Cancers Using Biotinylated -Labeled HMTV Probe;Stained with NBT/ BCIP (Blue)and Counter Stained by Nuclear Fast Red (Red). A-Breast Cancer with negative HMTV –ISH reactions (20X).B-Positive HMTV-ISH reaction with strong score and high signal intensity (40X).

IV. The Evaluation Results of BRCA Tumor Suppressor Genes Among Malignant Breast Tumors:

The signaling results of immunohistochemical reactions for BRCA1 and BRCA2 antigenic detection were observed as brown discoloration at the specific antigenic sites of these reactions with their specific primary antibodies (Figure 3& 4).

I.Results of BRCA1 - IHC Signal Scoring :

The positive- signal results of BRCA1 immunohistochemical reactions were 56.7% (17 out of total 30). While , no tissue in the control group has showed such IHC signals (Table 2). The statistical Pearson Chi-Square analysis shows significant difference between the patients and control groups regarding BRCA1 immunohistochemical results (<0.05).

Table (2) : Frequency distribution of immunohistochemistry results of BRCA1 protein according to the signal scoring.

				GROUP		Total	Valid Percent	Cumulative Percent
The Marker				Patients	Control			
BRCA-1		Positive	Count	17	0	17		
			%	56.7%	0.0%	28.3%	56.7	56.7
		Negative	Count	13	30	43		
			%	43.3%	100.0%	71.7%	43.3	100.0
	Total		Count	30	30	60		
			%	100.0%	100.0%	100.0%	100.0	

		Value	df	Asymp. Sig. (2-sided)
BRCA-1	Pearson Chi-Square	7.300	1	.0020

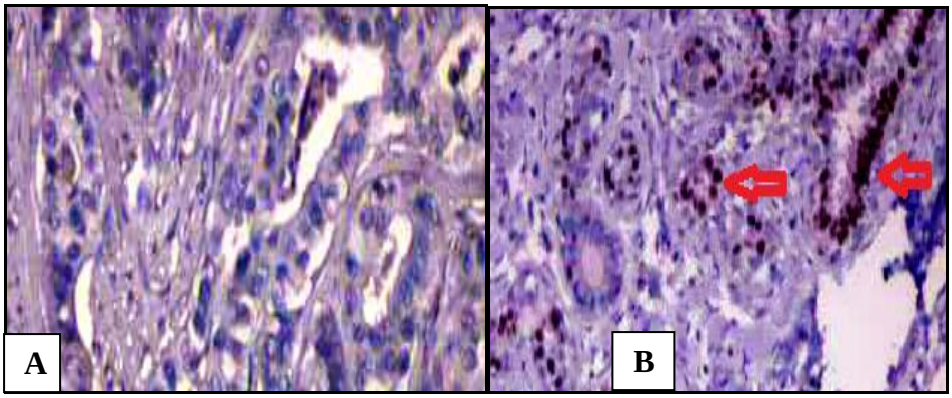


Figure (3):Infiltrative Ductal Carcinoma Showing The Results Of Immunohistochemical Staining Of BRCA1 Protein Expression Using Biotinylated -Labeled Anti- BRCA1 Protein Antibody, Stained By DAB-Chromogen (Brown) and Counter Stained By Mayer's Hematoxyline (Blue). A. Breast Cancer with negative BRCA1 –IHC reactions(40X) B. Positive BRCA1 –ICH reaction with low score and high signal intensity (40X).

II.Results of BRCA2- IHC Signal Scoring:

The BRCA2 was detected by IHC in46.7% % (14 out of total 30) of breast carcinoma group, while, no tissue in the control group has showed such IHC signals (Table 3). The statistical Pearson Chi-Square analysis shows significant difference between the patients and control groups regarding BRCA2 immunohistochemical results (<0.05).

Table (3) : Frequency distribution of immunohistochemistry results of BRCA2 protein according to the signal scoring.

				GROUP		Total	Valid Percent	Cumulative Percent
The Marker				Patients	Control			
BRCA-2		Positive	Count	14	0	14		
			%	46.7%	0.0%	33.3%	46.7	46.7
		Negative	Count	16	30	46		
			%	53.3%	100.0%	66.7%	53.3	100.0
	Total		Count	30	30	60		
			%	100.0%	100.0%	100.0%	100.0	

		Value	df	Asymp. Sig. (2-sided)
BRCA-2	Pearson Chi-Square	8.400	1	0.004

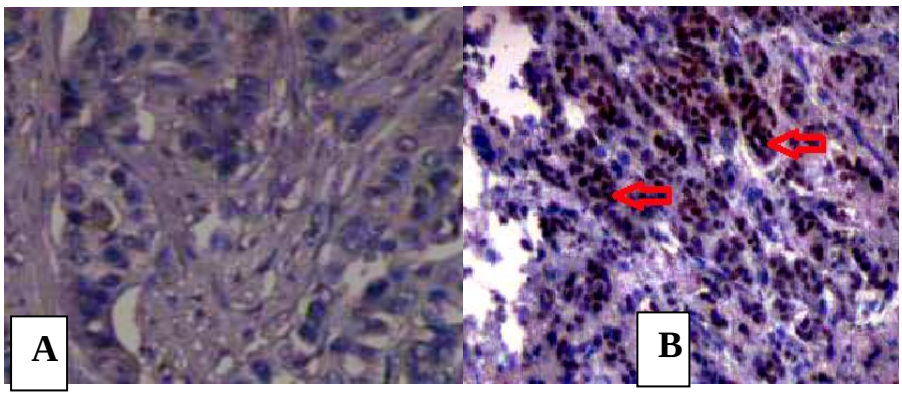


Figure (4): Infiltrative Ductal Carcinoma Showing The Results Of Immunohistochemical Staining Of BRCA2 Protein Expression Using Biotinylated **-Labeled Anti- BRCA2 Protein Antibody, Stained By DAB-Chromogen (Brown) and Counter Stained By Mayer's Hematoxyline (Blue).** A. Breast Cancer with negative **BRCA2** –IHC reactions(40X) B. Positive**BRCA2** –ICH reaction with moderate score and high signal intensity (40X).

V.Co-existence of HMTV infection with either BRCA1 or BRCA2 – Over expression in Breast Malignant Tumors.

Table (4) show the association of HMTV infection with expression of BRCA1 & BRCA2 proteins in malignant breast tumors group. Among 17 breast cancer tissues that showed BRCA1 protein expression, 61.5 % (8 out of 13) have additional impact by infection with HMTV .While 14 breast cancer tissues that showed BRCA2 protein overexpression ,53.8% (7 out of 13) have additional impact by infection with HMTV. Statistically, the association of expression of BRCA1&BRCA2 among breast cancer tissues with HMTV infection showed significantly higher differences when compared to their HMTV- negative counter parts(p=0.028 & p=0.026,respectively) .

Table (4) : The Co-existence of HMTV infection with either BRCA1 or BRCA2 –expression in Breast Malignant Tumors .

P	HMTV-ISH Test				Positive Immune Surface Markers
	Positive (n=13)		Negative (n=17)		
	%	N	%	N	
0.028	61.5	8/13	29.4	5/17	**BRCA 1 expression**
0.026	53.8	7/13	35.2	6/17	**BRCA2 expression**

Discussion

The origin of the HMTV is still unknown but it is possible to originate from mice by zoonosis and as proposed by Stewart TH*et al.*,[35] and by Szabo*et al.*,[36]. Epidemiologic studies had showed a correlation between breast cancer incidence and the feral mouse Musdomesticus, that carrying more copies of MMTV than other mouse species[35]. In addition, successful MMTV infection of human cells[24, 37, 38] and studies of viral sequences in human breast cancer in different geographic locations[23],have supported such hypothesis. However, the potential of these viral particles in the causality of the described breast cancers as well as their infectivity and transformation potential were under meticulous investigations[39].

Several researchers have reported a controversial data with a wide range of prevalence of MMTV-like env gene sequence in BC tissues from patients in several countries, including zero % in Japan[40], Austria[41] and the United Kingdom[42], 0.8% in Vietnam[23], Mexico has reported a prevalence of 4.2%[31], 17% inChina[43], 31% in Argentina[23], 40% in United States[44], 42% in Australia[17], and 74% in Tunisia[23].

Sequencing of envelope gene and immunological detection as well as serological reactivity against the envelope protein were commonly used to trace the presence of the human mammary tumor virus (HMTV)[16].

While they are usually not present in the human genome, different methodologies are available allowing distinguishing the exogenous sequences which are constituting 95% to 99% homology to MMTV[45].

The presence of such retroviral particles in normal human milk samples[45],as well as a low prevalence of the viral sequences has been found in normal tissues, have ruling out either the germ line transmission of endogenous retroviral sequences or the polymorphisms in such sequences[46].

The current technology and the developed methodologies allow us to distinguish exogenous sequences that are not present in the human genome that are 95% to 99% homologous to MMTV[45].A low prevalence or absence of the viral sequences has been reported in normal tissue, ruling out the germline transmission of or polymorphisms in endogenous retroviral sequences[46].

We observed in this study a percentage of positive- in situ hybridization reactions for the detection of envelope gene of human mammary tumor virus (HMTV) in 43.3% of the examined breast cancer samples, with a higher detection rates among women aged 46–55 years, and these results are compatible to those reported in other studies. These results are supporting those reported by a Mexican study where the tissues obtained from Mexicanbreast cancer patients have revealed MMTV-like sequences prevalence of 4.2%[31].This retrovirus, and since the beginning of this century, has been designated as human mammary tumor virus (HMTV)[31].In addition, HMTV-env was detected in the DNA of 7.6% (7 of 92) from a reference-group women and in the biopsy-group 20.6% had HMTV sequences in their milk cells[33].

Our results support the finding as well as suggestions of other researchers that the presence of the HMTV sequences is associated with increased breast cancer risk[33].

The capsid proteins on the outside of the virus particle are believed to connect the virus to the host cell receptor and likely to penetrate into the infected cells. The envelope contains ITAM (immunoreceptor activation motif based on tyrosine) which is able to stimulate certain hormones so as to contribute in the initiation of such breast carcinogenesis. The Env as well as ITAM proteins in human breast cancer are likely oncoproteins[47].In addition, the proviral structure of HMTV is replication-competent while its long terminal repeat has several hormone-responsive elements as well as the open reading frame for superantigen[1, 16].

However, the receptor that is used by HMTV to penetrate into the cells of such human breast cancers is not yet known, and mechanisms allowing this virus to infect is not clearly understood, too. It was hypothesized that the virus effect might depend on the site of insertion of the retrovirus[20].

In this study, no envelope gene of human mammary tumor virus (HMTV) was detected in the examined normal breast tissue. This result is in disagreement with the study that was made on patients with NPC by Cedro-Tanda*et al.*,[20]

However, these results raise a question regarding the increased presence of HMTV in normal breast tissues especially when the possibility of contamination can be dismissed for the appropriate technical strictions which were followed in achieving these methods[20].

Thepresently observed as well as the previously reported findings regarding the differences in the prevalence of HMTV (MMTV-like) genetic sequences as well as the differences in breast cancer rates among different populations could be related to differences in their geographic distribution[29].

Regarding transmission in humans, we don't believe that HMTV has passed down through the germ line in our series of breast cancer female patients, since it should then be present in all cells of a given patient, although this possibility remains a latent question.

A possibility that the observed results of positivity in both tumor and unaffected tissues of the evaluated patients who have presented a similarity among their amplified viral sequences might indicate two independent HMTV infections or an HMTV infection of common origin[20].

HMTV-env was detected in the DNA of 7.6% (7 of 92) women, among them 20.6% had HMTV sequences in their milk cells[33]. This difference between both groups was statistically significant (p: 0.015). The higher percentage of women with HMTV sequences in their breast milk DNA was not correlated with any of

the demographic parameters previously examined including age at time of milk donation, time after delivery, number of live births, or age at first pregnancy[48, 49].

The mammary gland undergoes molecular changes in response to hormonal influences[33] and gestational breast cancers that develop during pregnancy or lactation are very aggressive and are related to hormonal changes, and have shown to have high incidence of viral HMTV sequences[50].

The risks of breast cancer associated with mutations in BRCA1 and BRCA2, particularly in relation to, families with multiple cases of breast cancers and those analyses with generally lower estimates obtained from patients with breast cancer collected without regard to family history or from a series of unaffected individuals have recently been of considerable discussion[51].

In this study, we observed a percentage of positive-IHC reactions for *BRCA1* and *BRCA2*immunohisto-chemical (IHC) reactions in 46.7% and 56.7% in BC tissues, respectively.

The frequency of mutations in *BRCA1* or *BRCA2* have been detected in 39%- 51% families[52]. In addition, the prevalence of BRCA1 mutations among U.K- patients with breast cancers has been estimated at 7.5% for those diagnosed before age 30 years, 5.1% for those from age 30 years through 39 years, and 2.2% for those from ages 40 years through 49 years[53].

There was also a slightly lower prevalence of patients with a family history of breast cancer diagnosed before age 60 years (8.1% of patients) among those analyzed for BRCA1 and BRCA2 mutations compared with those not tested (10.2%)[54].

Other researchers found that 4.1% of cases diagnosed under 35 years-of-age carried a mutation in BRCA1. This is comparable with other published estimates in this age-group – 6.3%[55], 6.2%[56] and 3.5%[57]cases under 36.Estimates of prevalence of BRCA2mutations in the same age group was somewhat higher than those previously reported: 8.3%compared with 2.4%[57]and 2.2%[58], but there is a substantial overlap between the confidence intervals of these estimates. In cases aged 35–44 they found 1.0% with BRCA1mutations and 1.0% with BRCA2mutationscompared with 1.9% and 2.2% reported by Peto*et al*.,[57].

Mutations in Dutch as well as Swedish families are due to three different, large genomic deletions in BRCA1 and duplication of exon 13 BRCA1 [59, 60].

The significant detection of HMTV along with BRCA- 1& BRCA-2genes expression production breast cancer patients are supporting the hypothesis of etiologic roles for that virus along with mutated and / or defected BRCA 1&2 genes in breast cancer development.[61-66]

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