Molecular Study of Human Mammary Tumor Virus in Iraqi Women with Breast Cancer

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Summary:

Background: Earlier reports related the presence of Mouse Mammary Tumor Virus -like gene sequences to human breast carcinoma. Mouse Mammary Tumor Virus -like gene is a retrovirus, namely, a virus containing reverse transcriptase which transcript its RNA to DNA in a process that enables genetic material from the retrovirus to become a part of the genes of an infected cell permanently. The virus that found in women was designated as Human Mammary Tumor Virus by the authors, who have investigated the presence of Human Mammary Tumor Virus sequences in a many human breast tissues and in many countries.

Objectives: Detect HMTV genome in Iraqi women of breast cancer.

Patients and Methods: Formalin Fixed, Paraffin Embedded Tissues were collected from 50-breast cancer women with their age range (23-82 years) compared to 5 normal breast tissues as a control group. DNA was extracted from and by Nested PCR, using specific primers for amplifying Envelop gene for HMTV were taken in Jovac Center in Amman/Jordan.

Results: There were no detected signals for HMTV in study cases.

Conclusions: The absence of HMTV DNA in breast tissues cancers needs a large sample size to correlate HMTV with breast carcinoma.

Keywords: Human Mammary Tumor Virus, Breast Cancer, Nested PCR.

Introduction:

Breast cancer is one of the most common female cancers in Iraq; which forms about one third of the registered female malignancies (1).

Viruses cause at least 15% of all human cancers (2); through 1995, viral etiology for some cases of human breast cancer has been suggested, like mouse mammary tumor virus (MMTV), which causes murine breast tumors (3). There is a considerapble evidence that the presence of MMTV-like gene sequences in humans is highly associated with breast carcinoma (3) (4) (5) (6) (7) (8) and it has been reported as a human murine mammary tumor virus (MMTV)-like virus or (HMTV). Mant et al. 2004 (7) studied that HMLVs are integral members of the MMTV family and both MMTVs and HMLVs are genetically distinct from human endogenous retrovirus. The first studies found MMTV-like gene sequences in 38% of breast cancer tissue samples from the USA but in 2% of normal breast tissue samples (3). The prevalence of MMTV –like gene sequences in women is as follows: North America 38% (3), Italy 38% (9), Australia 38% (10), Argentina 31% (11), Tunisia 74% (9), and China 16.8% (12). However, in Vietnamese populations the prevalence of MMTV-like gene sequences was 0.8% and it was not detectable in breast cancer cell lines or biopsies (10) Mant et al. 2004(7) did not find any positive samples in South London, UK. However, Ford et al.,2004(13)reported that progression from normal breast to breast cancer pathology is associated with an increasing prevalence of MMTV-like sequences in men and women.

To verify the HMTV reports, a retrospective study using Iraqi breast cancer patients compare to healthy women as Formalin Fixed Paraffin Embedded Tissues (FFPET) were performed. A nested PCR, based on env of MMTV (non-endogenous highly MMTV-like sequences (here collectively called HMTV), was designed by Dr. Peatriz Pogo.

Patients and Methods:

Patients and samples: Tumor biopsies from cases of breast cancer as FFPET were collected from 50 newly diagnosed women who were selected randomly with 5 benign conditions . The Patients were recruited from Medical City Teaching Hospital in Baghdad from October 2010 to June 2011. The practical part work was made by JOVAC CENTER in Amman –Jordan

DNA Purification: The DNAs were extracted according to PureLink Genomic DNA Mini Kit (Invitrogen Inc., Carlsbad, CA) instruction.

The DNA quality was tested by amplifying a 260-bp sequence of the β-globin gene as a positive control (5). As in Table (1)

The semi- nested PCR was done for no DNA contained mouse mitochondrial DNA as a negative control (14) .As in Table (1).

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Table 1: Oligonucleotide Primers sequence by Integrated DNA Technology Company

Item detected	Primers used	Annealing Temp	Product bp
β-globin Gene	GH-20	55°C	260-bp
	(5’-GAAGAGCCAAGGACAGGTAC-3’) and		
	PCO4		
	(5’-CAACTTCATCCACGTTCACC-3’).		
Mouse Mitochondrial DNA	First round	55 °C	286bp.
	Mt15982F (5’-AGA CGCA CCTA CGGT GAAGA-3’)		
	mt16267R (5’-AGAG TTTTGG TTCAA CGGAA CATGA-3’)		
	Semi Nested		
	mt16115F (5’-TGCCAAA CCCC AAAAA CACT-3’)	55 °C	153bp
	mt16267R (5’-AGAG TTTTGG TTCAA CGGAA CATGA-3’)		
HMTV Env gene	First round 5L (5’-CCAGATCGCCTTTAAGAAGG-3’)	58ºC	595bp.
	3L (5’-TACAGGTAGCAGCACGTATG-3’)		
	The nested PCR		
	1XXX(5’ACTGCACTAGTCCCCCATAC-3’)		260bp.
	3F (5’-ATCGCTGCATAGTCGAGGC-3’)		

The nested PCR for HMTV env sequences detection as in Table (1): Each PCR reaction was done using 2 μl of DNA, the Illustra PureTag Ready-To-Go PCR beads (GE Healthcare Inc., Piscataway, NJ), and using 1.5 μl of the primers as in Table (2). The reactions were performed in a Thermocycler [Lab Net Company] for 35 cycles under the following conditions: denaturation 95ºC for 15 sec, annealing 58ºC for 30 sec and extension 72ºC for 30sec.

DNA quantity tested by spectrophotometer absorbance assay of 260 nm and 280nm ratio.

Gel loading: Nine (9ul) from the amplicons were loaded to the wells with (1ul) of DNA gel loading Buffer X10. Ten (10 ul) of DNA Ladder were loaded.

Table 2: PCR Master Mix were prepare with PureTag Ready-To-Go PCR beads

Each tube contain	Final vol. 25μl
Forward Primers	1.5
Revers Primers	1.5
DNA template	2
Sterile high-quality water	20

Results:

DNA Extraction: According to the results obtained by this study, the volume of extracted DNA from FFPET had a ratio of OD (1.6-1.9) for 30 cases and 5 control samples which were considered acceptable, so we preferred to use it in PCR. As Table (3) bellowed:

Table 3: DNA Extracted from FFPET in study groups used for PCR amplification

Sample No.=30 cases No.=5 controls	DNA concentration µg/ml	Mean Value µg/ml
FFPET	7.28- 18.64	12.96

The volumes of extracted DNA for the rest of 20 samples were under assay condition for PCR test, it had a ratio of OD ranged from (0.2- 0.5). The damage nature and limited amount of DNA obtained from fixed tissue sections, DNA products was not enough to be used in PCR amplification. As Table (4) bellowed:

Table 4: Unfavorable DNA Extracted from FFPET for PCR amplification

Sample No.=20 cases	DNA concentration µg/ml	Mean Value µg/ml
FFPET	0.25- 4.57	2.41

β-globin Gene results: Strong signals of β-globin from (30) and (5) control samples of FFPET (control for DNA preservation) amplified bands were identified from the DNA of Iraqi cases (tests and control). Primers flanking β-globin gene were used as a positive control for DNA preservation. As in Figure (1).

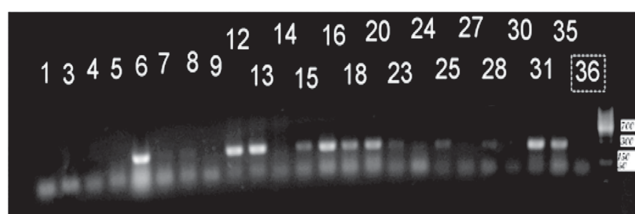

Figure 1: β-globin detection Results the +ve lans for 260bp
Strong Signal 6, 12,15,16,18,29,31,35
Weak Signal 23,24,25,28
No Signal 1, 3,4,5,7,8,9,14,36

Mice Mitochondrial DNA Results: Primers of Mice Mitochondrial DNA gene were used as a negative control for DNA preservation (expected PCR product size was 286bp for first round and 153bp for second round).

HMTV results: Primers HMTV env gene was used to detect the presence of the virus (expected PCR product size was 595bp for first round and 260bp for second round); HMTV –DNA bands were not detected among study group.

As in figure (2) and figure (3)

Figure 2: HMTV detection results
HMTV 1st Round - NO signal :(1-11)

Figure 3 : HMTV detection results
HMTV 2nd Round - NO signal :(1-11)

Discussion:

Our investigation along with Professor Pogo led us to think about HMTV that may play a role in the Pathogenesis of Breast Cancer among Iraqi women for many reasons, these are: 1- The increasing rate of breast cancer in Iraqi patients. 2- The prevalence of breast cancer in Iraqis among other cancers (15). 3- Iraq is one of the countries with a prevalent of House Mice; According to International Union for Conservation of Nature and Natural Rescores (IUCN) Red List –the native of these mice is Iraq (16). 4-According to Dr. Ruddy in 2010 mentioned that HMTV may play a role as a potential cofactor of Iraqi and Kuwaiti women; who are suffering from a very aggressive, fast-growing breast cancer and are presenting with only slight delay in diagnosis (due to cultural fears), and that it is the type of cancer they have that is to blame, not the women or their culture. (17)

According to the results obtained by this study; the purity of

extracted DNA from FFPET ranged from (1.6-1.9) for 30 cases and 5 control samples. While the purity of extracted DNA for additional 20 samples were ranged from (0.2- 0.5). As for formalin is known to fix protein in tissue samples including all proteins on each cellular level, which makes the reach for DNA difficult, and incomplete this agrees with Sambrook, (18). Protein contamination may be due to incomplete inactivation of Proteinase K. In addition, it is unsuitable for molecular techniques as slow degradation of DNA occurs with time as well as type of fixative employed (19).Both Al-Khalidi, (2006) (20) and Al-mansour, (2012) (21) demonstrated a similar results regarding DNA extraction .

The strong signals of β-globin from 30 and 5 control samples of FFPET indicated that the quality and quantity of the purified DNAs were adequate. While the other 20 samples gave weak, nonspecific bands of PCR round that mean a bad tissue processing, indicating that the qualities of purified DNAs were inadequate beside degraded nucleic acid fragments of the tissue samples primed the nonspecific product. Another explanation by previous researchers is that samples may contain PCR inhibitors that came either from the tissue cells or from the samples processing procedure and low DNA concentration. (22)

Despite of a very sensitive technique was taken HMTV env gene was not detected in human breast cancer tissue. These results could be explained by processing of FFPET the samples freshly prepared samples or due to small samples size, which gave less chance to detect the virus. Alternatively, the absence of HMTV DNA in both breast tissue samples of carcinoma and control indicated that this Virus may have no role in breast cancer development or progression among Iraqi women. These results are compatible with Japanese investigation (23) besides Sweden findings (8).

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