Detection Of Mouse Mammary Tumor Virus-Like Sequence (MMTV-Like Sequence) In The Breast Cancer Of Iraqi Women Samples By Nested PCR.

الكشف عن فايروس مشابه بفايروس سرطان ثدي الفئران في سرطان الثدي عند النساء العراقيات باستخدام تقنية تفاعل انزيم البلمرة المتداخل

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الخلاصة

خلفية البحث : إن اكتشاف فيروس سرطان ثدي الفئران في عترات الفئران ذات الاستعداد العالي للإصابة بالسرطان قادر على إحداث الأورامِ الثديية، وتعريفه كفيروس RNA من نوع B، يعد دليل على فرضيةِ على وجود عامل مماثل مرتبط بسرطان الثدي في الإنسان أجريتْ الدراسةُ الحاليةُ للكشفِ عن فيروس سرطان ثدي الإنسان في (فيروس المشابه لفيروس سرطان ثدي الفئران) باستخدام تقنية تفاعل أنزيم البلمرة المتسلسل.

الهدف: الكشف الجزيئي عن وجود فايروس شبيه بفايروس ثدي الفئران في سرطان الثدي عند النساء العراقيات.

المنهجية: الدراسةُ الحاليةُ عملت على نماذج الأنسجة المثبتة بالفورمالين لسرطان الثدي لنِساءِ عراقياتٍ، والتي جَمعتْ مِنْ مستشفى الصدر التعليمي في وحدة النسيج المرضي ووحدة الأورام، وعِدّة مختبرات خاصة في محافظةِ النجف، النماذج كانت مخزونة للسَنَواتِ (2008,2009,2010 و2011). النماذج تُضمّنتْ ، 54 نموذج لسرطان الثدي ، 21 نموذج التليف ألغدي، 18 نموذج لالتهاب ثدي ، 19 نموذج لحالات توسع القنوات الثديية. صنّفتْ العيناتُ إلى مجموعات حسب الأعمار، وكانت أكثر مجموعةِ عُمرية 36-45 (42.57 %). هذه الدراسةِ أجريتْ في مركز ماونت سيناي الطبي / نيويورك / الولايات المتحدة الأمريكية لأن برايمرات فيروس سرطان ثدي الإنسان (فيروس المشابه لفيروس سرطان ثدي الفئران) تصنع عندهم ، وهذه البرايمراتُ مهمة جداً في التقنياتِ الجزيئيةِ. تم التعامل مع نماذج الأنسجة المثبتة بالفورمالين لسرطان الثدي في مختبرِ علْمَ الأمراض / مركز ماونت سيناي الطبي، لإعادة الصبّ والتقطيع لاستخدامها في بروتوكول تفاعل أنزيم البلمرة المتداخل وكذلك استخدام صبغة الهيماتوكسلين والايوزين لتأكيد التشخيص. اما الحامض النووي المستخلص فقد تم تقييمه كميا ونوعيا بواسطة جهاز النانودروب وباستخدام جين البيتا كلوبين على التوالي.

النتائج: أظهرت النتائجَ بأنّ جين غلاف الفيروس المشابه لفيروس سرطان ثدي الفئران باستخدام تفاعل أنزيم البلمرة المتداخل بالنسب المئويةِ التاليةِ: نماذج سرطان الثدي 57.89 % (22 من 38) ، ونماذج التليف ألغدي22.22% (4 من 18)، ونماذج التهاب الثدي 7.14 % (1 من 14) حيث كان هناك اختلاف معنوي ($p <0.001$). كان هناك اختلاف معنوي ($p <0.001$) لنتائج تفاعل أنزيم البلمرة لفيروس ثدي الإنسان طبقاً للمجاميع العمرية.

الاستنتاجات: تم الكشف عن وجود هذا الفايروس في سرطان الثدي عند النساء العراقيات.

التوصيات: اعتماد التقنيات الحديثة في التشخيص الجزيئي وكما هو معمول به في المختبرات العالمية وذلك من خلال فحص الحامض النووي المستخلص من حيث الكمية والنوعية ثم البدء بالكشف الجزيئي بعد إجراء الحسابات الرياضية بدقة.

Abstract

Background: Since the discovery of MMTV in the high cancer strains of mice capable of initiating mammary tumors , and its subsequent identification as a B-type RNA virus, several evidence has been accumulated supporting the attractive hypothesis that a similar agent is associated with human breast cancer. The current study conducted to detection the MMTV-LIKE SEQUENCE(MMTV-like virus) by nested PCR.

Objectives: molecular detection of a virus-like MMTV in the breast cancer of Iraqi women.

Methods: The current study tested breast cancer FFPT specimens of Iraqi women, which collected from AL-Sader teaching hospital histopathology and neoplasm units , and several privet histopathology labs in AL-Najaf province, the specimens were stored for the years (2008,2009,2010 and 2011). The specimens were included of BC 54 , fibroadenoma 21, mastitis 18, and ductectesia 19 samples. The samples classified to age groups, the most age group was 36-45 (42.57%). This study was conducted at the MSMC/Tisch cancer institute/NY/USA because the MMTV-LIKE SEQUENCE (MMTV-like virus) primers manufactured by them , and the primers were very important in the molecular techniques. The FFPT passed through the pathology lab /MSMC , for recasting and sectioning for nested PCR protocol and Hematoxylin and eosin staining to confirm the diagnosis. The DNA extraction was started from the FFPT which were tested for quantity and quality by NanoDrop system and β-glubine PCR respectively.

Results: The findings of the MMTV-LIKE SEQUENCE-env gene appeared as following percentages : BC 57.89% (22 of 38) , fibroadenoma 22.22% (4 of 18), and mastitis 7.14% (1 of 14) there was a highly significant difference ($p<0.001$). There was a highly significant ($p<0.001$) of The MMTV-LIKE SEQUENCE PCR results according to the age groups.

Conclusions: presence of MMTV-like virus in the breast cancer of Iraqi women.

Recommendations: the modern techniques should be dependent in the molecular detection as it find in the world labs, for example the test of extracted nucleic acid by quantity and quality and then start the molecular detection after doing of definite math measurements.

Keywords: BC: breast cancer, MMTV: mouse mammary tumor virus

INTRODUCTION

Mouse mammary tumor virus (MMTV) belongs to the family of Retroviridae, subfamily: Orthoretrovirinae, genus: Betaretrovirus, which is responsible for over 95% of breast cancer in mice [(1)]. There was an interest in trying to identify human viruses that might cause breast cancer, oncogenic viruses considered as one of the breast cancer risk factors and it is actually one of the oldest areas of breast cancer research since (MMTV) was identified over 60 years ago. The oncogenic viruses and breast cancer has recently gotten the attention of the researchers who proposed that there is a certain type of wild mouse that resides mostly in Europe and North America, but not in Asia, may be transmitting the MMTV to human populations, and hence explain why breast cancer rates are lower in Asian compared to Western countries. In humans, MMTV-like virus has been described in between 14% and 74% of breast cancers [(2)] but other studies report no detection of the virus[(3)]. (MMTV) sequences have been reported to be present in human breast cancers, but it is unclear whether they have any causal role, the possible role of MMTV-like sequences in human breast cancer remains controversial. The majority of recent reports linking the MMTV and human breast cancer have come from one research group, including sequences of two complete pro-viral structures from human breast cancer tissue , others reported the presence of MMTV-like sequences in lymphomas. Several groups of investigators have reported that the MMTV-like sequences in up to 30% of human breast cancers [(4)].

STUDY AIM

The current study was applied to main aim, which was to determine presence of MMTV-LIKE SEQUENCE in the breast cancer tissues of Iraqi women formalin-fixed paraffin tissues (FFPT). The primers of the virus and other research practices and facilities, which were very important to completed the research, in Iraq were unavailable , for this reason, part of the practical aspect of the research conducted in the MSMC/Tisch cancer institute /NY/USA on Iraqi women FFPT samples.

MATERIALS AND METHODS

This study was started on May 2011 up to June 2012, including collection and processing of samples was carried out in the departments of microbiology and pathology / faculty of medicine/ university of Kufa and the teaching laboratories of AL- Najaf city.

Collection of patient'sFFPT samples.

Patient's history was collected at the neoplasm unit in Al-Sader Teaching Hospital, and then started to find the patient's reports for further information depending on the block's digit such as, patient's history , types of the breast cancer (BC), grading, and staging. Patients were clinically interviewed and examined using a triple assessment technique, i.e. clinical breast examination, mammography and/or ultrasonography, and fine needle aspiration cytology (FNAC).

After mastectomy, 112 FFPT samples were collected from the histology unit, in Al-Sader Teaching Hospital, and/or from privet labs in AL-Najaf province .
The FFPT classified as the followings:(54) blocks of breast cancer (BC), (21) blocks of fibroadenoma, (18) blocks of mastitis and (19) blocks of duct ectasia.

Processing of the FFPT samples

The FFPT samples processed started at the pathology lab. of the MSSM to cut of slices from blocks about 8 um by using sterile microtome , and put the slice in the 1.5ul eppendroff tubes for PCR protocol.

Extraction of human DNA genome.

It is the first step in the PCR protocol. The DNA extracted according to the Invitrogen company **(pureLink™,Genomic DNA mini kit, ca: k182001/USA), in which xyline and ethanol method wasused.**The purified DNA was stored at -20°C or use DNA for immediately.The evaluation of nucleic acid was by quantity and quality (NanoDrop instrument and β-globin respectively).Eighty samples were positive with β-glubine PCR which then tested for MMTV-like env gene by nested PCR .

Test the patient's samples by MMTV-like sequencenested PCR.

Positive β-globin PCR samples were used to MMTV-like sequence PCR by used of (GH healthcare,illustraTmpuretaq Tm ready to go Tm PCR beads, 0.2ml tubes/plates/UK, ca.27955702).

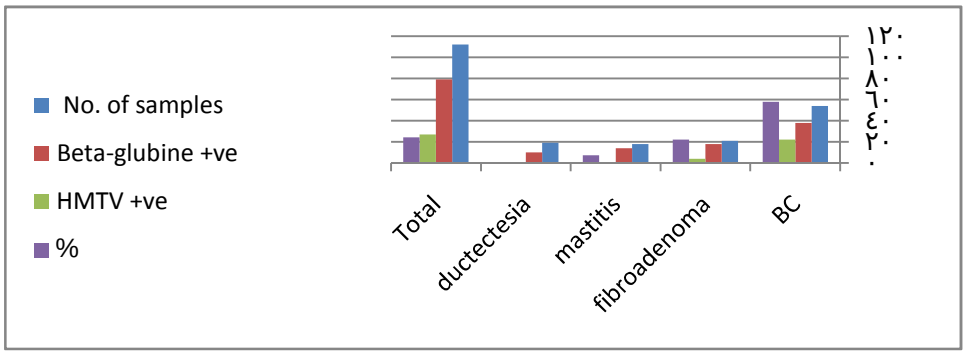
- Nested PCR was used to amplified of MMTV-LIKE SEQUENCEenv region.
- 2ul of master mix of the 1st MMTV-LIKE SEQUENCE primers (3L &5L) to amplified of Env gene was added.
- The three negative control tubes made by added primers plus water (2ul primers + 23 ul water).
- Positive control tube made by added 2ul of primers, 1 ul of positive control, and 22ul water.
- The samples volume was added, and complete the volume to 25 ul.
- PCR condition of MMTV-LIKE SEQUENCE was: 95°C (5 minutes), 95°C (30 seconds) , 58°C (30 seconds), 72°C (30 seconds), Go to 2 repeat 35 cycles and 72°C (7 minutes).
- Then the 2nd MMTV-LIKE SEQUENCE PCR was done.
- 5ul from each 1st MMTV-LIKE SEQUENCE PCR tube in new PCR tubes .
- 2ul of master mix of the 2nd set MMTV-LIKE SEQUENCE primers (3F &1XXX) to amplified of Env gene was added.
- 18ul of water was added to complete the PCR volume.
- Additional three negative control tube made as the 1st MMTV-LIKE SEQUENCE PCR (2ul primers + 23ul water).
- Positive control tube made by added 2ul of primers, 1ul of positive control, and 22ul water.
- PCR condition of 2nd set MMTV-LIKE SEQUENCE PCR
- gel electrophoresis instrument was done as previously described.
- The PCR results showed on 2% agarosegel .

Table (1) Results of MMTV-LIKE SEQUENCE nested PCR

Tissue types	No. of cases	β -glubine positive	MMTV-LIKE SEQUENCEnegative		MMTV-LIKE SEQUENCE positive	
			No.	%	No.	%
BC	54	38	16	42.10	22	57.89
Fibroadenoma	21	18	14	77.77	4	22.22
Mastitis	18	14	13	92.85	1	7.14
Ductectesia	19	10	10	100	0	0
Total	112	80	53	66.25	27	33.75

(X^2=20.5, P<0.001=0.0001334, df=3)

The total MMTV-like sequence positive were 27 (33.75%) as the following BC (%57.89, 22 of 38), fibroadenoma (%22.22, 4 of 18), mastitis (%7.14, 1 of 14), ductectaia (%0, 0 of 10), there was a significant differences (P<0.001) (**Table 1) (Histogram 1) (Figure 1).**



Histogram (1) results of theMMTV –like sequence nested PCR

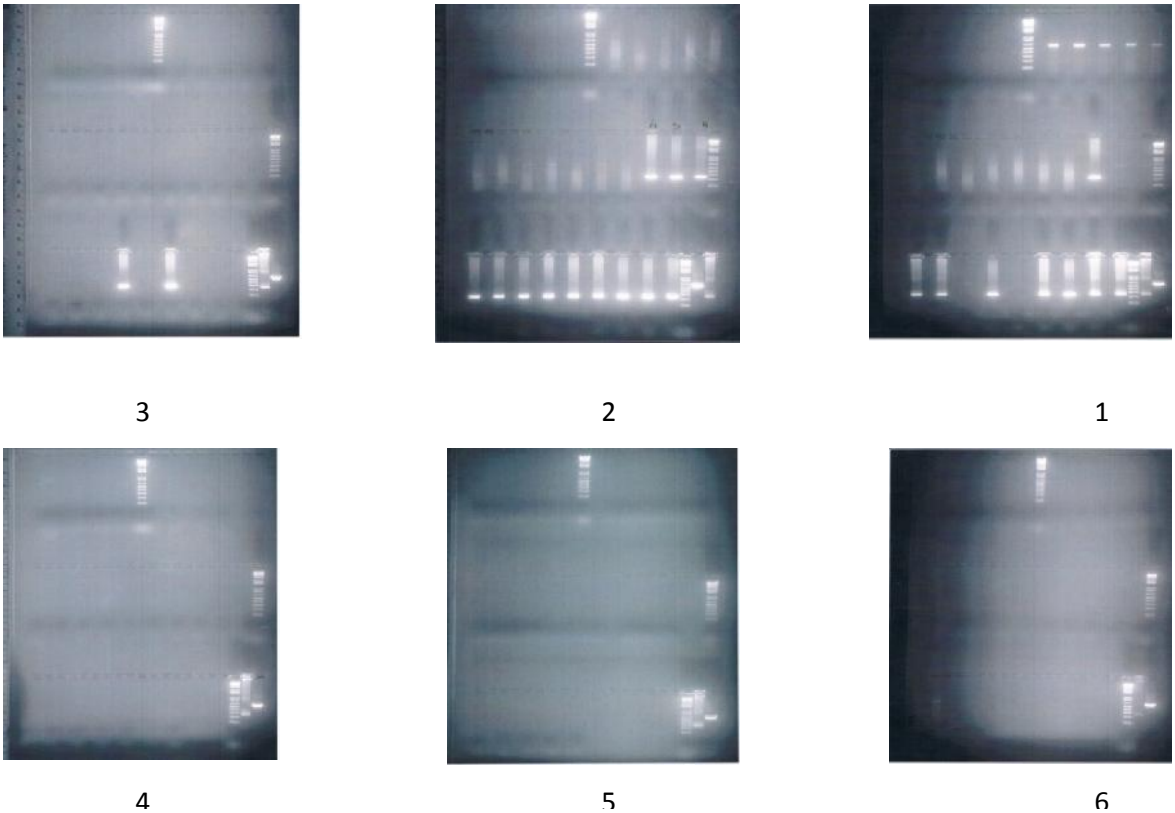


Figure (1) MMTV-like sequence results by 2% agarose gel electrophoresis:**1.** 12 samples of BC nested PCR results appeared positive .**2.** BC and fibroadenoma nested PCR results, include 12 samples (10 BC and 2 fibroadenoma samples were MMTV-like env gene positive).**3**. fibroadenoma nested PCR hybridization results, include 12 samples (only 2 fibroadenoma samples were MMTV-like env gene positive).**4**. 4 samples of fibroadenoma& 8 samples of mastitis nested PCR hybridization results, include 12 samples (only 1 mastitis sample was MMTV-like envgene positive).**5.** 7 samples of mastitis nested PCR hybridization results (all samples were MMTV-like envgene negative)**. 6**. ductectasia nested PCR hybridization results, include 19 samples (all samples were MMTV-like env gene negative).

Table (2) Number of MMTV-LIKE SEQUENCE positive samples according to the patient's age.

Age	BC	%	Fibroadenoma	%	Mastitis	%	ductectesia
20-30	0	0%	0	0%	1	100%	0
31-41	6	27.27%	4	100%	0	0%	0
42-51	8	36.36%	0	0%	0	0%	0
52-61	4	18.18%	0	0%	0	0%	0
62-70	4	18.18%	0	0%	0	0%	0
Total	22	57.89	4	22.22	1	7.14	0

(X^2=34.85, P<0.001= 0.0004939,df=12)

According to the patient's age, , the most age appeared positive -MMTV-LIKE SEQUENCE PCR was (45-51)(36.36%) **there was a significant differences** (P<0.001) **(Table 2).**

DISCUSSION

The current study have not considered the possible means of transmission of MMTV-like sequences, but the study aimed to show the prevalence of MMTV-like *Env* gene sequences in the invasive breast cancer tissues in the Iraqi women breast cancer. There are some of risk factors may have an oncogenic roles in the breast cancer, these include early-age menarche, late-age menopause, postmenopausal obesity and use of hormone therapy, most of which relate to estrogens and growth hormones in females. Therefore , hormone-responsive viruses have become major suspects as etiological agents for human breast cancer. MMTV is one of several viruses that consider the prime candidate viruses as causes of human breast cancer. Particularly, MMTV have hormone responsive elements that appear to be associated with enhanced replication of these viruses in the presence of corticosteroid and other hormones[(5)].MMTV has features such as , long terminal repeat (LTR) encodes certain carcinogenic proteins , reverse transcription, activates several oncogenes, and uses lymphocytes to infect mammary glands (without these cells, the virus cannot be transmitted to the mammary glands), also MMTV has been studied for many years in order to understand carcinogenic viruses and to determine any correlation with human breast cancer. With the emergence of DNA analysis in the 1990, there has been evidence that MMTV may be transmitted to human and shows to have an homologous strain apparent in human breast cancer tumors. While there **is** evidence supporting MMTV may be a cause of human breast cancer, there are also data that contradict this theory and it continues to be an issue of great controversy [(6)].The current study have shown that the prevalence of MMTV-LIKE SEQUENCE*Env* sequences in the invasive breast cancer tissues from the Iraqi women with breast cancer is much higher than in normal breast tissues, the MMTV-LIKE SEQUENCE positive results were, BC 57.89% (22 of 38) ,fibroadenoma 22.22% (4 of 18), and mastitis 7.14% (1 of 14) (Table 9) the results was statistically significant ($P<0.001$). The history of detection of the MMTV-like sequences in the last two decades, started when Caroline Ford *et al*. in 2004 find that the percentage of female breast cancer samples positive for MMTV-like env sequences increased from 23% of invasive ductal carcinoma (IDC) grade I tumors to 34% of IDC grade II tumors to 38% of IDC grade III tumors, also the prevalence of MMTV-like sequence in premalignant breast lesions were 20-28% [(7)] , this study results show similar to our study results. Wang et at. on 2004 was the first demonstrator that the sag gene sequences isolated from human breast cancer were able to perform similar functions to those in the mouse , the results indicated that 10 human partial 3'LTR isolates were highly homologous to the MMTV LTR sag gene but not to the endogenous virus LTR, this similarity between the sag gene of the mouse and human does not mean "causation" [(8)].James F. Holland said that the fundamental observation of MMTV-like *env* sequences in breast cancer has been confirmed by Etkind *et al.* , Ford *et al.*, and Levine *et al.* and by another laboratory known to us that has not yet published its results. Other workers using different methods have not reproduced our results, but none has then reported a faithful replication of our exact methodology . A recent report using *Insitu* polymerase chain reaction localized the product and its expression by reverse transcription-polymerase chain reaction to epithelial cells, whereas stromal cells were negative [(9)].

Mariana *et al.*in 2006 found the results clearly indicated that the transcriptional profile of the cells expressing MMTV-LIKE SEQUENCE sequences was enriched in genes involved in inflammation process, this results supports the hypothesis that a viral infection may play a role in breast cancer pathogenesis [(10)] . Moreover few researchers investigated the production of viral particles in primary cultures of human breast cancer form ascites or pleural effusions of patients with metastatic breast cancer contained viral sequences in their DNA, expressed Env protein, and showed retroviral particles by electron microscopy, the particles have morphologic features similar to the β-retroviruses sedimenting at buoyant densities of 1.12 to 1.18 g/mL in sucrose gradients and showed reverse transcriptase activity, the sequence homologies were, respectively, 85% to 95% compared with the MMTV and MMTV-LIKE SEQUENCE proviruses we have previously described. These results clearly show that breast

cancer cells in primary cultures produced MMTV-LIKE SEQUENCE viral particles that are similar to the mouse virus and which may play a role in human breast cancer pathogenesis [11] .In 2007StanislavIndik and his colleagues demonstrated the productive infection with MMTV of human breast cells, their study hypothesized the linking MMTV and human disease and might further substantiate the notion that MMTV may be involved in human diseases such as breast cancer and primary biliary cirrhosis [12] .In 2010 Stella et al. find expression of MMTV-LIKE SEQUENCE proteins in MSSM cells and the MMTV-LIKE SEQUENCEenv protein was detected by monoclonal AbP1, P2, P3, P4, and Su as well as by Gp36 but not by Gp52 [13] .

All the above studies were able to detect the virus in the BC samples on the other side, some researchers failed to detected such as Witt *et al*. found that MMTV-like env gene sequences were not detectable in breast cancer tissue of Austrian patients [14].In 2007 Bindra A. et al. searched for DNA of an exogenous MMTV-like virus, despite they having a very sensitive technique , they did not detected MMTV-LIKE SEQUENCE DNA in human breast cancer tissue [15]. Daniel et al. on 2011 find that the MMTV is unlikely to be a causative agent for female breast cancer and their research was inconsistent with previous reports on Australian breast cancer specimens but consistent with another negative reports on Australian women [16].Our study conducted on FFPT for breast cancer as a study group, and fibroadenoma as a comparative group, while mastitis and ductectesia as a control group (free of malignancy), these tissues clinically consider as an abnormal tissues, so, the presence of positive MMTV-LIKE SEQUENCE results among the study and control groups give a clue for the role virus with pathogenicity rather than malignancy. Our results were differs from previous studies, such as, the results of samples tested in different countries , showed Tunisia 74%, Australia 42% , Italy 38%, Argentina 31%, United States 36% and Vietnamese woman breast cancer samples linked less than one percent [17] .

Caroline Ford said that the PCR screening of 45 breast cancer samples from Australian women and 120 from Vietnamese women showed the prevalence of MMTV-like gene sequences of 42.2% (19 of 45) and 0.8% (1 of 120), respectively and a difference that was statistically significant ($P < 0.0001$) [18].The Env gene of MMTV genome have very important role in the breast tissue tumorigenesis, this idea agreed with the findings of Ross S. *et al*. who said, because the focus over the past 25 years has been on retroviral integration as the mechanism of tumor induction, including recent high-throughput analyses of integration sites, much less is known about the early steps in non-acute transforming retrovirus-induced cancers. Studies on the betaretrovirusesJaagsietke sheep retrovirus, which is related to MMTV and causes lung adenocarcinoma, have shown that its Env protein behaves as an oncogene , although it is also possible that insertional mutagenesis is required for transformation [19].The MMTV env gene-like sequence was found in 15 (33%) of the human breast cancers analyzed, whereas the same sequence was detectable neither in normal tissues nor in other types of tumor. Sequence analysis revealed 96% homology with the MMTV genome, but no other significant similarities with the human genome [20].

Yager J. in (2006) said that an association between the risk of breast cancer and persistently elevated blood levels of estrogen has been found consistently in many studies. Several endocrine-associated risk factors are regularly associated with an increased relative risk of breast cancer in postmenopausal women. One of these factors is obesity, which is probably related to an increased production of estrogen by aromatase activity in breast adipose tissue. Another factor is an elevated blood level of endogenous estrogen (relative risk, 2.00 to 2.58). An increased relative risk is also associated with higher-than-normal blood levels of androstenedione and testosterone, androgens that can be directly converted by aromatase to the estrogens estrone and estradiol, respectively. Elevated urinary levels of estrogens and androgens are also associated with an increased risk of breast cancer in postmenopausal women [21].On the other hand, Levine P. *et al*found that the possible role for hormone stimulation is suggested by the higher percentage of MMTV-related sequences in gestational breast cancer, which is associated with both increased hormonal stimulation and a poorer prognosis [22].

Mouse mammary tumorigenesis caused by MMTV has provided a rich source of interesting genes that play a role in mammary development and tumorigenesis. The geographic differences were compatible with studies of MMTV-like sequences in wild mice, particularly, species of house mouse, The current study finds that the Iraqi women who appeared MMTV-LIKE SEQUENCE positive, they might be infected with MMTV-like virus via house mice which have highly distribution in the widely Iraq areas, this idea agreed with Faedo M. *et al* who suggest that the MMTV is prevalent in wild-caught house mice in an agricultural area in southern Australia. It is unknown if a similar prevalence occurs in metropolitan regions, and further research to address prevalence of MMTV in urban house mice, and other species contacting (or feeding upon) mice, is required. Epidemiological surveys of the animal host are important because they define the virology of the wild population [(23)].

Beatrize Pogo, professor of medicine, hematology, and medical oncology at MSMC, named this human breast cancer virus the "human mammary tumor virus, MMTV-LIKE SEQUENCE." Many people refer to it as the "Pogo Virus." Beatrice Pogo's laboratory detected MMTV-like in human breast cancer samples, recently Pogo's laboratory found a higher prevalence (62%) of MMTV-like env gene sequences in gestational breast cancer [(24)].

Although Some studies assumed that the available evidence for an infectious etiology of human breast cancers is unconvincing. Amongst the many cognate arguments against an infective hypothesis for sporadic cases of human breast cancer are the facts that:

1. human tissues lack the appropriate cell-surface receptor for entry of MMTV.
2. unlike all other virally caused human malignancies, immunodeficiency does not predispose to an increased incidence, or prevalence, of human breast cancers.
3. reports of PCR amplification of MMTV sequences from breast cancers have been robustly disputed by four independent laboratories. Indeed positive PCR results may be readily explained by the mis-amplification of host genomic DNA [(25)].

CONCLUSIONS

1. This study may corroborate and consolidate the hypothesis linking MMTV-like sequences and breast cancer.
2. For best DNA extraction, modified manual method by using of xylineand ethanol method is preferable.
3. The NanoDrop thermo-scientific 8000 was the best instrument for DNA quantitation.

RECOMMENDATIONS

1. FFPT used in genomic testing should be stored in suitable conditions to avoid DNA degradation.
2. Avoid and eradicate house mouse (*musdomesticus*) because it is theoretically, very liable in transmission of MMTV to the mouse , and it may play a role in the transmission of MMTV-like virus to the human theoretically.
3. This study recommends further detection of MMTV –like sequences in other human cancers, such as lymphoma.
4. To prove that a virus causes malignances, the MMTV –like sequences requires working on cell lines to predict the cytopathic effects on the breast tissues.

REFERENCES

1. Harpreet J.; Margaret F.; Jacqueline F.; Amie L.; Railya M.; Paul C.; Anna D. and William DR. (2010). DNA of Mouse Mammary Tumor Virus-Like Virus Is Present in Human Tumors Influenced by Hormones. *Journal of Medical Virology* 82:1044–1050 .
2. Amarante MK. and Watanabe MA. (2009). The possible involvement of virus in breast cancer. *J Cancer Res ClinOncol* .135:329–337.
3. Fukuoka H.; Moriuchi M.; Yano H.; Nagayasu T. and Moriuchi H. (2008). No association of mousemammary tumor virus-related retroviruswith Japanese cases of breast cancer. *J Med Virol. 80:1447–1451.*

4. Mant C.; Hodgson S.; Hobday R.; D'Arrigo C. and Cason J. (2003). A viral aetiology for breast cancer: time to re-examine the postulate. *Intervirol*.47:2–13.
5. Lawson JS.; Günzburg WH.and Whitaker NJ. (2006).Viruses and human breast cancer.*Future Microbiol.*1(1):33-51.
6. Chelsea C.; Michael D.; Kaitlin M.; Amy S.; Samantha T. and Allan A. (2007).Viral Etiology in Breast Cancer?The Georgetown Undergraduate Journal of Health Sciences. Vol. 4, No. 2.
7. Ford C.;Faedo M.; Crouch R.; Lawson JS. and Rawlinson WD. (2004). Progression from normal breast pathology to breast cancer is associated with increasing prevalence of mouse mammary tumor virus-like sequences in men and women. *Cancer research*. 64(14). 4755 – 4759.
8. Wang Y.; Jiang J.; XuD.; Li Y.; QuCh.; Holland JF.and Pogo B., (2004).A Mouse Mammary Tumor Virus-Like Long Terminal Repeat Superantigen in Human Breast Cancer.*Cancer Res June* .15(64):4105.
9. Holland JF. and Pogo B. (2004). Mouse mammary tumor-like viral infection and human breast cancer. *Clin Cancer Res*. 10:5647–9.
10. Fernandez-Cobo M.; Melana S.; Holland J. and Pogo B. (2006). Transcription profile of human breast cancer cell line expressing MMTV-like sequences. *Infectious Agents and Cancer. 1:7.*
11. Melana S.; Irene N.; Michael S.; Andrea A.; Jennifer H.; Holland JF. and Pogo B. (2007). Characterization of Viral Particles Isolated from Primary Cultures of Human Breast Cancer Cells.*Cancer Res* . 67: 8960.
12. Indik S.; Günzburg WH.; Kulich P.; Salmons B. and Rouault F. (2007). Rapid spread of mouse mammary tumor virus in cultured human breast cells. *Retrovirology*. 4:73–88.
13. Melana S.; Nepomnaschy I.; Hasa J.; Djougarian A.; Holland J. and Pogo B. (2010). Detection of human mammary tumor virus proteins in human breast cancer cells. *J Virology methods*. 163 : 157-161.
14. Witt A.; Hartmann B.; Marton E.; Zeillinger R.; Schreiber M. and Kubista E. (2003). The mouse mammary tumor virus-like env gene sequence is not detectable in breast cancer tissue of Austrian patients. *Oncol Rep*. 10:1025-9.
15. Bindra A.; Muradrasoli S.; Kisekka R.; Nordgren H.; Warnberg F. and Blomberg J. (2007). Search for DNA of exogenous mouse mammary tumor virus-related virus in human breast cancer samples. *J Gen Virol.* 88, 1806-1809.
16. Daniel J.; Melissa C.; Graham G. and John L. (2011). N o evidence of MMTV-like env sequences in specimens from the Australian breast cancer family study. *Breast Cancer Res Treat*. 125:229-235.
17. Levine PH.; Pogo B.; Afifa K.; Stephanie C.; Karen W.; Melana S.; Nejib M. and Holland J. (2004). "Increasing Evidence for a Human Breast Carcinoma Virus with Geographic Differences" *Cancer*. 15,101(4):721-6
18. Ford C.; Rawlinson WD. and Tran D. (2003). MMTV-like gene sequences in breast tumors of Australian and Vietnamese women. *Clin Cancer Res*. 9: 1118-20.
19. Ross SR.; John WS.; Elad K.; Laura C.; Stacy H.; Phyllis G. and John GM. (2006). An Immunoreceptor Tyrosine Activation Motif in the Mouse Mammary Tumor Virus Envelope Protein Plays a Role in Virus-Induced Mammary Tumors. *J Virol*. 80(18): 9000–9008.
20. Zammarchi F.; Pistello M. and Piersigilli A. (2006). MMTV-like sequences in human breast cancer: a fluorescent PCR/laser microdissection approach. *J Pathol.* 209:436–44.
21. Yager JD. and Davidson NE. (2006). Estrogen carcinogenesis in breast cancer. *N Engl J Med*. 354:270–82.
22. Levine PH.; Heather AY.;LaQuisha M.; Helen R.; James FH. and Pogo B. (2009). Increased Detection of Breast Cancer Virus Sequences in Inflammatory Breast Cancer. *Advances in Tumor Virology* :1.

23. Faedo M.; Hinds LA.; Singleton GR. and Rawlinson WD. (2007). Prevalence of mouse mammary tumor virus (MMTV) in wild house mice (Musmusculis) in southeastern Australia. *J Wildl Dis*. 43:668–74.
24. Wang Y.; Melana SM.; Baker B.; Bleiweiss I.; Fernandez-Cobo M.; MandeliJF.; Holland JF. and Pogo B. (2003). High prevalence of MMTV-like env gene sequences in gestational breast cancer. *Med Oncol*.20(3):233-6.
25. Mant C. and Cason J. (2004). A human murine mammary tumour virus-like agent is an unconvincing aetiological agent for human breast cancer. *Rev Med Virol.* 14:169-177.