Studying the relationship between human papillomavirus (HPV) and breast cancer in Iraqi patients

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ABSTRACT

Viruses such as HPV have been identified in benign and cancerous breast tissue and as etiologies. Breast cancer has been considered, but there is controversial information about viral induction of breast cancer. The purpose of this study Investigation of the presence of human papillomavirus in Iraqi breast cancer patients.

Methodology:

In this case-control study, sections of paraffin blocks were taken from 100 female patients with breast cancer and 53 breast tissues from

Patients with fibrocystic disease were screened for the presence of HPV DNA as controls. Real-time PCR to amplify sequences

GP6+/GP5+ conserved HPV was used

Results:

HPV DNA sequences were found in 3 out of 53 benign breast tissue samples, but none of the breast cancer samples were found.

unknown.

Conclusion:

Our results do not confirm the role of HPV in breast cancer

Keywords: human papillomavirus, breast cancer, real-time polymerase chain reaction

Material and Method

Breast cancer is one of the most common types of cancer in the world. This cancer has been a health problem in the whole world and is the main factor The mortality rate is considered among women. Several factors Such as age, length of childbearing, infertility, obesity, etc. This relationship is known to this cancer from a clinical point of view It is a heterogeneous disease, mainly due to its broad spectrum From genetic changes such as loss or gain Chromosomes (or chromosomes), genome rearrangements and mutations Give. From screening genome assembly of cancerous tissue, information There is an infinite number to get of them Identifying successful diagnostic and prophylactic factors However, there are limitations in using There are also effective targeted molecular therapies

Breast cancer is a multi-stage disease that occurs in Virus DNA can be processed in one or several stages Involved in the pathogenesis of disease (3 and 4). (human papillomaviruses) They may play a role in the development of breast cancer. Hypotheses that HPV can cause breast cancer For participation, obtained from

experiments in which the cellular class Human breast, after complete genome transmission of HPV types 16 and 18 Become immortal However, there is no evidence that HPV It can infect human breast tissue in the body, it does not exist

Papillomaviruses (PVs) are a small group of non-viral viruses The DNA covering is two strands that are part of the family They are papillomaviruses. These viruses infect the squamous epithelium Pollution in different areas. According to available information) Nearly 200 types of human papillomavirus (HPV) have been identified. he is Vs causes a group of epithelial hyperplasia and is classified into two groups mucosal and cutaneous These groups depend on the strength of the lesion in causing the progressive malignancy to HPV is divided into two groups, low-risk and high-risk Low-risk mucous membranes such as HPV-6 and HPV-11, warts and high-risk HPV mucous membranes HPV 16-HPV and 18-HPV cause intraepithelial lesions Squamous cell carcinoma can progress to the stage of squamous cell carcinoma The attackers also advanced. HPV with oral malignancies is associated with the reproductive system. In fact, more than 99% of cervical cancer cases are caused by high-risk HPV Clinical studies provide controversial information about the presence of HPV presented in breast cancer. Mainly European studies Focus on HPV types 16 and 18, while Information obtained from Japanese and Chinese women, evidence from The relationship between breast cancer and rare types of HPV such as HPV 33 appears

The purpose of this study is to verify the presence of HPV in Iraqi female patients were diagnosed with breast cancer Solar The mean age of patients who underwent surgery was 48 years (33 years as a minimum and 56 years as a maximum). Age group Selected normal people are also in the cancer age group It was Using a microtome, very thin sections 10 4 µm paraffin blocks were prepared in 1.5 mL Eppendorf microtubules are missing RNase/DNase was ignored. Once per sample of glove Depreciation, new razor blade and applicator have been used.

After de-caffeination with xylene and absolute ethanol, DNA is used with Using the extraction kit, Indianapolis Diagnostics-Roche According to the agenda provided by the extraction company The extraction method was as follows:

Deparaffin in tissue lysing solution 100 µl, 16 µl of 10% SDS and 40 µl of proteinase K at 55 They were incubated until the tissues were completely lysed. after, after Add 325 µL of binding buffer and 325 µL The absolute ethanol mixture was taken on the column. So three times Wash to remove excess material with the help of washing buffer Take the DNA attached to the column with 50 µl of eluted buffer They were washed and kept in a 20° refrigerator until polymerase chain reaction The percentage is kept In this study, the Time-Real PCR method was used Green fluorescent dye called I Green SYBR, all samples It has been investigated. This fluorescent color is capable of lumen Small double-stranded DNA and fluorescent light The amount of light produced is directly proportional to the amount It has a PCR product. Molecule I Green SYBR has an inhibitory effect It does not affect PCR and can be used in place of probes Only fluorescent is used. To assess the validity of the DNA extraction, first all samples (HMBS, the human Homo sapiens HMBS gene for Hydroxymethylbeline synthase accession GenBank no. (1. M95623 as an internal control with specific prefixes

was PCR polymerase chain reaction mixture with A volume of 20 µl contains 0.2 µmol of each Direct (forward) and reverse (HMBS) primers (1 µl each), 0.1 µmol of template DNA (5 SYBR-Green PCR Master 10 µl f) µl Residual water distillation f) Takara Bio, Osto, Shiga, Japan) mix It has been prepared. The time heating program of the device is this too Has been appointed. Initial denaturation of typical DNA molecules at 95 °C for 1 minute, then 40 repeat cycles 95°C for 10 seconds and 60°C for 30 seconds and 72 °C for 34 seconds Taken at the end of the beating cycles, the melting temperature curve is The aim was to check the specificity of the copied part. To amplify the L1 HPV target gene sequence from a reaction mixture of A volume of 20 µl contains 0.5 µmol

of each Direct and inverse primers (GP6 + / GP5) 1 µl of each (11), (0.1 µmol of template DNA (5 µl) and Residual water and SYBR-Green PCR Master Mix 10 µl Distillation was used. Timetable Warmth to breed Initial denaturation at 94 °C for 0.5 min then
45 downhill cycles at 95° for 10 seconds, 50 to 60 degrees Celsius for 20 seconds and 72 degrees The degree was done for 34 seconds. face contact stage Touch down from 60°C to 50°C each The reduction cycle was set at 0.5 °C. then in Keep the final temperature cycle constant at 72°C for 5 minutes They were kept until the construction of the tendons was completed. Melting curve by keeping the PCR products from a temperature of 60 °C to 95 °C for one degree Celsius degrees per second Primers and probes for both control and target genes Purchased from Pioneer Corporation, Alameda, California. Sequence them according to Table 1.

Findings

Of 66 breast cancer paraffin block samples and 54 block samples Paraffinization of non-cancerous (fibrocystic) breast tissue, after DNA extraction and RCR of the HMBS gene, two samples The cancerous sample and the non-cancerous sample were not suitable and were disposed of The results of HMBS gene amplification in a number of samples became in the figure 1 appears

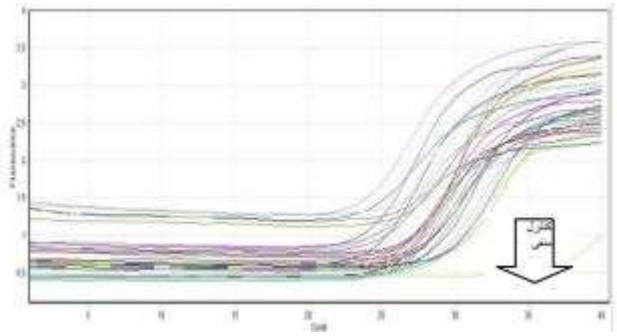

Figure 1 - The results of HMBS gene amplification from DNA extracted from some Patient plus negative control

To determine the optimal temperature for the binding of HPV L1 primers, out of six DNA samples extracted from uterine cancer tissues infected with 16-HPV was used. To ensure the type of virus of their genome The sequence was determined. In Figure 2, the optimal temperature for six samples HPV was determined at 60°C

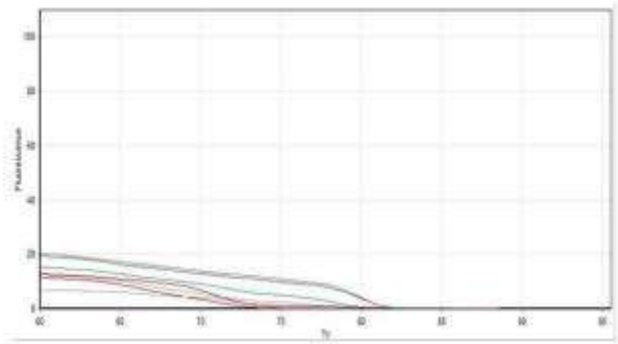

Figure 2 - Evaluation of the optimal binding temperature of HPV L1 primers in six 16-HPV positive control sample.

Positive samples for HMBS gene were selected and
Specific primers of HPV L1 gene were amplified in four steps along with positive and negative control. In figure 3 curve

It shows proliferation of several cancerous and non-cancerous samples which is only one of the positive samples of patients and controls Negative and positive are observed.

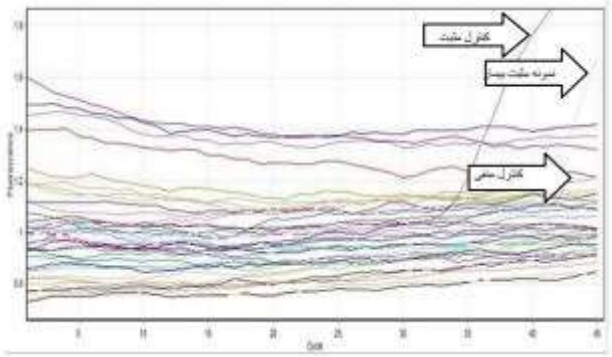

Figure 3 - Proliferation curves of patient samples in the Real test PCR time. As can be seen, only one positive control From the samples, the amount of fluorescent emitted from them with increasing values Products increase during PCR reaction cycles.

Discuss

The vast majority of breast cancers are carcinomas which is from the cells covering the milk ducts of the glands They are of mammary origin. A complex set of several steps. including genetics, environment and nutritional factors in shape change Malignant, these cells have a role that together can It has changed the main pathways regulating the regular growth of cells and as a result, their proliferation is out of control and leads to become carcinogenic Real understanding of these cellular pathways plays a role It is important in cancer biology (1. Most of the molecular events involved in the development of breast cancer are unknown Remains. In the early studies of the related similarity between cancer Breast and lesions caused by intraepithelial neoplasia (cervical intraepithelial neoplasia III: CIN III) uterine reported. These studies are of interest to search for HPV The title is one of the factors in the occurrence of breast cancer (12) For the first time in 1992, Leonardo Di and his colleagues They brought up HPV and breast cancer. They are HPV-16 DNA in 29.4% of 17 breast cancer samples by PCR method observed (13). In 1999, YU and his colleagues in A study on 72 patients, the relationship between HPV-33 and cancer Breast in Asian populations (China and Japan) to print delivered They found the presence of 33-HPV DNA in 34.1% of patients with Invasive ductal carcinoma (Invasive ductal carcinoma) They identified benign lesions in 5%. The presence of a virus in Benign lesions are hypothesized to be in the pathogenesis of breast cancer involved, but found no other serotypes They did not (14. Hennig and colleagues detected HPV-16 in 19 of 41 patients with Breast carcinoma (46% who have a history of CIN III lesions had, they reported (15). In another study, they reported HPV-16 DNA was identified in 32 cases out of 38 patients with III CIN (84%). they did All patients with breast cancer according to the lesions III CIN were positive for HPV-16. But nothing positive They did not find it for HPVs 11, 18 or 33 and his colleagues in 2004 using two sets 101, HPV-18 and HPV-16 viruses E6 region for primers The paraffin block was examined. They are in 25 patients Detection of breast carcinoma HPV DNA (24.75%) They did, but in 21 cases of benign breast tissue, nothing They did not observe a virus. Out of 25 positive cases, 14 cases positive") 40) HPV-18 for case 10 and HPV-16 for %) 56) were (16. In another study by Kan and his colleagues, 50 breast cancer samples by PCR method for HPV types 16, 18 and 33 put the 18-HPV genome in two female breast cancer samples Australians observed. 24 cases out of 50 samples 48% were positive for HPV Villiers, Damine, and Kan et al., in breast tumors HPV as a strong volunteer oncovirus to cause cancer They introduced the breast. Also,

Widschwendter et al HPV DNA in breast cancer tissues of patients with a history They diagnosed uterine cancer (9. Yasmeen and his colleagues in 2007 (18) and Akil et al His colleagues suggested in 2008 (19) that infection High-risk HPV can cause cellular invasion and metastasis in Breast cancer through 1-Id, which is one of the family members. Transcription factors are helical-circular-helix, induction

(18 and 19.) In the research of Ruiz-Mendizabal and his colleagues from 67 patients Mexican with breast cancer and 40 breast tissue samples from patients Non-cancerous cells that were analyzed by PCR method Only 3 positive cases of HPV types 16, 18 and 33 were observed Their type was determined by determining their genome sequence (20. Unlike the mentioned researches, there are no other cases of reviews It showed the presence of HPV in cancerous and control tissues. In a study by Hachana and his colleagues in 2010 with PCR and hybridization in situ methods on Tunisian patients did, none of the 123 breast cancer samples They were not positive for low-risk and high-risk types of papilloma virus (21) In the study of Lindel and his colleagues on 81 Swiss patients who They had no positive sample of breast cancer by PCR method HPV DNA was not found (22). (In another study by Hedau and his colleagues on 252 Indian patients, no positive samples from HPV DNA was not detected by PCR method (23. (in review). CremouxP and his colleagues also from 50 breast cancer samples French patients have no HPV DNA detected by PCR method It didn't work (24. In this research, unlike the methods used for the study This is from Real Time PCR method and using color I Green SYBR fluorescence of the samples was examined we gave. This technique is used to detect RNA and DNA can be done, it has high efficiency compared to normal PCR and One of the advantages of this method is DNA probe specificity and sensitivity Above is the analysis of the results. Real Time PCR method compared to other similar methods

Nested PCR is probably due to working in a closed space There is less pollution. Among other advantages of this method, It is possible to observe the reaction and its results from moment to moment It provides a review of the reproduction process. in the order possible Optimizing the reaction, such as determining the most suitable concentration of DNA and It enables the primer as well as the number of replication cycles. Since in this method the process of PCR can be seen

The possibility of stopping the reaction is also provided at the desired time and in Non-proliferation or going to the stationary phase can terminate the reaction

and avoided wasting time and energy (25. Another advantage of this method compared to ordinary PCR, There is no need for gel electrophoresis to analyze PCR products which increases the possibility of contamination and creating false positive results decreases. Due to the high sensitivity of the technique used in the research Now, our results showed that the genome of this virus in the samples There is no cancer, but in three cases of non-cancerous samples was observed. This result, the hypothesis presented regarding its role It strengthens the pathogenesis of breast cancer to some extent. Likely The virus genome cannot be observed in cancer samples Related to using the hit and run mechanism (run and hit (for Induction of cancer in this virus. According to the report presented in 1987 by Campo, Papillomaviruses use the mechanism of hit and escape (run and hit).

They use esophageal cancer induction. Their study on Bovine models showed that bovine papilloma viruses in The early stages of carcinogenesis in the foregut (in cows). They are necessary, but to progress to malignant state, presence

There is no need for them (26. In total, there are several dangerous factors related to progress Breast cancer, such as age, family history, history of breast cancer They have been introduced in the individual and..., but despite this, 50-80% of the cases Risk factors have not been identified yet. Studies have been done Also DNA connection The relationship between human papillomavirus DNA and breast cancer from 0 It shows up to 86% variable and until now the mechanism of reaching The virus has not been identified in

the breast either (3. It seems that there are several other factors among them nutritional and environmental and population genome heterogeneity, differences in Sampling method, selected sample type, technical sensitivity - Laboratory tests, the type of diagnostic method used and so on Possible contaminations during the sensitive PCR reaction It can be one of the important factors in creating variable results

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