Journal of the Egyptian National Cancer Institute xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of the Egyptian National Cancer Institute

journal homepage: www.sciencedirect.com

Full length article

# Epstein-Barr virus infection and breast invasive ductal carcinoma in Egyptian women: A single center experience

Noha ED Hassab El-Naby [a], Hameda Hassan Mohamed [b], Asmaa Mohamed Goda [b], Ahmed El Sayed Mohamed [c,*]

[a] Department of Pathology, Sohag University, Faculty of Medicine, Egypt
[b] Department of Medical Microbiology and Immunology, Sohag Faculty of Medicine, Sohag University, Egypt
[c] Department of Clinical and Radiation Oncology, Sohag University, Faculty of Medicine, Egypt

ARTICLE INFO

*Article history:*
Received 14 October 2016
Received in revised form 25 February 2017
Accepted 25 February 2017
Available online xxxx

*Keywords:*
Epstein-Barr virus
Breast invasive
Ductal carcinoma
Nested PCR
Immunohistochemistry

ABSTRACT

*Background:* A controversy of the role of Epstein-Barr virus (EBV) infection in breast carcinomas has been reported in the literature.
*Objectives:* We carried on this research to explore possible association between EBV infection and breast invasive ductal carcinoma (IDC) in Egyptian women attending our center.
*Study design:* This study carried out at Sohag university hospital on 84 paraffin embedded samples of breast tissue, of them 42 breast IDC as the case group and 42 breast fibroadenomas as the control group. Nested PCR and immunohistochemistry (IHC) done separately for all samples to identify the Epstein-Barr nuclear antigen-1 (EBNA-1) gene and EBV latent membrane protein-1 (LMP-1) respectively, in breast cancer cells and controls.
*Results:* Specimen considered positive when both (EBNA-1) gene and LMP-1 were detected using PCR and IHC separately for the same sample, this was achieved by 10/42 (23.81%) of breast IDC (case group) and 6/42 (14.29%) of breast fibro-adenomas (control group) (P-value = 0.4). Nodal involvement was the only parameter that demonstrated a significant statistical relationship with EBV presence in cancerous tissue with p-value = 0.003.
*Conclusion:* Our research could not find a significant statistical association between EBV infection and breast IDC in Egyptian women attending our center, but, there might be an association between the existence of EBV and tumor aggressiveness.
© 2017 National Cancer Institute, Cairo University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

## Introduction

Breast cancer is the most common malignancy of females in many populations and the second leading cause of death in the world [1]. According to National Cancer Institute (NCI) Egypt registry data, female breast cancer ranked first among tumors and IDC the most frequent pathological subtype.[2]

The identification of a viral agent for breast cancer has eluded researchers for decades [3,4]. As for many decades, oncogenic viruses have been hypothesized as having potential causal roles in breast cancer. The main candidate viruses include EBV, high risk human papilloma virus (HPV), mouse mammary tumor virus (MMTV) – like envelope DNA and Cytomegalovirus (CMV) [5–7]. Co-infection with more than one of these viruses has been argued; as the probability of a single patient to be infected with two or more distinct types of viruses is increasing [8,9].

Co-infection of EBV and HPV seems to be present in a significantly higher proportion in breast cancer than in normal breast epithelial cells. The Glenn group reported that HPV and EBV coexist in several human cancers; and the presence of these viruses in breast cancer is associated with young age at diagnosis and, possibly, an increased breast cancer grade [10,11].

The first report on the role of EBV in breast cancer has been described by Labrecque et al. 1995 [12]. Then accumulated reports and observations have strengthened this role; for example the high incidence of breast cancer in Mediterranean countries with endemic EBV infection, EBV -associated lymphomas in the breast and the morphological similarities between breast medullary

Peer review under responsibility of The National Cancer Institute, Cairo University.
* Corresponding author at: Sohag University, Faculty of Medicine, Clinical Oncology Dept., 4 AlTaawon st., Sohag 82519, Egypt.
*E-mail addresses:* noha_eldabie@yahoo.com (Noha ED Hassab El-Naby), dr_hd_66@yahoo.com (H. Hassan Mohamed), asmaa_gouda@med.sohag.edu.eg (A. Mohamed Goda), dr_ahmed_sayed76@yahoo.com, ahmed_khalifa@med.sohag.edu.eg (A. El Sayed Mohamed).

http://dx.doi.org/10.1016/j.jnci.2017.02.002
1110-0362/© 2017 National Cancer Institute, Cairo University. Production and hosting by Elsevier B.V.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article in press as: El-Naby NEH et al. Epstein-Barr virus infection and breast invasive ductal carcinoma in Egyptian women: A single center experience. J Egyptian Nat Cancer Inst (2017), http://dx.doi.org/10.1016/j.jnci.2017.02.002

carcinoma and EBV-associated nasopharyngeal carcinoma [3]. Nevertheless; data on viral presence and oncogenic mechanisms are still inconsistent and detailed mechanisms of interactions between infectious agents and host cells have yet to be fully elucidated [13]. But it has been suggested that cell cycle proteins could be the target of EBV- transformation mechanism, like other oncogenic viruses [14]. EBV uses its viral proteins; the actions of which mimic several growth factors, transcription factors, and anti-apoptotic factors, to usurp control of the cellular pathways that regulate diverse homeostatic cellular functions [15]. For example; the Epstein–Barr virus (EBV) nuclear antigen (EBNA)-1 promotes the accumulation of chromosomal aberrations in malignant B cells by inducing oxidative stress and activation of the DNA damage response [16,17]. In addition, EBV-positive neoplasms show genetic alterations that are distinct from those exhibited by EBV-negative neoplasms; for example EBV-positive gastric adenocarcinoma that displays recurrent PIK3CA mutations, extreme DNA hypermethylation, and amplification of JAK2, CD274 and PDCD1LG2 [18]. A recent work on DNA cytosine deaminases suggested that APOBEC3B -a newly defined source of DNA damage- has a role in breast cancer development. It showed that viral infections causing innate immune responses and/or splice variants may be contributing factors for its action [19]. In support of this idea; recent studies on head/neck cancer have linked human papilloma virus infection to APOBEC3B upregulation and implicated APOBEC3B mutagenesis in activation of PIK3CA kinase which is mutated in a large proportion of breast cancers [20].

Molecular techniques are the most definitive assays in establishing viral presence in cancerous tissue in comparison to other tests based on host antibody assessment. Up to date, no standard method has been generally accepted for EBV detection in cancer tissues, PCR and IHC have been considered as the most sensitive methods [21].

We conducted this study to explore possible relationship between EBV infection and breast IDC using both IHC and PCR techniques.

## Materials and methods

### *Samples and data collection*

Specimens obtained from pathology department laboratory at Sohag university hospital. Biopsies obtained by incisional and/or excisional method. Specimens were formalin fixed, and paraffin embedded. Collectively we have obtained84 paraffin embedded blocks; 42 IDC not otherwise specified (NOS) (as case group) and 42 fibroadenomas (as control group). Clinical data obtained from medical records of those patients. These data include: age, histopathological grade, tumor size, hormonal receptor status and TNM categories besides to other factors.

The study has been carried out under Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Informed consent taken from participants and the study approved by ethical committee of Sohag faculty of medicine.

### *Detection of EBV by nested PCR*

#### *DNA extraction*

Using a microtome; 8 sections of 5–10 μm thickness cut up from blocks and immediately placed into 2 ml microcentrifuge tube. Paraffin dissolved and DNA extraction done using QIAamp® DNA FFPE Tissue (Qiagen, USA) according to the manufacturer instructions.

#### *Testing DNA integrity by standard PCR*

The integrity of the extracted DNA confirmed by standard PCR using β-globin primers G073 (5′GAAGAGCCAAGGACAGGTAC-3′) and G074 (5′CAACTTCATCCACGTTCACC-3′). Reaction volume of 25 μl (supplied by Invitrogen; Groningen, the Netherlands, UK) used containing 2.5 μL of 1X PCR reaction buffer, 1 μL DNA solution, 1 μL MgCl2, 2 μL of each of the gene-specific primer, 0. 125 μL Taq-DNA polymerase, 2.5 μL deoxynucleoside triphosphates mix (dNTPs), and 16 μL PCR water. PCR amplifications performed on a T-Gradient thermal cycler (Biometra, USA). PCR amplification conditions as follows: initial heating at 95 °C for 10 min; followed by (denaturation at 95 °C for 5 min, annealing at 58 °C for 30 s; extension at 72 °C for 30 s) for 35 cycles then final extension at 72 °C for 5 min. Samples with negative β-globin gene excluded from the study.

#### *Nested PCR*

Two primer sets described by Cinque et al. were used. The first round for amplifying a 297 bp fragment (EB3 5′-AAG GAGGGTGGTTTGGAAAG), (EB4 5′-AGACAATGGACTCCCTTAGC); while the second one using a primer set that binds within the first round product generating a 209 bp fragment (EB1 5′-ATCGTGGTCAAGGAGGTTCC, EB2 5′-ACTCAATGGTGTAAGACGAC). The first cycle conducted using a reaction volume of 25 μl (supplied from Invitrogen; Groningen, the Netherlands, UK) containing 2.5 μL of 1X PCR reaction buffer, 1 μL DNA solution, 1 μL MgCl2, 2 μL of the first set gene-specific primer, 0. 125 μL TaqDNA polymerase, 2.5 μL deoxynucleoside triphosphates mix (dNTPs), and 16 μL PCR water. The cycling conditions as follows: initial heating at 95 °C for 3 min., followed by 35 cycles of 95 °C for 30 s, followed by 55 °C for30 s, then 72 °C for 30 s, and a final extension at 72 °C for 5 min. The PCR product of the first cycle used as the template for the second cycle where 1 μl of it added to complete the 25 μl volume mixture as mentioned before; then placed in the thermal cycler under the same cycling condition of cycle 1. PCR amplicons separated by electrophoresis on a 1% agarose gel and stained with ethidium bromide with a marker (DNA Ladder 100 bp, 10 bands) from (KOMA BIOTECH, Seoul, Korea) to assess PCR product size; then the bands photographed by a gel documentation system (Ingenius, Syngene, USA) (Figs. 1 and 2).

Positive control is genomic DNA which isolated from EBV-positive Hodgkin's lymphoma case. Negative control is PCR with the omission of the DNA template (Figs. 1 and 2).

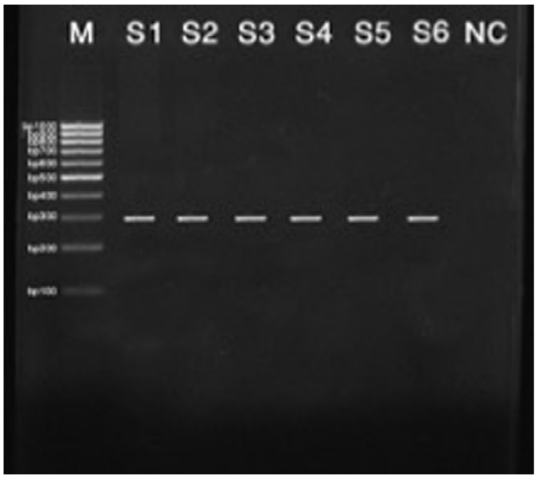


**Fig. 1.** agarose gel electrophoresis showing the amplified PCR product of β globin gene (297 bp) in S1–S5 indicating intact DNA of the corresponding specimens. NC: negative control; M: marker.

Please cite this article in press as: El-Naby NEH et al. Epstein-Barr virus infection and breast invasive ductal carcinoma in Egyptian women: A single center experience. J Egyptian Nat Cancer Inst (2017), http://dx.doi.org/10.1016/j.jnci.2017.02.002

Download English Version:

https://daneshyari.com/en/article/8787486

Download Persian Version:

https://daneshyari.com/article/8787486

Daneshyari.com