



## SEARCH FOR PROTEIN EBNA-1 P79 ANTI EBV IN SERUM OF PATIENTS REACHES ANY OF BREAST CANCER AND FROM WESTERN ALGERIA

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### ABSTRACT

Breast cancer is in most countries, the most common cancer in women. It is the leading cause of death worldwide, there is a multifactorial disease where multiple events can be the cause of this disease, it is noted as an example genetic factors such as BRCA1 and BRCA2 genes and here dietary environmental factors, the notion of cancer virusis back in for cein the field of scientific research since the virus-induced cancers increasingly studied. Several families of viruses can induce cancer as the Epstein-Barr virus (EBV). The aim of the present study was to determine if there is a greater association of EBV infection with breast cancer patients as compared patients with nasopharyngeal cancer. We measured the levels of anti-EBNA-1 P 79 in serum of these patients stored using Western blot technique. Patients with nasopharyngeal cancer were used as control group for comparison to serological analysis. Our study included 24 patients with breast cancer and 6 patients with nasopharyngeal cancer in western Algeria, from January 2009 to June 2009 in gynecology (CHUO), at the Pasteur Institute of Sidiferruj and Biology Laboratory of Development and Differentiation. 19 of 24 sera (79.16%) showed a positive serological profile, the 6 sera of patients reaches any of nasopharyngeal cancer (100%) were positive. The expression of EBNA-1P79 is seen in a significant proportion of sera from breast cancer, also for patients with nasopharyngeal cancer. Our results are consistent with those found in the literature.

**KEYWORDS:** *Breast carcinoma, Epstein-Barr virus (EBV), EBNA-1 P79, Western Blot*

### INTRODUCTION

Breast cancer is in most countries the most common cancer in women. each year worldwide, more than one million new cases, or 30% of cases of female cancers in industrialized countries and 14 % case in developing countries. It is also the leading cause of cancer death in women with 410,000 deaths annually.<sup>1</sup> More than 9,000 new cases of breast cancer are registered each year in Algeria, it remains the main reason for consultations in oncology. The mortality from

breast cancer is about 3500 Cases per year.<sup>2</sup> Cancer is the leading cause of death worldwide, there is a multifactorial disease in which multiple events can be the cause of this disease, there genetic factors, environmental factors and infectious by certain germs. So, cancer is a poorly understood disease of various origins, and also recently, the concept of carcinogenesis by virus returns in force in the field of scientific research since the virus-induced cancers are increasingly studied. About 15 to 20% of cancers are induced by viral infections, the percentage is even higher in resource-poor countries where the conditions for infection are

more prevalent. Indeed, in recent years the viral origin of cancer has emerged in the understanding of the malignancy, and multiple investigations are proposed in response to assumptions made. Research is conducted in the framework of epidemiological studies to understand the possible link between the virus and the development of cancer. Several families of viruses are capable of inducing cancer as the Epstein-Barr virus (EBV), hepatitis virus (HBV), hepatitis C (HCV), the human lymphoma virus T cells (HTLV-I), the human papilloma virus (HPV) and some retroviruses such as MMTV and others. EBV has been detected in breast milk in 1991.<sup>3</sup> Furthermore, it has been demonstrated by techniques conventional PCR dans 20 à 50% of breast cancer.<sup>4</sup> Viral load was quantified by Q-PCR in breast cancer biopsies and isolated tumor cells by microdissection.<sup>5</sup> The results show a low copy number, which explains the difficulty to highlight the presence of the virus. In addition, the distribution of the viral genome is highly heterogeneous within the same tumor and a tumor to another. On the other hand, it was detected by RT-PCR in EBV positive tumors, transcripts latent genes EBNA 1a was found in 80% of cases and two transcripts of viral oncogenes and LMP1 BARF1 were detected in 20% and 75% of cases. The results obtained using the microdissections well confirmed the presence of EBV in breast tumor cells and the detection of transcripts of latent genes shows that the viral genome is latent form. Furthermore, the possibility that the virus contribute to the progression of this tumor has been suggested.<sup>5</sup> The purpose of this study is to determine if there is a greater association between breast cancer and infection with EBV. We examined the presence of anti-EBV EBNA 1P79 in serum samples from patients with breast cancer and patients with nasopharyngeal cancer used as controls. Among different antigens known EBV expression, EBNA-1 is essential for the maintenance of the viral episome and is expressed in all known forms of viral latency.<sup>6</sup>

## PATIENTS AND METHODS

The study included twenty-four patients in western Algeria, admitted to the gynecology department, wing Medical Oncology at the University Hospital of Oran, over a period from January to June 2009. The study was conducted at Institut Pasteur in Sidi Ferrudj (Algiers) and the laboratory of

Developmental Biology and differentiation (University of Oran Es Senia). All patients were followed for breast cancer.

These patients were screened at random from the general population, they have been the subject of a survey carried using a questionnaire raising clinical and socioeconomic parameters. It is important to note that all these patients were consenting to the collection of biological samples, and signing the informed consent.

### *Samples*

#### *Taking biopsies*

Upon removal of the breast at the operative block, we proceed with the removal of the tumor samples. Part of the samples were immediately frozen at -80 ° C for use at a later molecular study on the other, has been set for the completion of the histologic study to determine the TNM classification (...) and SBR grade (grading of Scarff-Bloom Richardson).

#### *Sample Sera*

In dry tubes we proceeded to levy 10ml de total blood on patients before ablation of tumor, the blood was centrifuged at 1800 rpm, and the sera were collected and stored at -20 ° C for serological study.

#### *Histological techniques*

The light microscopic examination is essential before any additional technique. It is feasible that after several stages: fixation, inclusion, microtome and staining slides.

#### *Serological study*

This study allowed us to look for the serological marker (EBV antibodies) directed against the viral antigens expressed by EBV, it is EBNA-1 P79. For this we used the Western blot technique. There are several anti-EBV antibodies directed against different constituents of the virus: the anti-VCA antibodies (Viral Capsid Antigen), anti-EA antibody (Early Antigen) and anti-EBNA-1.

#### *Serological techniques: Western Blot*

Western blot test is a qualitative test invitro for the detection of human antibodies directed against EBV. The kit contains strips with antigen extracts of EBV separated by electrophoresis. The blot strips were in the first step, incubated in the diluted serum of patients. The test was performed using the Biorad reagents.

## RESULTS

### *Results of the histological study*

The results of the histological study of fixed sections were classified and displayed according to the categories of prognostic factors: clinical, morphological and biological.

### *Anatomical location*

A predominance of the left breast with 62.5% over the right breast. (Figure 1)

### *Contraception*

The percentage of women taking contraceptives is higher than the one that did not take contraceptives. (Figure 2)

### *Age of patients*

From the statements made, standardized forms, breast cancer incidence by age of the patients is shown in Figure 3 and shows that the most affected age group is between 40 and 50 years (46%) with average of  $44 \pm 11.93$  years. (Figure 3)

### *Histopathology type*

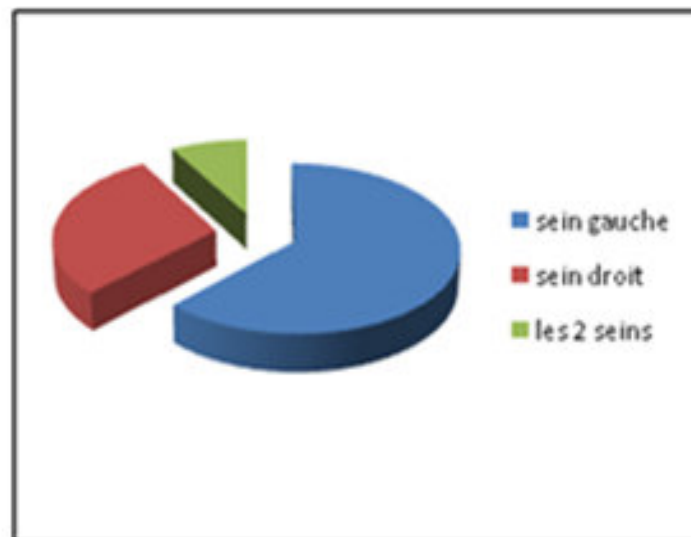
It appears from this study that the histological type more ITC is widespread With 73.33% against 20% of invasive lobular carcinoma, Infiltrating ductal carcinoma type comédo carcinoma represents 46,66% .(Figure 4)

### *Tumor grade*

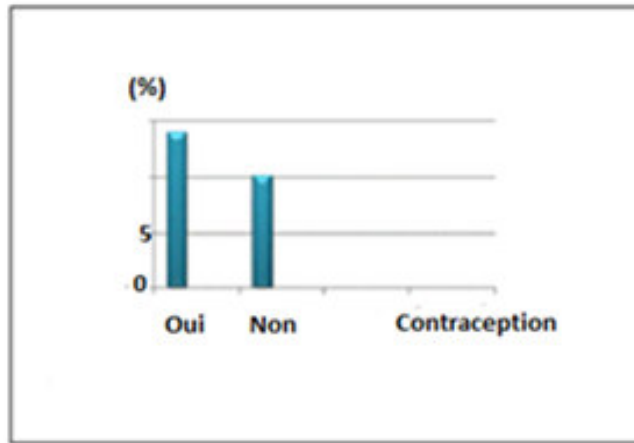
The SBR grade III represents 55% from the grade II SBR and SBR with 23% I 22%.( Figure 5)

## RESULTS OF THE SEROLOGICAL STUDY

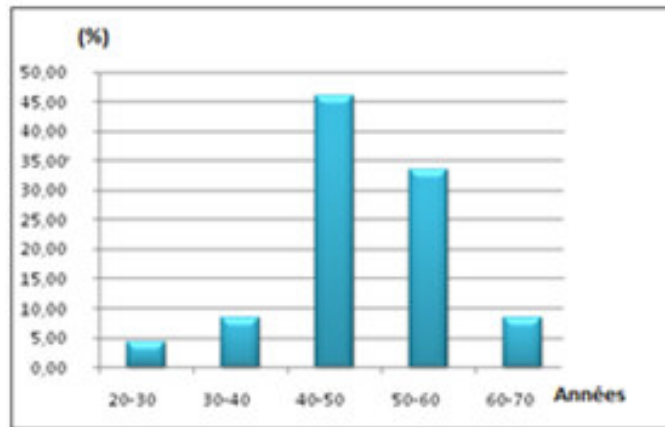
In this part of the work, we explored the serological markers directed against different viral proteins:the anti EBNA by the Western blot technique. These results show no significant difference between them, the sera investigated, they all posted positive serological profiles . On the 24 tested sera of patients, 19 of 24 patients had positive serological profile for EBNA antibodies, 79, 16% (Figure 6). Positivity was estimated with respect to a molecular size marker (EBNA position 79) (Figure 7). The sera of patients with nasopharyngeal carcinoma (controls) all displayed a positive profile (100%).



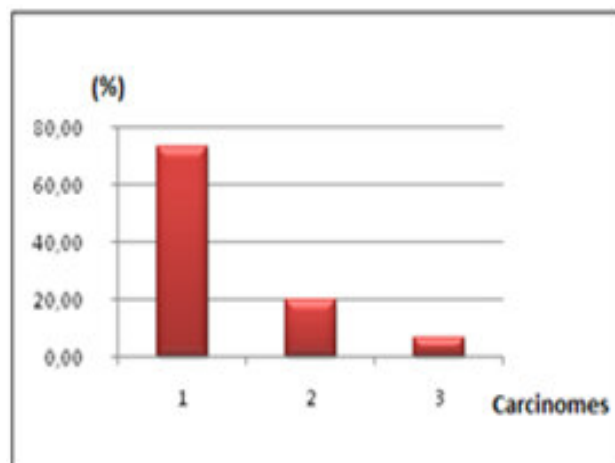
**Figure 1**  
*Representation according to the based location of the tumor*



**Figure 2**  
*Graphical representation contraceptive*

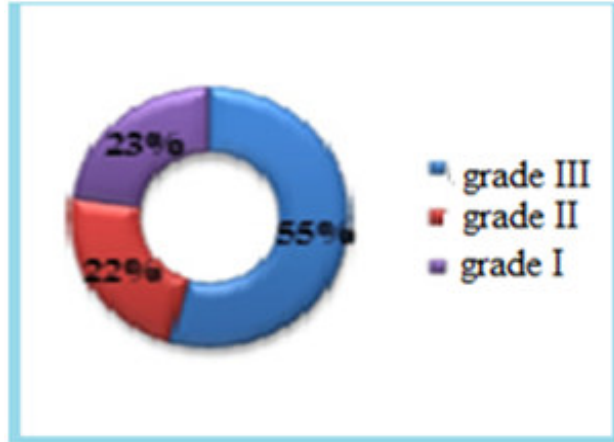


**Figure 3**  
*Graphic representation of the based on breast cancer incidence by age*



**Figure 4**  
*Graphic representation the histopathological types*

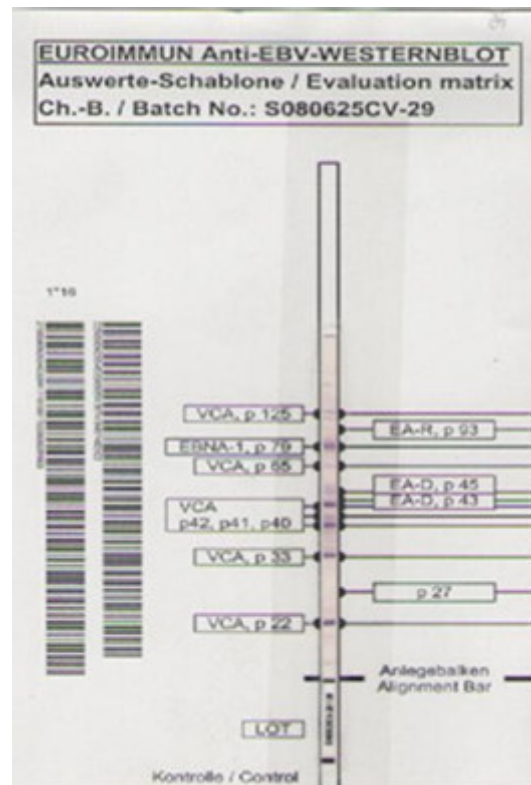
- 1 infiltrating ductal carcinoma
- 2 invasive lobular carcinoma
- 3 Tumor residue



**Figure 5**  
*Representation of grades appeared in target population.*



**Figure 6**  
*Representation of three blots EBNA*



**Figure7**  
*Size marker for Western all the containing patient blot*

## DISCUSSION

We tried to make a clarification as to the presence of tumor serum markers involved in breast cancer. Our study included 24 patients all breast cancer patients and from the west Algerian. The results obtained from the histological study show a predominant involvement of the left breast with 62.5% over the right breast. The age of onset of breast cancer between 28 and 73ans. Among the 24 patients, most of them were aged between 40 and 50 years with an average of 45.83%. Infiltrating ductal carcinoma was found in 11 patients with a percentage of 73.33% against 20% of invasive lobular carcinoma found in 3 patients, invasive ductal carcinoma comedo type of grade III was found in 10 patients, or 46, 66%. It appears from this study that the most common histological type in our study is the ICC. The results of the serological study by Western blotting showed a positive profile for the anti EBNA-1 P79 -EBV antibody in both groups of patients. Previous studies have looked for serologic evidence of infection in breast cancer patients<sup>7,8</sup>. This study has some limitations. While the first of our sample size

is small and the results cannot be generalized to the general population. Second, we searched the expression of a single viral protein: EBNA-1, ideally, we should have an inquiry for multiple proteins in the various forms of viral latency observed in other tumors associated with EBV.

## CONCLUSION

The expression of EBNA-1P79 is seen in a significant proportion of sera from breast cancer, also for patients with nasopharyngeal cancer. To demonstrate the specificity of the antibody, we must reproduce our results using another monoclonal antibody directed against EBNA-1. The role of EBV in breast cancer progression, even in a small subset of breast cancer would be of paramount importance. Further research is needed, first by increasing the sample size by studying other proteins present in the various forms of viral latency observed in other tumors associated with EBV, to define the exact role of EBV in the etiology or progression of breast cancer because it would help to design.

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