

Serodiagnosis of Human Herpesvirus 8 in Women with Breast Cancer

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ABSTRACT

Human herpesvirus 8 (HHV-8) is a common virus in the Mediterranean region. It has been linked to a number of malignancies, and it is believed to be the causative agent in certain cases. The aim of this study was to evaluate the distribution and possible association between HHV-8 and breast carcinoma, and to assess the risk factors associated with an HHV-8 infection. A total of 90 blood samples were collected from the study group. Forty-five of these patients were recently diagnosed with breast cancer and presented at the Oncology Centre in Basrah, Iraq, with ages ranging from 28 to 68 years old. Forty-five apparently healthy females matched for age and free from malignancy made up the control group, with ages ranging from 25 to 70 years old. The HHV-8 immunoglobulin G (IgG) antibody detection was done using an enzyme-linked immunosorbent assay with previously stored sera. HHV-8 was detected in 31.1% of the women with breast cancer, and a statistically significant difference was determined between the breast cancer patients and the control group. The highest HHV-8 seropositivity (17.8%) was seen in the 51–60 years old age group, and statistically significant differences were found between the patient and control groups with regard to the different age groups. Invasive ductal carcinoma was the most common type of breast malignancy in the women, with the majority of the patients classified as stage II. The histopathological types had a significant effect on the outcome proportion of the HHV-8 IgG antibodies. Of the 14 breast cancer patients with blood transfusion histories, 6.7% were HHV-8 IgG antibody positive, indicating a significant difference with regard to the blood transfusion history. Diabetes mellitus was determined to be one risk factor associated with a high seropositivity of HHV-8, and it occurred at a rate of 13.3% among those women with breast cancer ($p < 0.05$). In addition, it was found that the sexual route may be a significant risk factor. However, the HHV-8 infected breast cancer patients showed no statistically significant association with the coexistence of breast cancer markers. Based on the results of this study, female breast cancer may be associated with HHV-8. A blood transfusion history, diabetes mellitus and marriage were found to be risk factors for acquired HHV-8 infections in breast cancer patients.

Keywords: Human herpesvirus 8, breast cancer.

INTRODUCTION

Several infectious viral agents exhibit significant associations with a variety of human

cancers¹, with approximately 15–20% of all human cancers being caused by viruses². These oncogenic viruses appear to act at the beginning of tumour



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development or during the latent period on cells that already exhibit genetic dysregulation³.

Most seroepidemiological studies have shown a global human herpesvirus 8 (HHV-8) seroprevalence ranging from 2–10%, with a viral endemicity exhibiting geographical variation⁴. HHV-8 has two modes of replication, latent and lytic stages⁵, and it seems to be under immunological control mechanisms producing no symptoms⁴. The HHV-8 target cells are variable and numerous, including antigen presenting cells (dendritic cells, monocytes/macrophages and B lymphocytes)⁶, endothelial and spindle cells⁷, epithelial cells⁸, dorsal root sensory ganglion cells⁹ and keratinocytes¹⁰. Several proteins have been reported to serve as HHV-8 entry receptors, which explains the wide cell tropism range¹¹.

The viral genome encodes several protein homologues of the host cellular proteins, such as viral interleukin 6, chemokine-like molecules, interferon regulatory factors, complement binding proteins, viral cyclin D and viral Bcl2, which play major roles in the pathogenesis and progression of the related diseases¹².

MATERIALS AND METHODS

Study population

A total of 90 blood samples were collected from breast cancer patients and healthy women after obtaining the approval of the ethics committee of the Ministry of Health. The patient study group was comprised of 45 females who were recently diagnosed with breast cancer, but not treated with chemotherapy, and who were undergoing consultation at the Oncology Centre in Basrah, Iraq. Their ages ranged from 28–68 years old. The control group was comprised of 45 apparently healthy females from the general population, including College of Medicine employees and medical personnel, and their ages ranged from 25–70 years old. They were matched for age and free from any type of cancer.

Blood collection and laboratory investigations

Three to five millilitres of blood were collected aseptically from both the patients and

healthy controls in sterile disposable plain tubes, and they were allowed to clot while standing oblique for one hour at room temperature. After centrifugation at 3,000 rpm for 5 minutes, the serum was separated and stored frozen at -20 C° until the serological examination was performed. The serological testing included the detection of anti-HHV-8 immunoglobulin G (IgG) antibodies via the anti-Kaposi's sarcoma-associated herpesvirus/HHV-8 IgG enzyme-linked immunosorbent assay (ELISA) kit (catalogue no. 15-501-000; Advanced Biotechnologies Inc., Eldersburg, MD, USA), and the detection of cancer antigen 15-3 (CA15-3) via an ELISA kit (product code 5625-300; Monobind Inc., Lake Forest, CA, USA).

The hormone receptors [oestrogen receptor (ER) and progesterone receptor (PR)] and the human epidermal growth factor receptor 2 (HER2/neu) were detected via immunohistochemistry.

RESULTS

The HHV-8 proportion was 31.1% among the women with breast cancer (Table 1), with a statistically significant difference ($P < 0.05$). In addition, the serological results were variable among the different age groups (Figure 1). Moreover, the highest positivity of IgG antibodies was found in the 51–60 years old age group in the breast cancer patients (17.8%). Statistically significant differences were observed among the different age groups ($P < 0.05$).

Ductal carcinoma was the predominant type of the breast cancer (Table 2), and it represented 37 (82.2%) out of the 45 total cases. All 14 of the HHV-8 IgG antibody positive cases exhibited ductal carcinoma (31.1%), and a significant difference was observed in relation to the histopathological types of breast cancer. With regard to the breast cancer stages, 8 (17.7%) of the women with breast cancer who had positive HHV-8 IgG results were in stage II (Figure 2).

Fourteen out of the 45 patients received blood transfusions, and 3 (6.7%) of them were positive for HHV-8 antibodies (Table 3). There was a significant difference among the patients with regard to their previous blood transfusion histories ($P < 0.05$).

The percentage of positive HHV-8 IgG results was three times higher in the 6 diabetic breast cancer patients (13.3%), when compared with the 2 healthy diabetic women (4.4%), which was a significant difference. Thus, diabetes mellitus may be risk factor for an HHV-8 infection (odds ratio = 4.4) (Table 4).

The women were classified into two groups according to their marital status, as summarised in Table 5. Most of the women involved in this study were married (regardless of parity, divorced or widowed), for a total of 76 out of 90 women. All 14 of the women with breast cancer with HHV-8 seropositive results were married (31.1%). Therefore, the association between the marital status and HHV-8 antibodies was significant ($P < 0.05$).

An analysis of the data was also done to study the association between HHV-8 and the breast

cancer tumour markers (Table 6). With regard to tumour marker CA15-3, 1 patient (7.1%) was positive for HHV-8 antibodies. With regard to the PR, ER and HER2/neu, 9 out of the 45 patients were examined, and there were no cases positive for the HHV-8 IgG antibodies. Statistically, the differences between the HHV-8 IgG antibodies and the different tumour markers were not significant.

DISCUSSION

Breast cancer is a major health burden worldwide, and it is the most common type of cancer in both developing and developed countries¹³. Among Iraqi women, it represents the most frequently diagnosed malignant tumour¹⁴. The oncogenic potential of HHV-8 makes it an important public health issue, because it poses unique mechanisms that influence cellular proliferation and the apoptotic control pathways¹⁵.

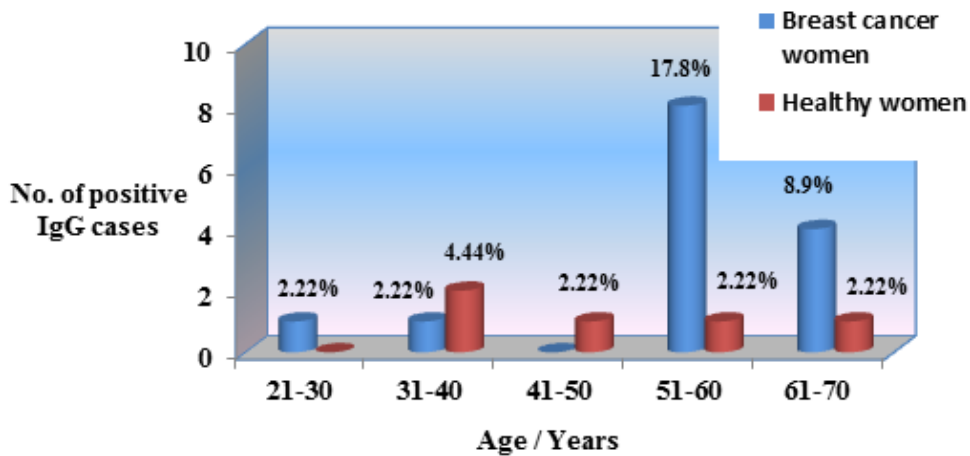


Fig. 1: Age distribution of the human herpesvirus 8 (HHV-8) immunoglobulin G (IgG) antibodies among the study groups
 $\chi^2 = 3.97$; $df = 1$; $P < 0.05$

Table 1: Detection of the human herpesvirus 8 (HHV-8) immunoglobulin G (IgG) antibodies in the study groups

Study groups	HHV-8 IgG antibodies		Total No. (%)
	Positive (%)	Negative (%)	
Breast cancer women	14 (31.1)	31 (68.9)	45(100)
Healthy women	5 (11.1)	40 (88.9)	45 (100)

$\chi^2 = 5.40$; $df = 1$; $P < 0.05$

No previous studies have compared the prevalence of HHV-8 infections in breast cancer patients to healthy women in Iraq; therefore, this study presents preliminary data and provides a valuable base for redirecting upcoming studies in near future. The results show a significant association between positive HHV-8 IgG antibodies and female patients with breast cancer. This may be due to several factors, including the fact that HHV-8

is endemic to our region, the infection is acquired through nonsexual routes, there is a high number of travellers between the neighbouring countries (especially trips for religious purposes), and the different HHV-8 subtypes. These results are in agreement with a study carried out in Taiwan that reported HHV-8 DNA positivity in 45.2% of breast cancer patients¹⁶.

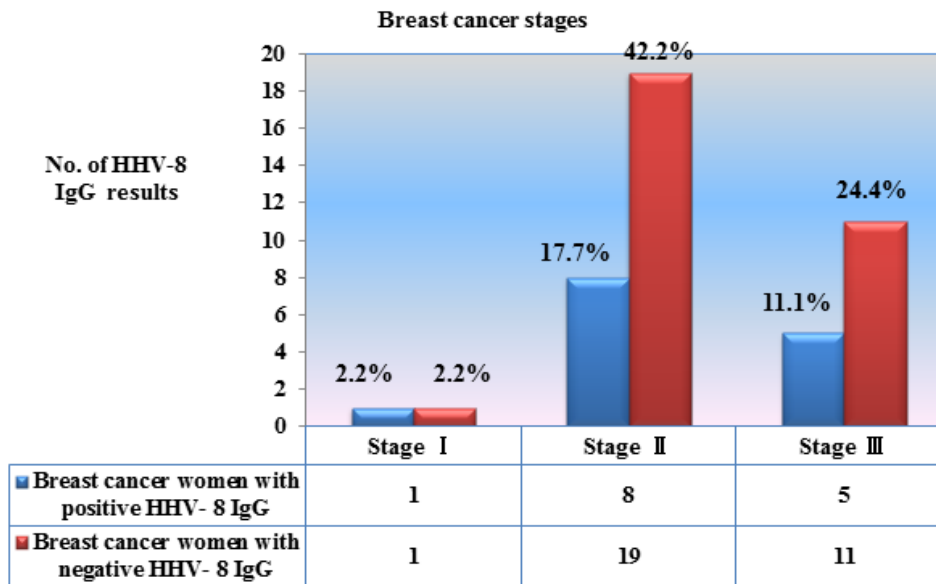


Fig. 2: Distribution of the human herpesvirus 8 (HHV-8) immunoglobulin G (IgG) antibodies with regard to the breast cancer staging
 $\chi^2 = 0.359$; $df = 2$; $P > 0.05$
 Fisher's exact test; $P = 0.877$

Table 2: Distribution of the human herpesvirus (HHV-8) immunoglobulin G (IgG) antibody results in relation to the histopathological types of breast cancer

Histopathological types	HHV -8 IgG antibodies response		Total No. (%)
	PositiveNo. (%)	NegativeNo. (%)	
Ductal carcinoma	14 (31.1)	23 (51.1)	37 (82.2)
Lobular carcinoma	—	4 (8.9)	4 (8.9)
Tubular carcinoma	—	3 (6.7)	3 (6.7)
Mucinous	—	1 (2.2)	1 (2.2)
Total No. (%)	14 (31.1)	31 (68.9)	45 (100)

χ^2 with Yates' correction = 2.806; $df = 1$; $P > 0.05$
 Fisher's exact test; $P = 0.0436$

The seropositivity of HHV-8 is divergent with age-specific groups. In contrast to several epidemiological studies carried out in different regions that have documented high rates of HHV-8 with progressing age¹⁷⁻¹⁹, this may be related to an uneven distribution across the population in various geographical areas, and that the paediatric age groups were excluded because all the participants were adult women. The study results are supported by a study carried out in Uganda showing a significant decline in the prevalence of HHV-8 with an increasing age among women²⁰. The present study shows a moderately significant association with regard to HHV-8 antibodies among the women with breast cancer in relation to age, which may be attributed to the disturbances in their immunological responses, in addition to the increased stress leading to the reactivation of silent HHV-8 infections or facilitating new HHV-8 infections.

The present study revealed a high percentage of ductal carcinoma when compared

with the other types of breast cancer, and that most of the patients were in stage II. Similar results were obtained from the Iraqi national breast cancer research unit documents²¹.

The blood transfusion history exhibited a positive relationship with the HHV-8 serostatus among the immunocompromised recipients. One possible hypothesis is that in affected countries there exists an active viral circulation, acute infections with lytic viral replication, and higher blood viral loads. On the contrary, in non-endemic regions, healthy immunocompetent individuals who are HHV-8 seropositive may harbour predominantly latent HHV-8 infected cells that would not undergo lytic replication unless exposed to specific stimuli. Nevertheless, if both the lytic and latent cycles occur concurrently, the viral load may be low and frequently below the detection threshold. Moreover, there may be a lack of a leukodepletion protocol in the blood components in the blood bank strategy, unless it is indicated for certain at-risk patients^{22,23}. These results

Table 3: Association between the human herpesvirus 8 (HHV-8) immunoglobulin G (IgG) antibodies and the blood transfusion history

Study groups (Total No.)	HHV -8 IgG	Blood transfusion		Total No. (%)
		Received No. (%)	Not received No. (%)	
Breast cancer women(45)	Positive	3 (6.7)	11 (24.4)	14 (31.1)
	Negative	11 (24.4)	20 (44.4)	31 (68.9)
Healthy women(45)	Positive	—	5 (11.1)	5 (11.1)
	Negative	4 (8.9)	36 (80)	40 (88.9)

$\chi^2 = 6.94$; $df = 1$; $P < 0.05$; odds ratio = 0.7

Table 4: Detection of human herpesvirus 8 (HHV-8) immunoglobulin G (IgG) antibody results in relation to diabetes mellitus

Study groups (Total No.)	HHV- 8 IgG	History of diabetes mellitus		Total No. (%)
		Diabetic women No. (%)	Not diabetic women No. (%)	
Breast cancer women(45)	Positive	6 (13.3)	8 (17.8)	14 (31.1)
	Negative	9 (20)	22 (48.9)	31 (68.9)
Healthy women(45)	Positive	2 (4.4)	3 (6.7)	5 (11.1)
	Negative	1 (2.2)	39 (86.7)	40 (88.9)

$\chi^2 = 10$; $df = 1$; $P < 0.05$; odds ratio = 4.4

correspond to the numbers from previous studies from China, Argentina and Uganda²²⁻²⁴.

The current study did not focus on the type of diabetes mellitus, nor whether an HHV-8 infection precedes the occurrence of DM or causes the reactivation of a pre-existing virus. Statistically, it revealed that there was a strong link and that diabetes mellitus can be considered as a probable risk factor for HHV-8. The same evidence was observed in several previous studies²⁵⁻²⁷. This can be explained by the fact that HHV-8 may be involved in the pathogenesis of diabetes mellitus via cytokine secretion, with subsequent induced inflammation mediated by natural killer cells or by direct infection

of the pancreatic b cells²⁸. In addition, HHV-8 infections induce endoplasmic reticulum stress in the b cells, thus affecting insulin actions and creating secretion defects. Other mechanisms suggest that HHV-8 promotes interferon production and the upregulation of major histocompatibility complex class I molecules, leading to an autoimmune attack²⁸.

Marriage is believed to be one marker of sexual activity and reproduction. The current study revealed a positive association between HHV-8 antibodies and the marital status. Similar findings were noticed in a number of previous studies^{18,29,30}. Conversely, one study carried out in male Zimbabwean factory workers showed that

Table 5: Relationship between the human herpesvirus 8 (HHV-8) immunoglobulin G (IgG) antibody results and the marital status

Study groups (Total No.)	HHV -8 IgG	Marital status		Total No. (%)
		Married No. (%)	Unmarried No. (%)	
Breast cancer women(45)	Positive	14(31.1)	—	14 (31.1)
	Negative	30 (66.7)	1 (2.2)	31 (68.9)
Healthy women(45)	Positive	4 (8.9)	1 (2.2)	5 (11.1)
	Negative	28 (62.2)	12 (26.7)	40 (88.9)

$\chi^2 = 12.18$; df = 1; P < 0.05; odds ratio = 4.03

Table 6: Association between the human herpesvirus (HHV-8) immunoglobulin G (IgG) antibody results and the different breast tumour markers

Tumour markers	+ / -	HHV -8 IgG antibodies*		Total No. (%) **
		Positive No. (%)	Negative No. (%)	
1- CA 15-3	Positive	1 (7.1)	4 (12.9)	45 (100)
	Negative	13 (92.9)	27 (87.1)	
2- ER	Positive	—	2 (6.5)	9 (20)
	Negative	1 (7.1)	6 (19.4)	
3- PR	Positive	—	5 (16.1)	9 (20)
	Negative	1 (7.1)	3 (9.7)	
4- Her-2 neu	Positive	—	5 (16.1)	9 (20)
	Negative	1 (7.1)	3 (9.7)	

Fisher's exact test; P = 0.444 (PR and HER2/neu); P = 1 (ER and CA15-3)

* Positive HHV-8 IgG cases = 14, while negative HHV-8 IgG cases = 31.

** Patients may have had more than two positive tumour markers.

the prevalence of HHV-8 among their wives was nearly the same, regardless of the serostatus of the husband³¹.

The seropositivity rate of CA15-3 among the patients was 11.11% (5 out of 45 patients), in contrast to a previous study that found a high level of CA15-3 (75.9%) in patients with breast cancer³². One explanation is that the majority of the women

were in stage II with no distant metastasis, and CA15-3 is good indicator of cancer metastasis^{33,34}. Statistically, there were no associations between an HHV-8 infection and the tumour markers.

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