

Epstein - Bar virus (EBV) - A cause of human breast cancer

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Abstract

Epstein-Barr Virus (EBV) has been widely proposed as a possible candidate virus for the viral etiology of human breast cancer, still the most common malignancy affecting females worldwide. Due to possible problems with PCR analyses (contamination), the lack of uniformity in the study design and insufficient mathematical/statistical methods used by the different authors, findings of several EBV (polymerase chain reaction (PCR)) studies contradict each other making it difficult to determine the EBV etiology for breast cancer. In this present study, we performed a re-investigation of some of the known studies. To place our results in context, this study support the hypothesis that EBV is a cause of human breast cancer.

Keywords

Epstein-Barr Virus (EBV), Human breast cancer, Causal relationship

1. Introduction

The etiology of human breast cancer (BC), one of the most commonly diagnosed cancer in women worldwide [1], is still not clear. Risk factors include cigarette smoking, obesity, hormone therapy, lifetime menstrual cycles, reproductive history, a family and personal history of BC, and others [2]. Viral infection has also been proposed to be associated with the development of breast cancer [3], [4], [5], [6]. The presence especially of the Epstein-Barr-virus (EBV) has been reported by several polymerase chain reaction (PCR) studies to be a pathogenic factor in breast cancer (BC). In this context it is important to note that the in situ hybridization (ISH) is able to differentiate between infection in other cells and viral infections in tumour cells and is regarded as superior to PCR. Still, the specificity and sensitivity of the in situ hybridization (ISH) depend on the target used. In particular, findings of polymerase chain reaction (PCR) studies of breast cancer and Epstein-Barr virus (EBV) vary too much, making it difficult to determine whether Epstein-Barr virus (EBV) is a cause or the cause of human breast cancer. Some of the known PCR studies of Epstein-Barr-virus and human breast cancer had positive results of

specimens tested [7], [8],[9],[10], [11], [12], [13], [14], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25], [26], [27], [28] whereas other studies [29], [30], [31], [32], [33], [34], [35], [36], [37], [44] did not. Due to the inconsistency of results in these different studies many investigators questioned the role of EBV as a primary etiologic agent for breast cancer in principle.

2. Material and methods

2.1. Study design of Bonnet et al.

Bonnet et al. [10] investigated 1999 the presence of the EBV genome by use of the polymerase chain reaction (PCR) in 100 consecutive primary invasive breast carcinomas, as well as in 30 samples of healthy breast tissue taken from next to the tumor as confirmed by pathologic examination. Bonnet et al. were able to detect the EBV genome by PCR in 51% of the tumors, whereas, in 90% of the cases studied, the virus was not detected in healthy tissues. The data as obtained by Bonnet et al. are presented by the 2 by 2-table (**Table 1**).

Table 1. The relationship between Epstein-Barr virus (EBV) and breast cancer (BC).

		Human breast cancer		
		yes	no	
EBV DNA	yes	51	3	54
	no	49	27	76
		100	30	130

2.2. Study design of Joshi et al.

Joshi et al. [38] investigated in the year 2009 the expression of Epstein-Barr virus Nuclear Antigen-1 (EBNA-1) in breast cancer tissue specimens by employing immunohistochemistry (IHC). Joshi et al. included 58 cases of malignant breast disease and 63 of benign breast disease (controls) in their study between January 2007 and March 2008. In 51 cases the immunohistochemistry (IHC) for EBV EBNA-1 was performed. In particular, 28 of the 51 cases (54.9%) were EBV EBNA-1 IHC positive. In contrast to this finding, EBV EBNA-1 expression by IHC was negative for all tested 30 controls. The data as obtained by Joshi et al. are presented by the 2 by 2-table (**Table 2**).

Table 2. The relationship between Epstein-Barr virus (EBV) and breast cancer (BC).

		Human breast cancer		
		yes	no	
EBV EBNA-1 (IHC)	yes	28	0	28
	no	23	30	53
		51	30	81

2.3. Study design of Lorenzetti et al.

Lorenzetti et al. [39] conducted in the year 2010 a study on 71 biopsies of breast carcinoma and in 48 non-neoplastic breast controls. EBV genomic DNA and EBNA1 expression was positive in 31% (22/71) of patients specifically restricted to tumor epithelial cells in breast carcinoma while all breast control samples were negative for both EBNA1 protein and viral EBV DNA. The data as obtained by Lorenzetti et al. are presented by the 2 by 2-table (**Table 3**).

Table 3. The relationship between Epstein-Barr virus (EBV) and breast cancer (BC).

		Human breast cancer		
		yes	no	
EBV DNA	yes	22	0	22
	no	49	48	97
		71	48	119

2.4. Study design of Zekri et al.

Zekri et al. [40] investigated in the year 2012 exactly 40 Egyptian and 50 Iraqi patients with primary invasive breast cancer (BC) in addition to 20 normal breast tissues as controls for each group. Zekri et al. were able to detect EBV-DNA in 18/40 (45%) and 14/50 (28%) of Egyptian and Iraqi women compared to 0/20 (0%) of their control groups. The data as obtained by Zekri et al. are presented by the 2 by 2-table (**Table 4**).

Table 4. The relationship between Epstein-Barr virus (EBV) and breast cancer (BC).

		Human breast cancer		
		yes	no	
ISH EBV DNA	yes	32	0	32
	no	58	40	98
		90	40	130

2.5. Study design of Morales-Sánchez et al.

Morales-Sánchez et al. [41] screened 86 tissues from Mexican women with breast cancer and 65 non-tumor adjacent-tissue cases by a standard polymerase chain reaction (PCR) for EBV in the year 2013. Additionally, a more sensitive nested PCR was used to confirm results. Only 4 of the 86 tumor samples were EBV positive by the more sensitive nested PCR. EBV was not found in the 65 non-tumor adjacent-tissue cases. The data as obtained by Morales-Sánchez et al. are presented by the 2 by 2-table (**Table 5**).

Table 5. The relationship between Epstein-Barr virus (EBV) and breast cancer (BC).

		Human breast cancer		
		yes	no	
EBV DNA	yes	4	0	4
	no	82	65	147
		86	65	151

2.6. Study design of Yahia et al.

Yahia et al. [42] investigated in the year 2014 exactly 92 biopsy specimens of breast carcinoma and 50 matched normal tissues adjacent to breast tumors from operated individuals in Sudan who had not yet received anti-cancer medications. EBV DNA EBNA-4 primers were detected in 10 (11%) of 92 patients with breast carcinoma while all control samples were negative when EBV DNA EBNA-4 primers were used. The data as obtained by Yahia et al. are presented by the 2 by 2-table (**Table 6**).

Table 6. The relationship between Epstein-Barr virus (EBV) and breast cancer (BC).

		Human breast cancer		
		yes	no	
EBV DNA	yes	10	0	10
	no	82	50	132
		92	50	142

In the following, Yahia et al. confirmed the presence of the EBV genome in malignant breast tissue while using the in situ hybridization technique (ISH). Using the in situ hybridization technique (ISH), Yahia et al. was detected EBV in all 18 biopsies examined while the presence of EBV was confined to the malignant cells. In contrast to this finding, all five histologically normal tissues examined by the in situ hybridization technique (ISH) showed no signal for EBV. The data as obtained by Yahia et al. are presented by the 2 by 2-table (**Table 7**).

Table 7. The relationship between Epstein-Barr virus (EBV) and breast cancer (BC).

		Human breast cancer		
		yes	no	
ISH EBV DNA	yes	18	0	18
	no	0	5	5
		18	5	23

2.7. Study design of Richardson et al.

Richardson et al. [43] investigated in the year 2015 cytomegalovirus (CMV) and EBV in paired samples of invasive human breast cancer tissue and normal breast tissue from 70 women using quantitative polymerase chain reaction (PCR). Quantitative PCR detected EBV in 9 (13%) of the paired normal specimens and 24 (34%) of the invasive human breast cancer tissue. Quantitative PCR detected cytomegalovirus (CMV) in 0 (0%) of the 70 tumour specimens and in 2 (3%) of the paired normal specimens. The data as obtained by Richardson et al. are presented by the 2 by 2-table2 (**Table 8**) and (**Table 9**).

Table 8. The relationship between Epstein-Barr virus (EBV) and breast cancer (BC).

		Human breast cancer		
		yes	no	
EBV DNA	yes	24	9	33
	no	46	61	107
		70	70	140

Table 9. The relationship between Cytomegalovirus (CMV) and breast cancer (BC).

		Human breast cancer		
		yes	no	
CMV DNA	yes	0	2	2
	no	70	68	138
		70	70	140

2.8. Statistical Analysis

All statistical analyses were performed with Microsoft Excel version 14.0.7166.5000 (32-Bit) software (Microsoft GmbH, Munich, Germany).

2.8.1. Conditio per quam

The formula of the conditio per quam [44] relationship

$$p(\text{EBV DNA} \rightarrow \text{Human breast cancer}) \quad (1)$$

was used to proof the hypothesis: An EBV infection (EBV DNA) is a conditio per quam of human breast cancer.

2.8.2. Exclusion relationship

The formula of the exclusion relationship [44]

$$p(\text{CMV DNA} \uparrow \text{Human breast cancer}) \quad (2)$$

was used to proof the hypothesis: A CMV infection (CMV DNA) excludes human breast cancer and vice versa.

2.8.3. The rule of three

Confidence intervals for proportions or a population mean of random variables which are not normally distributed in the population can be constructed while relying on the central limit theorem as long as the sample sizes and counts are big enough (i. e. a sample size of 30 and more). The formula, justified by the central limit theorem, is

$$p_{\text{Crit}} = p_{\text{Calc}} \pm \left(z_{\text{Alpha}/2} \times \left(\sqrt{\frac{1}{N} \times p_{\text{Calc}} \times (1 - p_{\text{Calc}})} \right) \right) \quad (3)$$

where p_{Calc} is the sample proportion of successes in a Bernoulli trial process with N trials yielding X successes and $N-X$ failures and z is the $1 - (\text{Alpha}/2)$ quantile of a standard normal distribution corresponding to the significance level α . For example, for a 95% confidence level $\alpha = 0.05$ and z is $z = 1.96$. The Agresti-Coull [45] formula is another approximate method for calculating binomial confidence intervals. The Clopper-Pearson interval [46] is of use too. A faster way to determine the lower and upper “exact” confidence interval for p_{Calc} can be based on the F distribution [47] too. In this study, we will use *the rule of three* [48] to calculate the confidence interval for p_{Calc} . Briefly sketched, the rule of three can be derived [49] from the binomial model. The rule of three defines that $3/N$ is an upper 95% confidence bound for a binomial probability p_{Calc} when in N independent trials no [50] events occur [51]. Under conditions where a certain event did not occur in a sample with N subjects (i. e. $p_{\text{Calc}} = 0$) the interval from 0 to $3/n$ is called a

95% classical confidence interval for the binomial parameter for the rate of occurrences in the population. According to the rule of the three the same interval is calculated for a sample sizes of 30-50 or more as

$$P_{\text{lower}} = \left(\frac{3}{N} \right) \quad (4)$$

By symmetry, the one-sided 95 percent confidence interval for only successes (i.e. $p_{\text{Calc}}=1$) is

$$P_{\text{lower}} = 1 - \left(\frac{3}{N} \right) \quad (5)$$

2.8.4. The mathematical formula of the causal relationship k

The mathematical formula of the causal relationship k [52] and the chi-square distribution [53] were applied to determine the significance of causal relationship between a *Helicobacter pylori* infection and human gastric cancer. A one-tailed test makes it much more easier to reject a null hypothesis (no causal relationship) while a two-tailed test makes it more difficult to reject a null hypothesis and is more conservative on this account. For this reason, in causal relationship testing, a two-tailed test is preferred. In general, a p value of $p < 0.05$ is considered as significant.

2.8.5. The chi square distribution

The chi-squared distribution [53] is a widely known distribution and used in hypothesis testing, in inferential statistics or in construction of confidence intervals. The critical values of the chi square distribution are visualized by **Table 10**.

Table 10. The critical values of the chi square distribution (degrees of freedom: 1).

	p-Value	One sided X ²	Two sided X ²
	0,1000000000	1,642374415	2,705543454
	0,0500000000	2,705543454	3,841458821
	0,0400000000	3,06490172	4,217884588
	0,0300000000	3,537384596	4,709292247
	0,0200000000	4,217884588	5,411894431
	0,0100000000	5,411894431	6,634896601
	0,0010000000	9,549535706	10,82756617
	0,0001000000	13,83108362	15,13670523
	0,0000100000	18,18929348	19,51142096
	0,0000010000	22,59504266	23,92812698
	0,0000001000	27,03311129	28,37398736
	0,0000000100	31,49455797	32,84125335
	0,0000000010	35,97368894	37,32489311
	0,0000000001	40,46665791	41,82145620

3. Results

3.1. An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer due to Bonnet et al.

Claims.

Null hypothesis:

An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer.

$$(p_0 \geq p_{Crit}).$$

Alternative hypothesis:

An infection of human breast by Epstein-Bar virus is not a *conditio per quam* of human breast cancer.

$$(p_0 < p_{Crit}).$$

Significance level (Alpha) below which the null hypothesis will be rejected: 0.05.

Proof.

The data for this test of an infection by Epstein-Bar virus of human breast and human breast cancer are provided by Bonnet et al. [10] and viewed in the 2×2 table (**Table 1**). The proportion of successes in the sample of a *conditio per quam* relationship $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer})$ is calculated [44] as

$$p(\text{EBV DNA} \rightarrow \text{Breast cancer}) = \frac{(51 + 49 + 27)}{130} = \frac{127}{130} = 0,976923077$$

The critical value p_{Crit} (significance level $\alpha = 0.05$) is calculated [44] approximately as

$$p_{Crit} = 1 - \frac{3}{130} = 0,976923077$$

The critical value is $p_{Crit} = 0,976923077$ and not greater than the proportion of successes of the sample $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer}) = 0,976923077$. Due to this evidence, we do not reject the null hypothesis in favor of the alternative hypotheses. The data as obtained by Bonnet et al. [10] do support the Null hypothesis that an infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer. In other words, *if* an infection of human breast by Epstein-Bar virus *then* human breast cancer.

Q. e. d.

3.2. An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer due to Joshi et al.

Claims.

Null hypothesis:

An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer.

$$(p_0 \geq p_{Crit}).$$

Alternative hypothesis:

An infection of human breast by Epstein-Bar virus is not a *conditio per quam* of human breast cancer.

$$(p_0 < p_{Crit}).$$

Significance level (Alpha) below which the null hypothesis will be rejected: 0.05.

Proof.

The data for this test of an infection by Epstein-Bar virus of human breast and human breast cancer are provided by Joshi et al. [38] and viewed in the 2×2 table (**Table 2**). The proportion of successes in the sample of a *conditio per quam* relationship $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer})$ is calculated [44] as

$$p(\text{EBV EBNA1} \rightarrow \text{Breast cancer}) = \frac{(28 + 23 + 30)}{81} = \frac{81}{81} = 1$$

The critical value p_{Crit} (significance level $\alpha = 0.05$) is calculated [44] approximately as

$$p_{Crit} = 1 - \frac{3}{81} = 0,962962963$$

The critical value is $p_{Crit} = 0,962962963$ and not greater than the proportion of successes of the sample $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer}) = 1$. Due to this evidence, we do not reject the null hypothesis in favor of the alternative hypotheses. The data as obtained by Joshi et al. [38] do support the Null hypothesis that an infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer. In other words, *if* an infection of human breast by Epstein-Bar virus *then* human breast cancer.

Q. e. d.

3.3. An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer due to Lorenzetti et al.

Claims.

Null hypothesis:

An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer.

$$(p_0 \geq p_{Crit}).$$

Alternative hypothesis:

An infection of human breast by Epstein-Bar virus is not a *conditio per quam* of human breast cancer.

$$(p_0 < p_{Crit}).$$

Significance level (Alpha) below which the null hypothesis will be rejected: 0.05.

Proof.

The data for this test of an infection by Epstein-Bar virus of human breast and human breast cancer are provided by Lorenzetti et al. [39] and viewed in the 2×2 table (**Table 3**). The proportion of successes in the sample of a *conditio per quam* relationship $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer})$ is calculated [44] as

$$p(\text{EBV EBNA1} \rightarrow \text{Breast cancer}) = \frac{(22 + 49 + 48)}{119} = \frac{119}{119} = 1$$

The critical value p_{Crit} (significance level $\alpha = 0.05$) is calculated [44] approximately as

$$p_{Crit} = 1 - \frac{3}{119} = 0,974789916$$

The critical value is $p_{Crit} = 0,974789916$ and not greater than the proportion of successes of the sample $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer}) = 1$. Due to this evidence, we do not reject the null hypothesis in favor of the alternative hypotheses. The data as obtained by Lorenzetti et al. [39] do support the null hypothesis that an infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer. In other words, *if* an infection of human breast by Epstein-Bar virus *then* human breast cancer.

Q. e. d.

3.4. An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer due to Zekri et al.

Claims.

Null hypothesis:

An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer.

$$(p_0 \geq p_{Crit}).$$

Alternative hypothesis:

An infection of human breast by Epstein-Bar virus is not a *conditio per quam* of human breast cancer.

$$(p_0 < p_{Crit}).$$

Significance level (Alpha) below which the null hypothesis will be rejected: 0.05.

Proof.

The data for this test of an infection by Epstein-Bar virus of human breast and human breast cancer are provided by Zekri et al. [40] and viewed in the 2×2 table (**Table 4**). The proportion of successes in the sample of a *conditio per quam* relationship $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer})$ is calculated [44] as

$$p(\text{EBV EBNA1} \rightarrow \text{Breast cancer}) = \frac{32 + 58 + 40}{130} = \frac{130}{130} = 1$$

The critical value p_{Crit} (significance level $\alpha = 0.05$) is calculated [44] approximately as

$$p_{Crit} = 1 - \frac{3}{130} = 0,976923077$$

The critical value is $p_{Crit} = 0,974789916$ and not greater than the proportion of successes of the sample $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer}) = 1$. Due to this evidence, we do not reject the null hypothesis in favor of the alternative hypotheses. The data as obtained by Zekri et al. [40] do support the null hypothesis that an infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer. In other words, *if* an infection of human breast by Epstein-Bar virus *then* human breast cancer.

Q. e. d.

3.5. An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer due to Morales-Sánchez et al.

Claims.

Null hypothesis:

An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer.

$$(p_0 \geq p_{Crit}).$$

Alternative hypothesis:

An infection of human breast by Epstein-Bar virus is not a *conditio per quam* of human breast cancer.

$$(p_0 < p_{Crit}).$$

Significance level (Alpha) below which the null hypothesis will be rejected: 0.05.

Proof.

The data for this test of an infection by Epstein-Bar virus of human breast and human breast cancer are provided by Morales-Sánchez et al. [41] and viewed in the 2×2 table (**Table 5**). The proportion of successes in the sample of a *conditio per quam* relationship $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer})$ is calculated [44] as

$$p(\text{EBV DNA} \rightarrow \text{Breast cancer}) = \frac{(22 + 49 + 48)}{119} = \frac{119}{119} = 1$$

The critical value p_{Crit} (significance level $\alpha = 0.05$) is calculated [44] approximately as

$$p_{Crit} = 1 - \frac{3}{119} = 0,98013245$$

The critical value is $p_{Crit} = 0,974789916$ and not greater than the proportion of successes of the sample $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer}) = 1$. Due to this evidence, we do not reject the null hypothesis in favor of the alternative hypotheses. The data as obtained by Morales-Sánchez et al. [41] are used as an argument against the EBV hypothesis in the viral etiology human breast cancer. Contrary to the published opinion, the data of Morales-Sánchez et al. [41] do support the Null hypothesis that an infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer. In other words, due to Morales-Sánchez et al. [41] *if* an infection of human breast by Epstein-Bar virus *then* human breast cancer.

Q. e. d.

3.6. An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer due to Yahia et al.

Claims.

Null hypothesis:

An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer.

$$(p_0 \geq p_{Crit}).$$

Alternative hypothesis:

An infection of human breast by Epstein-Bar virus is not a *conditio per quam* of human breast cancer.

$$(p_0 < p_{Crit}).$$

Significance level (Alpha) below which the null hypothesis will be rejected: 0.05.

Proof.

The data for this test of an infection by Epstein-Bar virus of human breast and human breast cancer are provided by Yahia et al. [42] and viewed in the 2×2 table (**Table 6**). The proportion of successes in the sample of a *conditio per quam* relationship p(Epstein-Bar virus DNA \rightarrow breast cancer) is calculated [44] as

$$p(\text{EBV DNA} \rightarrow \text{Breast cancer}) = \frac{(10 + 82 + 50)}{142} = \frac{142}{142} = 1$$

The critical value p_{Crit} (significance level $\alpha = 0.05$) is calculated [44] approximately as

$$p_{Crit} = 1 - \frac{3}{142} = +0,978873239$$

The critical value is $p_{Crit} = +0,978873239$ and not greater than the proportion of successes of the sample $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer}) = 1$. Due to this evidence, we do not reject the null hypothesis in favor of the alternative hypotheses. The data as obtained by Yahia et al. [42] do support the null hypothesis that an infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer. In other words, due to Yahia et al. [42] *if* an infection of human breast by Epstein-Bar virus *then* human breast cancer.

Q. e. d.

3.8. An infection of human breast by Epstein-Bar virus is not a *conditio per quam* of human breast cancer due to Richardson et al.

Claims.

Null hypothesis:

An infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer.

$$(p_0 \geq p_{Crit}).$$

Alternative hypothesis:

An infection of human breast by Epstein-Bar virus is not a *conditio per quam* of human breast cancer.

$$(p_0 < p_{Crit}).$$

Significance level (Alpha) below which the null hypothesis will be rejected: 0.05.

Proof.

The data for this test of an infection by Epstein-Bar virus of human breast and human breast cancer are provided by Richardson et al. [43] and viewed in the 2×2 table (**Table 8**). The proportion of successes in the sample of a *conditio per quam* relationship $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer})$ is calculated [44] as

$$p(\text{EBV DNA} \rightarrow \text{Breast cancer}) = \frac{(24 + 46 + 61)}{140} = \frac{131}{140} = 0,935714286$$

The critical value p_{Crit} (significance level $\alpha = 0.05$) is calculated [44] approximately as

$$p_{Crit} = 1 - \frac{3}{140} = 0,978571429$$

The critical value is $p_{Crit} = 0,978571429$ and is greater than the proportion of successes of the sample $p(\text{Epstein-Bar virus DNA} \rightarrow \text{breast cancer}) = 0,935714286$. Due to the data as provided by Richardson et al. [43], we do reject the null hypothesis in favor of the alternative hypothesis. The data as obtained by Richardson et al. [43] do not support the Null hypothesis that an infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer.

Q. e. d.

3.9. An infection with cytomegalovirus (CMV) excludes human breast cancer and vice versa due to Richardson et al.

Claims.

Null hypothesis:

An infection with cytomegalovirus (CMV) excludes human breast cancer.

$$(p_0 \geq p_{Crit}).$$

Alternative hypothesis:

An infection with cytomegalovirus (CMV) does not exclude human breast cancer.

$$(p_0 < p_{Crit}).$$

Significance level (Alpha) below which the null hypothesis will be rejected: 0.05.

Proof.

The data for this test of an exclusion of an infection with cytomegalovirus (CMV) and human breast cancer are provided by Richardson et al. [43] and viewed in the 2×2 table (**Table 9**). The proportion of successes in the sample of an exclusion relationship $p(\text{Cytomegalovirus DNA} \uparrow \text{breast cancer})$ is calculated [44] as

$$p(\text{Cytomegalovirus DNA} \uparrow \text{Breast cancer}) = \frac{(2 + 70 + 68)}{140} = \frac{140}{140} = 1$$

The critical value p_{Crit} (significance level $\alpha = 0.05$) is calculated [44] approximately as

$$p_{Crit} = 1 - \frac{3}{140} = 0,978571429$$

The critical value is $p_{Crit} = 0,978571429$ and is not greater than the proportion of successes of the sample $p(\text{Cytomegalovirus DNA} \uparrow \text{breast cancer}) = 1$. Due to the data as provided by Richardson et al. [43], we do not reject the null hypothesis in favor of the alternative hypothesis. The data as obtained by Richardson et al. [43] do support the null hypothesis that an infection of tissues investigated with cytomegalovirus excludes human breast cancer and vice versa. In the Cytomegalovirus, the cure of human breast cancer can be found.

Q. e. d.

3.10. Epstein-Bar virus is a cause of breast cancer due to Bonnet et al.

Claims.

Null hypothesis: (no causal relationship)

There is no causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma.

($k = 0$).

Alternative hypothesis: (causal relationship)

There is a causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma.

($k > 0$).

Conditions.

Alpha level = 5%.

The two tailed critical Chi square value (degrees of freedom = 1) for alpha level 5% is 3.841458821.

Proof.

The data for this hypothesis test are provided by Bonnet et al. and illustrated in the 2×2 table (**Table 1**). The causal relationship k (EBV DNA , Breast cancer) is calculated [44], [52] as

$$k_{\text{Calc}}(\text{EBV DNA, Breast cancer}) = \frac{((130 \times 51) - (54 \times 100))}{\sqrt[2]{(100 \times 30) \times (54 \times 76)}} = +0,350542604$$

The value of the test statistic $k_{\text{Calc}} = +0,350542604$ is equivalent to a calculated [44], [52] chi-square value of

$$\chi^2_{\text{Calculated}} = N \times k_{\text{Calculated}} \times k_{\text{Calculated}}$$

$$\chi^2_{\text{Calculated}} \equiv 130 \times \frac{((130 \times 51) - (54 \times 100))}{\sqrt[2]{(100 \times 30) \times (54 \times 76)}} \times \frac{((130 \times 51) - (54 \times 100))}{\sqrt[2]{(100 \times 30) \times (54 \times 76)}}$$

$$\chi^2_{\text{Calculated}} = 130 \times 0,350542604 \times 0,350542604$$

$$\chi^2_{\text{Calculated}} = 15,9744152$$

The chi-square statistic, uncorrected for continuity, is $X^2_{\text{Calculated}} = 15,9744152$ and as such equivalent to a two sided p-value of p-value = 0,000064204332405. The test statistic Chi-square_{Calculated} exceeds the critical Chi-square value of 3.841458821 (**Table 10**). Consequently, we reject the null hypothesis and accept the alternative hypotheses. There is statistically significant causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma ($k = +0,350542604$, p-value (two sided) = 0,000064204332405). The result is significant at $p < 0.05$.

Q. e. d.

3.11. Epstein-Bar virus is a cause of breast cancer due to Joshi et al.

Claims.

Null hypothesis: (no causal relationship)

There is no causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma.

($k = 0$).

Alternative hypothesis: (causal relationship)

There is a causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma.

($k > 0$).

Conditions.

Alpha level = 5%.

The two tailed critical Chi square value (degrees of freedom = 1) for alpha level 5% is 3.841458821.

Proof.

The data for this hypothesis test are provided by Joshi et al. [38] and illustrated in the 2×2 table (**Table 2**). The causal relationship k (EBV DNA , Breast cancer) is calculated [44], [52] as

$$k_{\text{Calc}}(\text{EBV DNA, Breast cancer}) = \frac{((81 \times 28) - (28 \times 51))}{\sqrt[3]{(51 \times 30) \times (28 \times 53)}} = +0,557463735$$

The value of the test statistic $k_{\text{Calc}} = +0,557463735$ is equivalent to a calculated [44], [52] chi-square value of

$$\chi^2_{\text{Calculated}} = N \times k_{\text{Calculated}} \times k_{\text{Calculated}}$$

$$\chi^2_{\text{Calculated}} \equiv 81 \times \frac{((81 \times 28) - (28 \times 51))}{\sqrt[3]{(51 \times 30) \times (28 \times 53)}} \times \frac{((81 \times 28) - (28 \times 51))}{\sqrt[3]{(51 \times 30) \times (28 \times 53)}}$$

$$\chi^2_{\text{Calculated}} = 81 \times 0,557463735 \times 0,557463735$$

$$\chi^2_{\text{Calculated}} = 25,17203108$$

The chi-square statistic, uncorrected for continuity, is $X^2_{\text{Calculated}} = 25,17203108$ and as such equivalent to a two sided p-value of p-value = 0,000000524371787. The test statistic $\text{Chi-square}_{\text{Calculated}}$ exceeds the critical Chi-square value of 3.841458821 (**Table 10**). Consequently, we reject the null hypothesis and accept the alternative hypotheses. There is statistically significant causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma ($k = +0,557463735$, p-value (two sided) = 0,000000524371787). The result is significant at $p < 0.05$.

Q. e. d.

3.12. Epstein-Bar virus is a cause of breast cancer due to Lorenzetti et al.

Claims.

Null hypothesis: (no causal relationship)

There is no causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k = 0$).

Alternative hypothesis: (causal relationship)

There is a causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k > 0$).

Conditions.

Alpha level = 5%.

The two tailed critical Chi square value (degrees of freedom = 1) for alpha level 5% is 3.841458821.

Proof.

The data for this hypothesis test are provided by Lorenzetti et al. [39] and illustrated in the 2×2 table (**Table 3**). The causal relationship $k(\text{EBV DNA}, \text{Breast cancer})$ is calculated [44], [52] as

$$k_{\text{Calc}}(\text{EBV DNA}, \text{Breast cancer}) = \frac{((119 \times 22) - (22 \times 71))}{\sqrt[3]{(71 \times 48) \times (22 \times 97)}} = +0,391576768$$

The value of the test statistic $k_{\text{Calc}} = +0,391576768$ is equivalent to a calculated [44], [52] chi-square value of

$$\chi^2_{\text{Calculated}} = N \times k_{\text{Calculated}} \times k_{\text{Calculated}}$$

$$\chi^2_{\text{Calculated}} \equiv 119 \times \frac{((119 \times 22) - (22 \times 71))}{\sqrt[3]{(71 \times 48) \times (22 \times 97)}} \times \frac{((119 \times 22) - (22 \times 71))}{\sqrt[3]{(71 \times 48) \times (22 \times 97)}}$$

$$\chi^2_{\text{Calculated}} = 119 \times 0,391576768 \times 0,391576768$$

$$\chi^2_{\text{Calculated}} = 18,24655147$$

The chi-square statistic, uncorrected for continuity, is $X^2_{\text{Calculated}} = 18,24655147$ and as such equivalent to a two sided p-value of $p\text{-value} = 0,000019407700006$. The test statistic $\text{Chi-square}_{\text{Calculated}}$ exceeds the critical Chi-square value of 3.841458821 (**Table 10**). Consequently, we reject the null hypothesis and accept the alternative hypotheses. There is statistically significant causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma ($k = +0,391576768$, $p\text{-value}$ (two sided) = $0,000019407700006$). The result is significant at $p < 0.05$.

Q. e. d.

3.13. Epstein-Bar virus is a cause of breast cancer due to Zekri et al.

Claims.

Null hypothesis: (no causal relationship)

There is no causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k = 0$).

Alternative hypothesis: (causal relationship)

There is a causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k > 0$).

Conditions.

Alpha level = 5%.

The two tailed critical Chi square value (degrees of freedom = 1) for alpha level 5% is 3.841458821.

Proof.

The data for this hypothesis test are provided by Zekri et al. [40] and illustrated in the 2×2 table (**Table 4**). The causal relationship $k(\text{EBV DNA}, \text{Breast cancer})$ is calculated [44], [52] as

$$k_{\text{Calc}}(\text{EBV DNA}, \text{Breast cancer}) = \frac{((130 \times 32) - (32 \times 90))}{\sqrt[3]{(90 \times 40) \times (32 \times 98)}} = +0,380952381$$

The value of the test statistic $k_{\text{Calc}} = +0,380952381$ is equivalent to a calculated [44], [52] chi-square value of

$$\chi^2_{\text{Calculated}} = N \times k_{\text{Calculated}} \times k_{\text{Calculated}}$$

$$\chi^2_{\text{Calculated}} \equiv 130 \times \frac{((130 \times 32) - (32 \times 90))}{\sqrt[3]{(90 \times 40) \times (32 \times 98)}} \times \frac{((130 \times 32) - (32 \times 90))}{\sqrt[3]{(90 \times 40) \times (32 \times 98)}}$$

$$\chi^2_{\text{Calculated}} = 130 \times 0,380952381 \times 0,380952381$$

$$\chi^2_{\text{Calculated}} = 18,86621315$$

The chi-square statistic, uncorrected for continuity, is $X^2_{\text{Calculated}} = 18,86621315$ and as such equivalent to a two sided p-value of $p\text{-value} = 0,000014021423911$. The test statistic $\text{Chi-square}_{\text{Calculated}}$ exceeds the critical Chi-square value of 3.841458821 (**Table 10**). Consequently, we reject the null hypothesis and accept the alternative hypotheses. There is statistically significant causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma ($k = +0,380952381$, $p\text{-value}$ (two sided) = $0,000014021423911$). The result is significant at $p < 0.05$.

Q. e. d.

3.14. Epstein-Bar virus is a cause of breast cancer due to Morales-Sánchez et al.

Claims.

Null hypothesis: (no causal relationship)

There is no causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k = 0$).

Alternative hypothesis: (causal relationship)

There is a causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k > 0$).

Conditions.

Alpha level = 5%.

The one tailed critical Chi square value (degrees of freedom = 1) for alpha level 5% is 2,705543454.

Proof.

The data for this hypothesis test are provided by Morales-Sánchez et al. [44] and illustrated in the 2×2 table (Table 5). The causal relationship $k(\text{EBV DNA}, \text{Breast cancer})$ is calculated [44], [52] as

$$k_{\text{Calc}}(\text{EBV DNA}, \text{Breast cancer}) = \frac{((151 \times 4) - (4 \times 86))}{\sqrt[2]{(86 \times 65) \times (4 \times 147)}} = +0,143409784$$

The value of the test statistic $k_{\text{Calc}} = +0,143409784$ is equivalent to a calculated [44], [52] chi-square value of

$$\chi^2_{\text{Calculated}} = N \times k_{\text{Calculated}} \times k_{\text{Calculated}}$$

$$\chi^2_{\text{Calculated}} \equiv 151 \times \frac{((151 \times 4) - (4 \times 86))}{\sqrt[2]{(86 \times 65) \times (4 \times 147)}} \times \frac{((151 \times 4) - (4 \times 86))}{\sqrt[2]{(86 \times 65) \times (4 \times 147)}}$$

$$\chi^2_{\text{Calculated}} = 151 \times 0,143409784 \times 0,143409784$$

$$\chi^2_{\text{Calculated}} = 3,105521278$$

The chi-square statistic, uncorrected for continuity, is $X^2_{\text{Calculated}} = 3,105521278$ and as such equivalent to a one sided p-value of $p\text{-value (one sided)} = 0,039013624479347$. The test statistic $\text{Chi-square}_{\text{Calculated}}$ exceeds the critical Chi-square value of 2,705543454 (Table 10). Consequently, we reject the null hypothesis and accept the alternative hypotheses. There is statistically significant causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma ($k = +0,143409784$, $p\text{-value (one sided)} = 0,039013624479347$). The result is significant at $p < 0.05$.

Q. e. d.

3.15. Epstein-Bar virus is a cause of breast cancer due to Yahia et al. et al.

Claims.

Null hypothesis: (no causal relationship)

There is no causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k = 0$).

Alternative hypothesis: (causal relationship)

There is a causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k > 0$).

Conditions.

Alpha level = 5%.

The two tailed critical Chi square value (degrees of freedom = 1) for alpha level 5% is 3.841458821.

Proof.

The data for this hypothesis test are provided by Yahia et al. [42] and illustrated in the 2×2 table (**Table 6**). The causal relationship $k(\text{EBV DNA}, \text{Breast cancer})$ is calculated [44], [52] as

$$k_{\text{Calc}}(\text{EBV DNA}, \text{Breast cancer}) = \frac{((142 \times 10) - (10 \times 92))}{\sqrt[3]{(92 \times 50) \times (10 \times 132)}} = +0,202910314$$

The value of the test statistic $k_{\text{Calc}} = +0,202910314$ is equivalent to a calculated [44], [52] chi-square value of

$$\chi^2_{\text{Calculated}} = N \times k_{\text{Calculated}} \times k_{\text{Calculated}}$$

$$\chi^2_{\text{Calculated}} \equiv 142 \times \frac{((142 \times 10) - (10 \times 92))}{\sqrt[3]{(92 \times 50) \times (10 \times 132)}} \times \frac{((142 \times 10) - (10 \times 92))}{\sqrt[3]{(92 \times 50) \times (10 \times 132)}}$$

$$\chi^2_{\text{Calculated}} = 142 \times 0,202910314 \times 0,202910314$$

$$\chi^2_{\text{Calculated}} = 5,846508564$$

The chi-square statistic, uncorrected for continuity, is $X^2_{\text{Calculated}} = 5,846508564$ and as such equivalent to a two sided p-value of $p\text{-value} = 0,015607987546856$. The test statistic $\text{Chi-square}_{\text{Calculated}}$ exceeds the critical Chi-square value of 3.841458821 (**Table 10**). Consequently, we reject the null hypothesis and accept the alternative hypotheses. There is statistically significant causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma ($k = +0,202910314$, $p\text{-value}$ (two sided) = 0,015607987546856). The result is significant at $p < 0.05$.

Q. e. d.

3.16. Epstein-Bar virus is a cause of breast cancer due to Yahia et al. et al. II

Claims.

Null hypothesis: (no causal relationship)

There is no causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k = 0$).

Alternative hypothesis: (causal relationship)

There is a causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k > 0$).

Conditions.

Alpha level = 5%.

The two tailed critical Chi square value (degrees of freedom = 1) for alpha level 5% is 3.841458821.

Proof.

Yahia et al. [42] used the in situ hybridization technique (ISH) to confirm the presence of the EBV genome in malignant breast tissues. The data for this hypothesis test are provided by Yahia et al. [42] and illustrated in the 2×2 table (**Table 7**). The causal relationship k (EBV DNA , Breast cancer) is calculated [44], [52] as

$$k_{\text{Calc}}(\text{ISH EBV , Breast cancer}) = \frac{((23 \times 18) - (18 \times 18))}{\sqrt[2]{(18 \times 5) \times (18 \times 5)}} = +1$$

The value of the test statistic $k_{\text{Calc}} = +1$ is equivalent to a calculated [44], [52] chi-square value of

$$\chi^2_{\text{Calculated}} = N \times k_{\text{Calculated}} \times k_{\text{Calculated}}$$

$$\chi^2_{\text{Calculated}} = 23 \times \frac{((23 \times 18) - (18 \times 18))}{\sqrt[2]{(18 \times 5) \times (18 \times 5)}} \times \frac{((23 \times 18) - (18 \times 18))}{\sqrt[2]{(18 \times 5) \times (18 \times 5)}}$$

$$\chi^2_{\text{Calculated}} = 23 \times 1 \times 1$$

$$\chi^2_{\text{Calculated}} = 23$$

The chi-square statistic, uncorrected for continuity, is $X^2_{\text{Calculated}} = 23$ and as such equivalent to a two sided p-value of $p\text{-value} = 0,000001620013982$. The test statistic $\text{Chi-square}_{\text{Calculated}}$ exceeds the critical Chi-square value of 3.841458821 (**Table 10**). Consequently, we reject the null hypothesis and accept the alternative hypotheses. There is statistically significant causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma ($k = +1$, $p\text{-value}$ (two sided) = 0,000001620013982). The result is significant at $p < 0.05$.

Q. e. d.

3.17. Epstein-Bar virus is a cause of breast cancer due to Richardson et al.

Claims.

Null hypothesis: (no causal relationship)

There is no causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k = 0$).

Alternative hypothesis: (causal relationship)

There is a causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma. ($k > 0$).

Conditions.

Alpha level = 5%.

The two tailed critical Chi square value (degrees of freedom = 1) for alpha level 5% is 3.841458821.

Proof.

The data for this hypothesis test are provided by Richardson et al. [43] and illustrated in the 2×2 table (**Table 8**). The causal relationship $k(\text{EBV DNA}, \text{Breast cancer})$ is calculated [44], [52] as

$$k_{\text{Calc}}(\text{EBV DNA}, \text{Breast cancer}) = \frac{((140 \times 24) - (33 \times 70))}{\sqrt{(70 \times 70) \times (33 \times 107)}} = +0,252430833$$

The value of the test statistic $k_{\text{Calc}} = +0,252430833$ is equivalent to a calculated [44], [52] chi-square value of

$$\chi^2_{\text{Calculated}} = N \times k_{\text{Calculated}} \times k_{\text{Calculated}}$$

$$\chi^2_{\text{Calculated}} \equiv 140 \times \frac{((140 \times 24) - (33 \times 70))}{\sqrt{(70 \times 70) \times (33 \times 107)}} \times \frac{((140 \times 24) - (33 \times 70))}{\sqrt{(70 \times 70) \times (33 \times 107)}}$$

$$\chi^2_{\text{Calculated}} = 140 \times 0,252430833 \times 0,252430833$$

$$\chi^2_{\text{Calculated}} = 8,920985556$$

The chi-square statistic, uncorrected for continuity, is $X^2_{\text{Calculated}} = 8,920985556$ and as such equivalent to a two sided p-value of p-value = 0,002819123393212. The test statistic $\text{Chi-square}_{\text{Calculated}}$ exceeds the critical Chi-square value of 3.841458821 (**Table 10**). Consequently, we reject the null hypothesis and accept the alternative hypotheses. There is statistically significant causal relationship between an infection of human breast by Epstein-Bar virus and human breast carcinoma ($k = +0,252430833$, p-value (two sided) = 0,002819123393212). The result is significant at $p < 0.05$.

Q. e. d.

4. Discussion

An infection with EBV virus is a very frequent event worldwide. In fact, only on very few individuals, EBV virus is able to exert an oncogenic effect while the most individuals are spared. The exact mechanism by which EBV transforms a cell into a cancer cell remains poorly understood. In fact, it is known that the molecular evidence for EBV as a cause or the cause of human breast cancer is still not generally [54] accepted. Some of the PCR analyses can lead to false positive or false-positive results. Epstein-Barr virus (EBV) is a virus which infects more than 90% of the population worldwide [55] and EBV persists for life in memory B cells [56], [57], [58] in the peripheral blood in its human host after the primary infection. Memory B cells can be found in breast cancer tissues too. Theoretically, it is possible that memory B cells with EBV DNA can be found in breast cancer tissues too with the consequence that false-positive results can be caused by the presence of EBV DNA containing memory B cells and other EBV-positive lymphocytes. A theoretical contamination of breast cancer tissues with EBV DNA containing i. e. memory B cells cannot be excluded. In particular, EBV DNA can be derived from surrounding stroma, infiltrating lymphocytes or tumor cells. Still, even if accept to some extent a possible contamination of breast cancer tissues with not tumor EBV DNA (i. e. containing memory B cells) why should there be more EBV DNA in breast cancer tissues than in healthy tissues. A significant difference does not make any sense, in this context, a causal relationship cannot be accepted. In fact, several published results of PCR analyses of the relationship between EBV and breast cancer have been inconsistent. PCR is a potentially sensitive technique as such. The discrepancies in EBV DNA detection efficiency may be due to technical PCR problems. The PCR technique cannot differentiate the source of EBV genome and is of limited value for studying tumors with memory cells or with B-lymphocyte infiltrates. To address such concerns the in situ hybridization technique (ISH) was used to investigate cellular localization of the virus itself. Using the in situ hybridization technique (ISH), several authors detected the EBV genome in breast cancer but could not detect the EBV virus genome in healthy breast tissue. The confinement of viral EBV DNA to tumor cells is demonstrated by several in situ hybridization studies and supports the hypothesis that EBV contributes to breast cancer aetiology.

In this study, we performed a re-analysis of several EBV DNA PCR studies, some of them ISH-based too. All studies investigated are supporting the hypothesis that an infection of human breast by Epstein-Bar virus is a *conditio per quam* of human breast cancer only the study of Richardson et al. [43] is not supporting this hypothesis. Contrary to expectation, the study of Richardson et al. [43] agrees with the other studies on the fact that EBV is cause of human breast cancer ($k = +0,252430833$, $p\text{-value (two sided)} = 0,002819123393212$). Thus far, there are some principle problems with the study of Richardson et al. [43]. How could Richardson et al. [43] detect in 9 of 70 control cases the EBV DNA? Technical errors, an insufficient kit or a contamination are some possible explanations. The majority of studies reanalyzed in this publication do support the alternative hypothesis that EBV virus is a cause of human breast cancer. The table (**Table 11**) summarizes these findings.

Table 11. The causal relationship between Epstein-Barr virus (EBV) and breast cancer (BC).

Study	Year	Sample N	k	p-value	
Bonnet et. al.	1999	130	+0,350542604	0,000064204332405	two sided
Joshi et al.	2009	81	+0,557463735	0,00000524371787	two sided
Lorenzetti et al.	2010	119	+0,391576768	0,000019407700006	two sided
Zekri et al.	2012	130	+0,380952381	0,000014021423911	two sided
Morales-Sánchez et al.	2013	151	+0,143409784	0,039013624479347	one sided
Yahia et al.	2014	142	+0,202910314	0,015607987546856	two sided
Yahia et al.	2014	23	+1,000000000	0,000001620013982	two sided
Richardson et al.	2015	140	+0,252430833	0,002819123393212	two sided

The best proof that Epstein-Barr virus (EBV) is a cause of human breast cancer (BC) were provided by Joshi et al. [38] and Yahia et al. [42] even if the sub-group studied by Yahia et al. [42] was small (N=23). Considering the analysis of its own data, Morales-Sánchez et al. [41] came to the conclusion that the data of their study do not support the involvement of EBV in the etiology of breast cancer. In fact, even the very critical study of Morales-Sánchez et al. [41] provides data of a significant causal relationship between Epstein-Barr virus (EBV) and breast cancer (BC) but only on one side ($k=+0,143409784$, p-value (one sided) = 0,039013624479347). All these results support the alternative hypothesis that EBV virus is a cause of human breast cancer.

5. Conclusion

The results of this study are in agreement with studies that detected the EBV virus in breast cancer. In conclusion, this study presents an unequivocal evidence that EBV is a cause of breast cancer. Especially the localisation of EBV in malignant cells and a significant causal relationship due to Yahia et al. [42] makes EBV a likely a cause of human breast cancer. A causal relationship between EBV and breast cancer is established and therapeutic implications might follow (i. e. EBV vaccination).

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