Epstein - Bar virus. A main cause of Hodgkin's lymphoma

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Abstract

Epstein-Barr virus (EBV), a herpes virus which persists in memory B cells in the peripheral blood for the lifetime of a person, is associated with some malignancies. Many studies suggested that the Epstein-Barr virus contributes to the development of Hodgkin lymphoma (HL) in some cases too. Despite intensive study, the role of Epstein-Barr virus in Hodgkin lymphoma remains enigmatic. It is the purpose of this publication to make the proof the Epstein-Barr virus is a main cause of Hodgkin's lymphoma (k=+0,739814235, p Value = 0,000000000000138).

Keywords

Epstein-Barr virus, Hodgkin lymphoma, Causal relationship

1. Introduction

Epstein-Barr [1] virus (EBV) is a ubiquitous human herpes virus which infects more than 90% of the world population. After the primary infection, EBV persists for life [2] in memory B cells in the peripheral blood of human host while well controlled by host's immune system. Similar to other herpes viruses, an EBV reactivation [3] reflected by aberrant IgG. IgM, IgA antibody responses can occur. The spectrum of diseases which are associated with Epstein-Barr virus includes Burkitt's [1] lymphoma (BL), nasopharyngeal [4] carcinoma, infectious mononucleosis [5] (IM), Hodgkin's [6] disease and many other too. Hodgkin's lymphoma (HL) itself, named after the English physician Thomas Hodgkin [7], who first described this malignancy in 1832, is characterized by the presence of a minority of malignant Hodgkin/Reed–Sternberg (HRS) cells [8] and the disruption of normal lymph node architecture. The Sternberg-Reed cells [9], [10] which are pathognomonic for Hodgkin lymphoma (HL) are described over a century ago and origin from B lymphocytes [11] . The detection of raised antibody titres to EBV [12] antigens in HL patients compared with other lymphoma patients provided the first evidence that EBV might be involved in the pathogenesis of HL. Finally, Weiss et el. [13] examined for the presence of Epstein-Barr virus (EBV) in tissue specimens of Hodgkin's disease and were able to detect EBV DNA in Hodgkin's disease. Still, we are far away from identifying a cause or the cause of Hodgkin's lymphoma.

2. Material and methods

2.1. Study design

Veronique Dinand et al. [14] conducted a case-control study to measure circulating EBV DNA in 30 children with Hodgkin lymphoma (HL) and in 70 controls, with prospective follow-up of the Hodgkin lymphoma cohort (2007-2012). Over the same time period, a cohort study monitored the HL cohort's response to therapy, EBV load and long-term remission status. Pre-treatment quantitative EBV-DNA polymerase chain reaction (PCR) was positive in 19 out of 30 children with Hodgkin's lymphoma cases while all 70 controls were tested EBV quantitative PCR negative. The highest EBV load was 430,000 copies/mL. Out of 19 quantitative EBV-DNA PCR positive children, one child died of advanced disease before starting chemotherapy. The data as obtained by Dinand et al. are presented by the 2 by 2-table (**Table 1**).

Table 1. The relationship between Epstein-Barr virus (EBV) and Hodgkin's lymphoma (HL).

		Hodgkin's lymphoma		
		yes	**no**	
EBV DNA	**yes**	19	0	19
	no	11	70	81
		30	70	100

2.2. Statistical Analysis

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

2.2.1. Conditio sine qua non

The formula of the conditio per quam [15] relationship

$$p\left(\text{EBV DNA} \rightarrow \text{Hodgkin's lymphoma}\right) \tag{1}$$

was used to proof the hypothesis: if EBV infection (EBV DNA) then Hodgkin's lymphoma.

2.2.2. 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

$$p_{Crit} = p_{Calc} \pm \left(z_{Alpha/2} \times \left(\sqrt[2]{\frac{1}{N} \times p_{Calc} \times \left(1 - p_{Calc}\right)} \right) \right) \tag{2}$$

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 – (Alpha/2) quantile of a standard normal distribution corresponding to the significance level alpha. For example, for a 95% confidence level alpha = 0.05 and z is z = 1.96. The Agresti-Coull [16] formula is another approximate method for calculating binomial confidence intervals. The Clopper-Pearson interval [17] 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 [18] too. In this study, we will use *the rule of three* [19] to calculate the confidence interval for p_{Calc}. Briefly sketched, the rule of three can be derived [20] 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 [21] events occur [22]. Under conditions where a certain event did not occur in a sample with N subjects (i. e. $p_{Calc} = 0$) 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

$$p_{lower} = \left(\frac{3}{N} \right) \tag{3}$$

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

$$p_{lower} = 1 - \left(\frac{3}{N} \right) \tag{4}$$

2.2.3. The mathematical formula of the causal relationship k

The mathematical formula of the causal relationship k [23] and the chi-square distribution [24] 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 < 0.05 is considered as significant.

2.2.3. The chi square distribution

The chi-squared distribution [24] 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 2**.

Table 2. The critical values of the chi square distribution (degrees of freedom: 1).

	p-Value	One sided X^2	Two sided X^2
The chi square distribution	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 lymph nodes by Epstein-Bar virus is a *conditio per quam* of Hodgkin's lymphoma

Claims.

Null hypothesis:

An infection of human lymph nodes by Epstein-Bar virus is a conditio per quam of Hodgkin's lymphoma.

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

Alternative hypothesis:

An infection of human lymph nodes by Epstein-Bar virus is not a conditio per quam of Hodgkin's lymphoma.

($p_0 < p_{Crit}$).

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

Proof.

The data of an infection by Epstein-Bar virus and Hodgkin's lymphoma are viewed in the 2 × 2 table (**Table 1**). The proportion of successes in the sample of a conditio per quam relationship p(Epstein-Bar virus DNA → Hodgkin's lymphoma) is calculated [15] as

$$p(\text{EBV DNA} \rightarrow \text{Hodgkin's lymphoma}) = \frac{(19+11+70)}{100} = \frac{100}{100} = 1$$

The critical value p_{Crit} (significance level alpha = 0.05) is calculated [15] approximately as

$$p_{Crit} = 1 - \frac{3}{100} = 0{,}97$$

The critical value is p_{Crit} = 0,97 and is less than the proportion of successes calculated as p(Epstein-Bar virus DNA → Hodgkin's lymphoma) =1. Due to this evidence, we do not

reject the null hypothesis in favor of the alternative hypotheses. The data as published by Dinand et al. [14] do support our Null hypothesis that an infection of human lymph nodes by Epstein-Bar virus is a conditio per quam of Hodgkin's lymphoma. In other words, *if* an infection of human lymph nodes by Epstein-Bar virus *then* Hodgkin's lymphoma.

Q. e. d.

3.2. Epstein-Bar virus is a main cause of Hodgkin's lymphoma

Claims.

Null hypothesis: (no causal relationship)

There is no causal relationship between an infection of human lymph nodes by Epstein-Bar virus and Hodgkin's lymphoma.

Alternative hypothesis: (causal relationship)

There is a causal relationship between an infection of human lymph nodes by Epstein-Bar virus and Hodgkin's lymphoma.

(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 illustrated in the 2 × 2 table (**Table 1**). The causal relationship k(EBV DNA , Hodgkin's lymphoma) is calculated [15], [23] as

$$k(\text{EBV DNA, Hodgkin's lymphoma}) = \frac{((100\times 19)-(30\times 19))}{\sqrt[2]{(30\times 70)\times(19\times 81)}} = +0{,}739814235$$

The value of the test statistic k=+0,739814235 is equivalent to a calculated [15] chi-square value of

$$\chi^2_{\text{Calculated}} = 100\times \frac{((100\times 19)-(30\times 19))}{\sqrt[2]{(30\times 70)\times(19\times 81)}} \times \frac{((100\times 19)-(30\times 19))}{\sqrt[2]{(30\times 70)\times(19\times 81)}}$$

$$\chi^2_{\text{Calculated}} = 100\times 0{,}739814235\times 0{,}739814235$$

$$\chi^2_{\text{Calculated}} = 54{,}7325102881$$

The chi-square statistic, uncorrected for continuity, is calculated as X^2 =54,7325102881 and thus far equivalent to a P value of 0,000000000000138. The calculated chi-square statistic exceeds the critical chi-square value of 3.841458821 (**Table 2**). Consequently, we reject the null hypothesis and accept the alternative hypotheses. There is a highly significant causal relationship between an infection of human lymph nodes by Epstein-Bar virus and Hodgkin's lymphoma (k=+0,739814235, p Value = 0,000000000000138). The result is significant at $p < 0.05$.

Q. e. d.

4. Discussion

In general, it is known that a great proportion of HL tissues is able to harbour EBV within tumour cells. Emerging evidence suggests that EBV is causality related to Hodgkin's lymphoma. There is a highly significant causal relationship between an infection of human lymph nodes by Epstein-Bar virus and Hodgkin's lymphoma (k=+0,739814235, p Value = 0,000000000000138). About 100*(19/30)=63 % of Hodgkin's lymphoma are caused by an Epstein Bar virus. The challenge is to unravel this complexity of the relationship between Epstein Bar virus and Hodgkin's lymphoma by detailed consideration of the function of EBV genes in the appropriate (tumor) cellular context. The hope is justified that this approach has revealed the most fundamental aspects of HL pathogenesis and that the same has paved the way for a more targeted and individual therapies for HL patients.

5. Conclusion

Epstein-Bar virus is a main cause of Hodgkin's lymphoma (k=+0,739814235, p Value = 0,000000000000138).

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