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Epstein Bar Virus—The Cause of Hodgkin's Lymphoma

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Abstract

Objective: Epstein-Barr virus (EBV), a herpes virus which persists in memory B cells in the peripheral blood for the lifetime of a person, is accused to be associated with several malignancies. Hodgkin's lymphoma (HL) has long been suspected to have an Epstein-Barr virus infection as a causal agent. Some recent studies identified an EBV latent infection to a high degree in Hodgkin's lymphoma. However, despite intensive study, the role of Epstein-Barr virus infection in Hodgkin lymphoma remains enigmatic. **Methods:** To explore the cause-effect relationship between EBV and HL and so to understand the role of EBV in HL etiology more clearly, a systematic review and re-analysis of studies published is performed. The method of the conditio per quam relationship was used to proof the hypothesis if Epstein-Barr virus infection (DNA) in human lymph nodes is present then Hodgkin lymphoma is present too. The mathematical formula of the causal relationship k was used to proof the hypothesis, whether there is a cause effect relationship between an Epstein-Barr virus infection (EBV DNA) and Hodgkin lymphoma. Significance was indicated by a p-value of less than 0.05. **Result:** The data analyzed support the Null-hypotheses that if Epstein-Barr virus infection (EBV DNA) is present in human lymph nodes then Hodgkin lymphoma is present too. In the same respect, the studies analyzed provide highly significant evidence that Epstein-Barr virus the cause of Hodgkin lymphoma. **Conclusion:** The findings of this study suggest that Epstein-Barr virus is the cause of Hodgkin's lymphoma besides of the complexity of Hodgkin's disease.

Keywords

Epstein-Barr Virus, Hodgkin's Lymphoma, Causal Relationship

1. Introduction

In 1964, Epstein [1], Barr, and Achong discovered viral particles in lymphoblasts

isolated from a patient with Burkitt's lymphoma, meanwhile known as Epstein-Bar virus. Historically, EBV was the first human cancer virus to be described. This fundamental discovery paved the way for further investigations into the oncogenic potential of viruses. Epstein-Barr virus (EBV), also called human herpes virus 4 (HHV-4), is a ubiquitous double-stranded DNA gamma-1 human herpes virus which infects more than 90% of the world population. EBV can be transmitted from person to person in several ways. 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. Primarily resting memory B cells in peripheral blood are the infected cells which provide a permanent reservoir for the virus. 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 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 and the disruption of normal lymph node [8] architecture. The Sternberg-Reed cells [9] [10] which are pathognomonic for Hodgkin lymphoma (HL) were described over a century ago and origin from B lymphocytes [11]. Several environmental factors have been discussed in the etiology of Hodgkin's disease [12]. Among them viruses like herpes simplex, cytomegalovirus [13] or EBV [14]. The detection of raised antibody titers to EBV [15] 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 al.* [16] 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.

2. Material and Methods

Hodgkin lymphoma (HL) is a deadly disease too. Identifying the cause of Hodgkin's lymphoma has the potential to spare a lot of lives.

2.1. Study of Veronique Dinad *et al.* (2007)

Dinand *et al.* [17] conducted a case control study to investigate the prevalence and significance of Epstein-Barr virus in Hodgkin's and Reed-Sternberg cells in children. EBV detection was performed by immunohistochemistry (IHC) and in situ hybridization (ISH). Dinand *et al.* [17] detected EBV by ISH in 126/135 (93.3%) out of 135 cases, and in none 0/25 (0%) of the control lymph node examined. The data as obtained 2007 by Dinand *et al.* are presented by the 2 by 2-table (**Table 1**).

Novel and modern laboratory techniques [18] such as Southern Blot hybridization, Immunohistochemistry (IHC), In-situ hybridization (ISH), Fluorescent

Table 1. Epstein-Barr virus (EBV) and Hodgkin's lymphoma according to Dinand *et al.* (2007).

		Hodgkin's lymphoma		Total
		yes	no	
EBV DNA (ISH)	yes	126	0	126
	no	9	25	34
	Total	135	25	160

ISH (FISH), RNA in situ hybridization (RNA ISH), Polymerase chain reaction (PCR), Nested PCR, Quantitative polymerase chain reaction (QPCR) have fueled us to change our understanding of the pathogenesis of cancer development. Immunohistochemistry (IHC), introduced by Coons [19] in 1941, is useful in distinguishing between benign and malignant cell populations. Still, a cross-reactivity with cellular proteins is possible which has impact on the specificity of this method. In situ hybridization (ISH) is a fundamental technique, described in the year 1969 by Joseph G. Gall [20] is used commonly for research purposes especially to distinguish virus in tumor cells from virus in non-tumor cells. Despite of numerous advantages, the use of the ISH technique is associated with certain and severe limitations. The skill of the personnel involved in performing and interpreting ISH has influence on the reproducibility and accuracy of this procedure. In situ hybridization (ISH) even if regarded as superior to PCR depend on the target used which has impact on the sensitivity and specificity of this methods. Even the In situ hybridization (ISH) can produce false positive or false negative results.

2.2. Study of Veronique Dinad *et al.* (2015)

Veronique Dinand *et al.* [21] conducted a 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 PCR was positive in 19 out of 30 children with Hodgkin 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 was positive children, one died of advanced disease before starting chemotherapy. The data as obtained 2015 by Dinand *et al.* are presented by the 2 by 2-table (**Table 2**).

2.3. Statistical Analysis

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

2.3.1. Bernoulli Trials

Among some discrete distributions like the hypergeometric distribution, the

Table 2. Epstein-Barr virus (EBV) and Hodgkin's lymphoma according to Dinand *et al.* (2015).

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

Poisson distribution et cetera the binomial distribution is of special interest. Sometimes, the binomial distribution is called the Bernoulli distribution in honor of the Swiss mathematician Jakob Bernoulli (1654 - 1705), who derived the same. Bernoulli trials are an essential part of the Bernoulli distribution. Thus far, let us assume two fair coins named as ${}_0W_t$ and as ${}_RU_t$. In our model, *heads* of such a coin are considered as success T (*i.e.* true) and labeled as +1 while *tails* may be considered as failure F (*i.e.* false) and are labeled as +0. Such a coin is called a *Bernoulli-Boole coin*. The probability of success of ${}_RU_t$ at one single Bernoulli trial t is denoted as

$$p\left({}_RU_t=+1\right)\equiv p\left({}_RU_t\right) \tag{1}$$

The probability of failure of ${}_R\underline{U}_t$ at one single Bernoulli trial t is denoted as

$$p\left({}_RU_t=+0\right)\equiv p\left({}_R\underline{U}_t\right)\equiv 1-p\left({}_RU_t\right) \tag{2}$$

Furthermore, no matter how many times an experiment is repeated, let the probability of a head or the tail remain the same. The trials are independent which implies that no matter how many times an experiment is repeated, the probability of a single event at a single trial remain the same. Repeated independent trials which are determined by the characteristic that there are always only two possible outcomes, either +1 or +0 and that the probability of an event (outcome) remain the same at each single trial for all trials are called *Bernoulli trials*. The definition of Bernoulli trials provides a theoretical model which is of further use. However, in many practical applications, we may by confronted by circumstances which may be considered as approximately satisfying Bernoulli trials. Thus far, let us perform an experiment of tossing two fair coins simultaneously. Suppose two fair coins are tossed twice. Then there are $2^2 = 4$ possible outcomes (the sample space), which may be shown as

$$\left(\left[{}_RU_t=+1\right],\left[{}_0W_t=+1\right]\right),\left(\left[{}_RU_t=+1\right],\left[{}_0\underline{W}_t=+0\right]\right),$$
$$\left(\left[{}_R\underline{U}_t=+0,{}_0W_t=+1\right]\right),\left(\left[{}_R\underline{U}_t=+0,{}_0\underline{W}_t=+0\right]\right)$$

This may also be shown as a 2-dimensional sample space in the form of a contingency table (**Table 3**).

In the following, the contingency table is defined more precisely (**Table 4**).

In general it is $\left(a+c\right)={}_0W_t$, $\left(a+b\right)={}_RU_t$, $\left(c+d\right)={}_0\underline{W}_t$, $\left(b+d\right)={}_R\underline{U}_t$ and $a+b+c+d=N={}_RW_t$. Equally, it is ${}_0W_t+{}_0\underline{W}_t={}_RU_t+{}_R\underline{U}_t={}_RW_t=N$. Thus far, if one fair coin is tossed n times, we have n repeated Bernoulli trials

Table 3. The sample space of a contingency table.

		Conditioned		Total
		Yes = +1	No = +0	
Condition	Yes =+1	$([{}_{R}U_t=+1],[{}_{0}W_t=+1])$	$([{}_{R}U_t=+1],[{}_{0}\underline{W}_t=+0])$	${}_{R}U_t$
	No = +0	$([{}_{R}\underline{U}_t=+0],[{}_{0}W_t=+1])$	$([{}_{R}\underline{U}_t=+0],[{}_{0}\underline{W}_t=+0])$	${}_{R}\underline{U}_t$
	Total	${}_{0}W_t$	${}_{0}\underline{W}_t$	${}_{R}W_t$

Table 4. The sample space of a contingency table.

		Conditioned		Total
		Yes = +1	No = +0	
Condition	Yes = +1	a	b	${}_{R}U_t$
	No = +0	c	d	${}_{R}\underline{U}_t$
	Total	${}_{0}W_t$	${}_{0}\underline{W}_t$	$N={}_{R}W_t$

and an n dimensional sample space with 2^n sample points is generated. In general, when given n Bernoulli trials with k successes, the probability to obtain exactly k successes in n Bernoulli trials is given by

$$p(k)=\binom{n}{k}\times p({}_{R}U_t=+1)^k\times\left(1-p({}_{R}U_t=+1)\right)^{n-k} \tag{3}$$

The random variable k is sometimes called a *binomial variable*. The probability to obtain k events or more (*at least k events*) in n trials is calculated as

$$\begin{aligned} p(k\geq X)&=p(k=X)+p(k>X)\\ &=\sum_{k=X}^{k=n}\left(\binom{n}{k}\times p({}_{R}U_t=+1)^k\times\left(1-p({}_{R}U_t=+1)\right)^{n-k}\right) \end{aligned} \tag{4}$$

The probability to obtain less than k events in n Bernoulli trials is calculated as

$$\begin{aligned} p(k<X)&=1-p(k\geq X)\\ &=1-\sum_{k=X}^{k=n}\left(\binom{n}{k}\times p({}_{R}U_t=+1)^k\times\left(1-p({}_{R}U_t=+1)\right)^{n-k}\right) \end{aligned} \tag{5}$$

2.3.2. Sufficient Condition (Conditio per Quam)

The formula of the conditio per quam [22]-[35] relationship was derived as

$$p(\text{EBV DNA}\rightarrow\text{Hodgkin's lymphoma})\equiv\frac{a+c+d}{N} \tag{6}$$

and used to proof the hypothesis: *if* presence of EBV infection (EBV DNA) *then* presence of Hodgkin's lymphoma.

2.3.3. Necessary Condition (Conditio Sine Qua Non)

The formula of the conditio per quam [22]-[35] relationship was derived as

$$p(\text{EBV DNA}\leftarrow\text{Hodgkin's lymphoma})\equiv\frac{a+b+d}{N} \tag{7}$$

and used to proof the hypothesis: *without* presence of EBV infection (EBV DNA) *no* presence of Hodgkin's lymphoma.

2.3.4. Necessary and Sufficient Condition

The necessary and sufficient condition relationship was defined [22]-[35] as

$$p\left(\text{EBV DNA} \longleftrightarrow \text{Hodgkin's lymphoma}\right) \equiv \frac{a+d}{N} \tag{8}$$

Scholium.

Historically, the notion sufficient condition is known since thousands of years. Many authors testified original contributions of the notion material implication only for Diodorus Cronus. Still, Philo the Logician (~300 BC), a member of a group of early Hellenistic philosophers (the Dialectical school), is the main forerunner of the notion material implication and has made some groundbreaking contributions [36] to the basics of this relationship. As it turns out, it is very hard to think of the "conditio per quam" relationship without considering the historical background of this concept. Remarkable as it is, Philo's concept of the material implications came very close to that of modern concept material implication. In propositional logic, a conditional is generally symbolized as "$p \rightarrow q$" or in spoken language "if p then q". Both q and p are statements, with q the consequent and p the antecedent. Many times, the logical relation between the consequent and the antecedent is called a **material implication**. In general, a conditional "if p then q" is false only if p is true and q is false otherwise, in the three other possible combinations, the conditional is always true. In other words, to say that p is a sufficient condition for q is to say that the presence of p guarantees the presence of q. In particular, it is impossible to have p without q. *If p is present, then q must be present too*. To show that p is not sufficient for q, we come up with cases where p is present but q is not. It is well-known that the notion of a necessary condition can be used in defining what a sufficient condition is (and vice versa). In general, p is a necessary condition for q if it is impossible to have q without p. In fact, the absence of p guarantees the absence of q. **Example (Condition: Our earth)**, without oxygen no fire. Table 5 may demonstrate this relationship.

In contrast to such a point of view, the opposite point of view is correct too. Thus far, there is a straightforward way to give a precise and comprehensive account of the meaning of the term necessary or sufficient condition itself. In other words, **if** fire is present **then** oxygen is present too. Table 6 may demonstrate this relationship.

Table 5. Without oxygen no fire (on our planet earth).

		Fire		Total
		Yes = +1	No = +0	
Oxygen	Yes = +1	a	b	${}_{R}U_{t}$
	No = +0	0	d	${}_{R}\underline{U}_{t}$
	Total	${}_{0}W_{t}$	${}_{0}\underline{W}_{t}$	$N = {}_{R}W_{t}$

Table 6. If fire is present then oxygen is present too (on our planet earth).

		Oxygen		Total
		Yes = +1	No = +0	
Fire	Yes = +1	a	0	${}_{R}U_{t}$
	No = +0	c	d	${}_{R}\underline{U}_{t}$
	Total	${}_{0}W_{t}$	${}_{0}\underline{W}_{t}$	$N={}_{R}W_{t}$

Especially, necessary and sufficient conditions are converses of each other. Still, *the fire is not the cause of oxygen* and vice versa. *Oxygen is note the cause of fire*. In this example before, oxygen is a necessary condition, *a conditio sine qua non*, of fire. A necessary condition is sometimes also called "an essential condition" or a conditio sine qua non. In propositional logic, a necessary condition, a condition sine qua non, is generally symbolized as "$p \leftarrow q$" or in spoken language "**without** p **no** q". Both q and p are statements, with p the antecedent and q the consequent. To show that p is not a necessary condition for q, it is necessary to find an event or circumstances where q is present (*i.e.* an illness) but p (*i.e.* a risk factor) is not. On any view, (classical) logic has as one of its goals to characterize the most basic, the most simple and the most general laws of objective reality. Especially, in classical logic, the notions of necessary conditions, of sufficient conditions of necessary and sufficient conditions et cetera are defined very precisely for a single event, for a single Bernoulli trial t. In point of fact, no matter how many times an experiment is repeated, the relationship of the conditio sine qua or of the conditio per quam which is defined for every single event will remain the same. Under conditions of independent trials this implies that no matter how many times an experiment is repeated, the probability of the conditio sine qua or of the conditio per quam of a single event at a single trial t remain the same which transfers the relationship of the conditio sine qua or of the conditio per quam et cetera into the sphere of (Bio-) statistics. Consequently, (Bio) statistics generalizes the notions of a sufficient or of a necessary condition from one single Bernoulli trial to N Bernoulli trials. However, in many practical applications, we may by confronted by circumstances which may be considered as approximately satisfying the notions of a sufficient or of a necessary condition. Thus far, under these circumstances, we will need to perform some tests to investigate, can we rely on our investigation.

2.3.5. The Central Limit Theorem

Many times, for some reason or other it is not possible to study exhaustively a whole population. Still, sometimes it is possible to draw a sample from such a population which itself can be studied in detail and used to convince us about the properties of the population. Roughly speaking, statistical inference derived from a randomly selected subset of a population (a sample) can lead to erroneous results. The question raised is how to deal with the uncertainty inherent in

such results? The concept of confidence intervals, closely related to statistical significance testing, was formulated to provide an answer to this problem.

Confidence intervals, introduced to statistics by Jerzy Neyman in a paper published in 1937 [37], specifies a range within a parameter, *i.e.* the population proportion π, with a certain probability, contain the desired parameter value. Most commonly, the 95% *confidence interval* is used. Interpreting a confidence interval involves a couple of important but subtle issues. In general, a 95% confidence interval for the value of a random number means that there is a 95% probability that the "true" value of the value of a random number is within the interval. 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 $n = 30$ and more). A formula, justified by the central limit theorem, is known as

$$p_{Crit} = p_{Calc} \pm \left(z_{\text{Alpha}/2} \times \left(\sqrt[2]{\frac{1}{N} \times p_{Calc} \times (1 - p_{Calc})} \right) \right) \tag{9}$$

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 *i.e.* 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 $z = 1.96$. A very common technique for calculating binomial confidence intervals was published by Clopper-Pearson [38]. Agresti-Coull proposed another different method [39] for calculating binomial confidence intervals. A faster and an alternative way to determine the lower and upper "exact" confidence interval is justified by the F distribution [40].

2.3.6. The Rule of Three

Furthermore, an approximate and conservative (one sided) confidence interval was developed by Louis [41], Hanley *et al.* [42] and Jovanovic [43] known as the rule of three. Briefly sketched, the rule of three can be derived from the binomial model. Let π_U denote the upper limit of the one-sided $100 \times (1 - \alpha)\%$ confidence interval for the unknown proportion when in N independent trials **no events occur** [43]. Then π_U is the value such that

$$\pi_U = \left(\frac{-\ln(\alpha)}{n} \right) \approx \left(\frac{3}{n} \right) \tag{10}$$

assuming that $\alpha = 0.05$. In other words, an one-sided approximate upper 95% confidence bound for the true binomial population proportion π, the rate of occurrences in the population, based on a sample of size n where no successes are observed ($p = 0$) is $3/n$ [43] or given approximately by $[0 < \pi < (3/n)]$. The rule of three is a useful tool especially in the analysis of medical studies. Table 7 will illustrate this relationship.

Under conditions where **a certain event did not occur** [41] in a sample with

n subjects (*i.e.* $\boldsymbol{p = 0}$) the interval from 0 to $(-\ln(\alpha)/n)$ is called a $100 \times (1 - \alpha)\%$ confidence interval for the binomial parameter for the rate of occurrences in the population.

Another special case of the binomial distribution is based on a sample of size n where **only successes are observed ($p = 1$)**. Accordingly, the lower limit of a one-sided $100 \times (1 - \alpha)\%$ confidence interval for a binomial probability π_L, the rate of occurrences in the population, based on a sample of size n where only successes are observed is given approximately by $[(1- (-\ln(\alpha)/n)) < \pi < +1]$ or (assuming $\alpha = 0.05$)

$$\pi_L = 1 - \left(\frac{-\ln(\alpha)}{n} \right) \approx 1 - \left(\frac{3}{n} \right) \tag{11}$$

Table 8 may illustrate this relationship.

To construct a two-sided $100 \times (1 - (\alpha))\%$ interval according to the rule of three, it is necessary to take a one-sided $100 \times (1 - (\alpha/2))\%$ confidence interval. In this study, we will use the rule of three [44] too, to calculate the confidence interval for the value of a random number.

Table 7. The one-sided approximate upper $100 \times (1 - \alpha)\%$ confidence bound where no successes ($p = 0$) are observed.

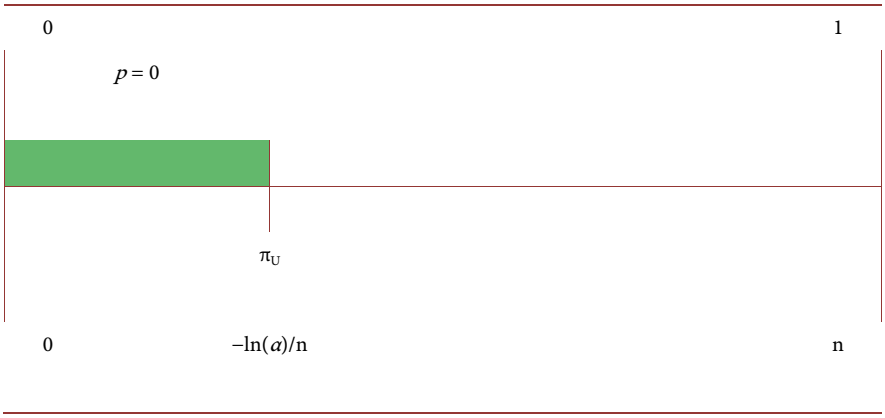
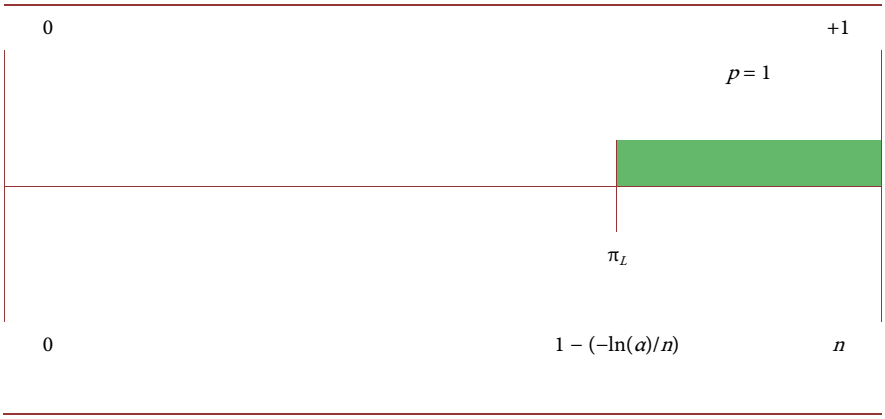


Table 8. The one-sided approximate upper $100 \times (1 - \alpha)\%$ confidence bound where only successes are observed.



2.3.7. Fisher's Exact Test

A test statistics of independent and more or less normally distributed data which follow a chi-squared distribution is valid as with many statistical tests due to the central limit theorem. Especially, with large samples, a chi-squared distribution can be used. A sample is considered as large when the sample size n is $n = 30$ or more. With a small sample ($n < 30$), the central limit theorem does not apply and erroneous results could potentially be obtained from the few observations if the same is applied. Thus far, when the number of observations obtained from a population is too small, a more appropriate test for of analysis of categorical data *i.e.* contingency tables is R. A. Fisher's exact test [45]. Fisher's exact test is valid for all sample sizes and calculates the significance of the p-value (*i.e.* the deviation from a null hypothesis) exactly even if in practice it is employed when sample size is small. Fisher's exact test is called exact because the same uses the exact hypergeometric distribution to compute the p-value rather than the approximate chi-square distribution. Still, computations involved in Fisher's exact test can be time consuming to calculate by hand.

2.3.8. Hypergeometric Distribution

The hypergeometric distribution, illustrated in a table (Table 9), is a discrete probability distribution which describes the probability of a events/successes in a sample with the size ${}_0W_t$, without replacement, from a finite population of the size N which contains exactly ${}_RU_t$ objects with a certain feature while each event is either a success or a failure. The formula for the hypergeometric distribution, a discrete probability distribution, is

$$p(a) = \frac{\binom{{}_RU_t}{a} \times \binom{N - {}_RU_t}{{}_0W_t - a}}{\binom{N}{{}_0W_t}} \tag{12}$$

The hypergeometric distribution has a wide range of applications. The Hypergeometric distribution can be approximated by a Binomial distribution. The elements of the population being sampled are classified into one of two mutually exclusive categories: **either** conditio sine qua non **or** no conditio sine qua non relationship. We are sampling without replacement from a finite population. How probable is it to draw specific $\boldsymbol{c}$ events/successes out of ${}_0W_t$ total draws from an aforementioned population of the size N? The hypergeometric distribution, as shown in a table (Table 10) is of use to calculate how probable is it to obtain $c = ({}_0W_t - a)$ events out of N events.

Table 9. The hypergeometric distribution.

		Conditioned		Total
		Yes = +1	**No = +0**	
Condition	**Yes = +1**	$\boldsymbol{a}$	$b = ({}_RU_t - a)$	$\boldsymbol{{}_RU_t}$
	No = +0	$c = ({}_0W_t - a)$	$N - {}_RU_t - {}_0W_t + a$	$N - {}_RU_t$
	Total	$\boldsymbol{{}_0W_t}$	$N - {}_0W_t$	$\boldsymbol{N}$

Table 10. The hypergeometric distribution and conditio sine qua non.

		Conditioned		Total
		Yes = +1	No = +0	
No Condition	Yes =+1	$c = ({}_0W_t - a)$	$N - {}_RU_t - {}_0W_t + a$	$N - {}_RU_t$
	No = +0	a	$b = ({}_RU_t - a)$	${}_RU_t$
	Total	${}_0W_t$	$N - {}_0W_t$	N

2.3.9. Statistical Hypothesis Testing

A statistical hypothesis test is a method to extract some inferences from data. A hypothesis is compared as an alternative hypothesis. Under which conditions does the outcomes of a study lead to a rejection of the null hypothesis for a pre-specified level of significance. According to the rules of a proof by contradiction, a null hypothesis (H_0) is a statement which one seeks to disproof. The related specific alternative hypothesis (H_A) is opposed to the null hypothesis such that if null hypothesis (H_0) is true, the alternative hypothesis (H_A) is false and vice versa. If the alternative hypothesis (HA) is true then the null hypothesis (H_0) is false. In principle, a null hypothesis that is true can be rejected (type I error) which lead us to falsely infer the existence of something which is not given. The significance level, also denoted as α (alpha) is the probability of rejecting a null hypothesis when the same is true. A type II error is given, if we falsely infer the absence of something which in reality is given. A null hypothesis can be false but a statistical test may fail to reject such a false null hypothesis. The probability of accepting a null hypothesis when the same is false (type II error), is denoted by the Greek letter β (beta) and related to the power of a test (which equals $1 - \beta$). The power of a test indicates *the probability by which the test correctly rejects the null hypothesis* (H_0) *when a specific alternative hypothesis* (H_A) *is true*. Most investigator assess the power of a tests using $\mathbf{1 - \beta} = 0.80$ as a standard for adequacy. A tabularized relation between truth/falseness of the null hypothesis and outcomes of the test are shown precisely within a table (Table 11).

In general, it is $1 - \alpha + \alpha = 1$ or $(1 - \alpha - \beta) + \alpha = 1 - \beta$. Figure 1 may illustrate these relationships.

2.3.10. The Mathematical Formula of the Causal Relationship *k*

The mathematical formula of the causal relationship k [22]-[35] defined as

$$k\left({}_RU_t, {}_0W_t\right) \equiv \frac{\left(\left(N \times a\right) - \left({}_RU_t \times {}_0W_t\right)\right)}{\sqrt[2]{\left({}_RU_t \times {}_R\underline{U}_t\right) \times \left({}_0W \times {}_0\underline{W}_t\right)}} \tag{13}$$

and the chi-square distribution [46] were applied to determine the significance of causal relationship between a EBV and HL. A one-tailed test makes it much 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 less than 0.05 is considered as significant. In this context, what is the necessary connection between a cause and effect? What

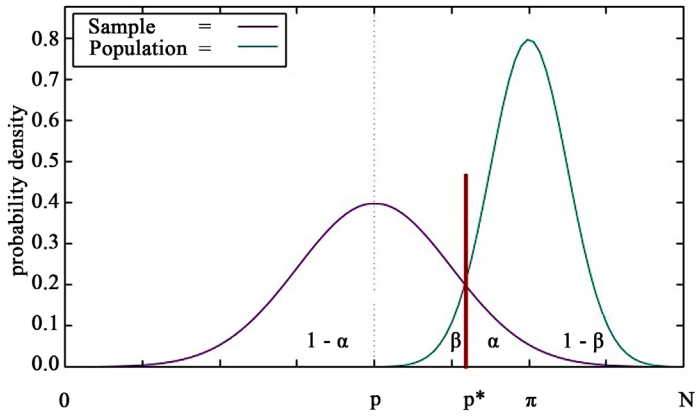


Figure 1. The relationship between error types.

Table 11. Table of error types.

		Null Hypothesis (H_0) is		Total
		True	False	
Null Hypothesis (H_0)	Accepted	$1-\alpha$	β	$1-\alpha+\beta$
	Rejected	α	$1-\beta$	$1+\alpha-\beta$
	Total	1	1	2

ties a cause and its own effect together? Is there a necessary connection between a cause and effect at all? Theoretically, it is neither justified nor necessary to reduce causation as such to an act of observation or measurement. Sill, case-control studies, experiments, observations et cetera can help us to recognize cause effect relationships. In this context it is necessary to stress out that **every single event (effect) has its own cause**, which is the logical foundation of the mathematical formula of the causal relationship k. It is therefore entirely clear that this is the fundamental difference to Pearson's methodological approach. Obviously, although under some certain specified circumstances Pearson's product-moment correlation coefficient [47] or Pearson's Phi [48] coefficient can yield the same numerical result as the mathematical formula of the causal relationship k, there is nothing truly exciting about such a coincidence. Nevertheless, when conducting experiments and analyzing data, views in which correlation and causation are brought very close together are incorrect and worthless. The mathematical formula of the causal relationship k is neither identical nor can the same mathematical formula be reduced to Pearson's product-moment correlation coefficient [47] or to Pearson's Phi [48] Coefficient (Mean Square Contingency Coefficient). In contrast to Pearson's product-moment correlation coefficient and to Pearson's Phi Coefficient (Mean Square Contingency Coefficient) the mathematical formula of the causal relationship k is defined and valid at every single Bernoulli trial t or at every single event. Sir Austin Bradford Hill (1897-1991), an English epidemiologist, proposed 1965 a set of nine criteria (Strength, Consistency, Specificity, Temporality, Biological gradient, Plausibility, Coherence, Experiment, Analogy) [49] to establish epidemiologic evidence of a

causal relationship (Bradford Hill criteria). In point of fact, Bredford's "fourth characteristic is the temporal relationship of the association" [49] and in last consequence the "*post hoc ergo propter hoc*" logical fallacy. Causation cannot be derived from the "*post hoc ergo propter hoc*" [35] logical fallacy. Consequently, the Mathematical Formula of the causal relationship k can neither be reduced to the Bradford Hill criteria nor is the same just a mathematization of Bradford Hill criteria.

2.3.11. The Chi Square Distribution

The chi-squared distribution [46] 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 12**.

2.3.12. The X^2 Goodness of Fit Test

A chi-square goodness of fit test can be applied to determine whether sample data are consistent with a hypothesized distribution. The chi-square goodness of fit test is appropriate when some conditions are met. A view of these conditions are simple random sampling, categorical variables and an expected value of the number of sample observations which is at least 5. The null hypothesis (H_0) and its own alternative hypothesis (H_A) are stated in such a way that they are mutually exclusive. In point of fact, if the null hypothesis (H_0) is true, the other, alternative hypothesis (H_A), must be false; and vice versa. For a chi-square goodness of fit test, the hypotheses can take the following form.

Table 12. 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

H_0: The sample distribution agrees with the hypothetical (theoretical) distribution.

H_A: The sample distribution does not agree with the hypothetical (theoretical) distribution.

The X^2 Goodness-of-Fit Test can be shown schematically as

$$\chi^2 \equiv \sum_{t=+1}^{t=+N}\left(\frac{\left(\text{Observed}_t - \text{Expected}_t\right)^2}{\text{Expected}_t}\right) \tag{14}$$

The degrees of freedom are calculated as $N-1$. If there is no discrepancy between an observed and a theoretical distribution, then $X^2 = 0$. As the discrepancy between an observed and a theoretical distribution becomes larger, the X^2 becomes larger. This X^2 values are evaluated by the known X^2 distribution.

The original X^2 values are calculated from an original theoretical distribution, which is continuous, whereas the approximation by the X^2 Goodness of fit test we are using is discrete. Thus far, there is a tendency to underestimate the probability, which means that the number of rejections of the null hypothesis can increase too much and must be corrected downward. Such an adjustment (*Yate's correction for continuity*) is used only when there is one degree of freedom. When there is more than one degree of freedom, the same adjustment is not used. Applying this to the formula above, we find the X^2 Goodness-of-Fit Test *with continuity correction* shown schematically as

$$\chi^2 \equiv \sum_{t=+1}^{t=+N}\left(\frac{\left(\left|\text{Observed}_t - \text{Expected}_t\right| - \left(\frac{1}{2}\right)\right)^2}{\text{Expected}_t}\right) \tag{15}$$

When the term $(|\text{Observed}_t - \text{Expected}_t|)$ is less than 1/2, the continuity correction should be omitted.

1) The X^2 Goodness of Fit Test of a Sufficient Condition

The theoretical (hypothetical) distribution of a sufficient condition is shown schematically by the 2 × 2 table (Table 13).

The theoretical distribution of a sufficient condition (conditio pre quam) is determined by the fact that $\boldsymbol{b = 0}$. The X^2 Goodness-of-Fit Test *with continuity correction* of a sufficient condition (conditio per quam) is calculated as

Table 13. The theoretical distribution of a sufficient condition (conditio pre quam).

		Conditioned		Total
		Yes = +1	No = +0	
Condition	Yes = +1	a	$\boldsymbol{b = 0}$	$(a + b)$
	No = +0	c	d	$(c + d)$
	Total	$(a + c)$	$(b + d)$	$(a + b + c + d)$

$$\chi^2(\mathrm{IMP}) \equiv \left(\frac{\left(\left| a-(a+b) \right| - \left(\frac{1}{2} \right) \right)^2}{(a+b)} \right) + \left(\frac{\left(\left| (c+d)-(c+d) \right| - \left(\frac{1}{2} \right) \right)^2}{(c+d)} \right) = \left(\frac{\left(\left| a-(a+b) \right| - \left(\frac{1}{2} \right) \right)^2}{(a+b)} \right) + 0 \tag{16}$$

or more simplified as

$$\chi^2(\mathrm{IMP}) \equiv \left(\frac{\left(\left| -b \right| - \left(\frac{1}{2} \right) \right)^2}{(a+b)} \right) + 0 \tag{17}$$

Under these circumstances, the degree of freedom is d.f. = $N-1=2-1=1$.

2) The X^2 goodness of fit test of a necessary condition

The theoretical (hypothetical) distribution of a necessary condition is shown schematically by the 2 × 2 table (Table 14).

The theoretical distribution of a necessary condition (conditio sine qua non) is determined by the fact that $c = 0$. The X^2 Goodness-of-Fit Test *with continuity correction* of a necessary condition (conditio sine qua non) is calculated as

$$\chi^2(\mathrm{SINE}) \equiv \left(\frac{\left(\left| (a+b)-(a+b) \right| - \left(\frac{1}{2} \right) \right)^2}{(a+b)} \right) + \left(\frac{\left(\left| (d)-(c+d) \right| - \left(\frac{1}{2} \right) \right)^2}{(c+d)} \right) = 0 + \left(\frac{\left(\left| d-(c+d) \right| - \left(\frac{1}{2} \right) \right)^2}{(c+d)} \right) \tag{18}$$

or more simplified as

$$\chi^2(\mathrm{SINE}) \equiv \left(\frac{\left(\left| -c \right| - \left(\frac{1}{2} \right) \right)^2}{(c+d)} \right) + 0 \tag{19}$$

Table 14. The theoretical distribution of a necessary condition (conditio sine qua non).

		Conditioned		Total
		Yes = +1	No = +0	
Condition	Yes = +1	a	b	$(a+b)$
	No = +0	$c = 0$	d	$(c+d)$
	Total	$(a+c)$	$(b+d)$	$(a+b+c+d)$

Under these circumstances, the degree of freedom is d.f. = $N-1=2-1=1$.

3) The X^2 goodness of fit test of a necessary and sufficient condition

The theoretical (hypothetical) distribution of a necessary and sufficient condition is shown schematically by the 2 × 2 table (Table 15).

The theoretical distribution of a necessary and sufficient condition is determined by the fact that $\boldsymbol{b=0}$ and that $\boldsymbol{c=0}$. The X^2 Goodness-of-Fit Test *with continuity correction* of a necessary and sufficient condition is calculated as

$$\chi^2\left(\text{Necessary AND Sufficient}\right) \equiv \left(\frac{\left(\left|(a)-(a+b)\right|-\left(\frac{1}{2}\right)\right)^2}{(a+b)}\right)+\left(\frac{\left(\left|(d)-(c+d)\right|-\left(\frac{1}{2}\right)\right)^2}{(c+d)}\right) \tag{20}$$

or more simplified as

$$\chi^2\left(\text{Necessary AND Sufficient}\right) \equiv \left(\frac{\left(\left|-b\right|-\left(\frac{1}{2}\right)\right)^2}{(a+b)}\right)+\left(\frac{\left(\left|-c\right|-\left(\frac{1}{2}\right)\right)^2}{(c+d)}\right) \tag{21}$$

Under these circumstances, the degree of freedom is d.f. = $N-1=2-1=1$.

3. Results

3.1. Epstein-Bar Virus Is a *Conditio sine qua* Non of Hodgkin's Lymphoma

Claims.

Null hypothesis:

An infection of human lymph nodes by Epstein-Bar virus is a conditio sine qua non of Hodgkin's lymphoma.

Alternative hypothesis:

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

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 X^2 Goodness-of-Fit Test *with continuity*

Table 15. The theoretical distribution of a necessary and sufficient condition.

		Conditioned		Total
		Yes = +1	No = +0	
Condition	Yes = +1	a	$\boldsymbol{b=0}$	$(a+b)$
	No = +0	$\boldsymbol{c=0}$	d	$(c+d)$
	Total	$(a+c)$	$(b+d)$	$(a+b+c+d)$

correction of a necessary condition (conditio sine qua non) known to be defined as p (Epstein-Bar virus DNA ← Hodgkin's lymphoma) is calculated as

$$\chi^2(\text{SINE}) \equiv \left(\frac{\left(|-c| - \left(\frac{1}{2} \right) \right)^2}{(c+d)} \right) + 0 = \left(\frac{\left(|-9| - \left(\frac{1}{2} \right) \right)^2}{(9+25)} \right) = 2.125$$

Under these circumstances, the degree of freedom is d.f. = $N-1=2-1=1$. The critical X^2 (significance level alpha = 0.05) is known to be 3.841458821 (Table 12). The calculated X^2 value = 2.125 and less than the critical X^2 = 3.841458821. Hence, our calculated X^2 value = 2.125 is not significant and we accept our null hypothesis. Due to this evidence, we do not reject the null hypothesis in favor of the alternative hypotheses. In other words, the sample distribution agrees with the hypothetical (theoretical) distribution. Our hypothetical distribution was the distribution of the necessary condition. Thus far, the data as published by Dinand *et al.* [17] do support our null hypothesis that an infection of human lymph nodes by Epstein-Bar virus is a conditio sine qua non of Hodgkin's lymphoma. In other words, *without* an infection of human lymph nodes by Epstein-Bar virus *no* Hodgkin's lymphoma.

Q.e.d.

3.2. 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.

($p_0 > p_{Crit}$).

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 [22]-[35] as

$$p(\text{EBV DNA} \to \text{Hodgkin's lymphoma}) = \frac{(126+9+25)}{160} = \frac{160}{160} = 1$$

The critical value p_{Crit} (significance level alpha = 0.05) is calculated [39]-[44] as

$$p_{Crit} = 1 - \frac{3}{160} = 0.981276673$$

The critical value is $p_{Crit} = 0.981276673$ 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.* [17] 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 is present *then* Hodgkin's lymphoma is present too.

Q.e.d.

3.3. Epstein-Bar Virus Is the 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 [22]-[35] as

$$k(\text{EBV DNA, Hodgkin's lymphoma}) = \frac{((160\times126)-(126\times135))}{\sqrt[2]{(126\times34)\times(135\times25)}} = +0.82841687$$

The value of the test statistic k = +0.82841687 is equivalent to a calculated [22]-[35] chi-square value of

$$\chi^2_{\text{Calculated}} = 160\times\left(\frac{((160\times126)-(126\times135))}{\sqrt[2]{(126\times34)\times(135\times25)}}\right)\times\left(\frac{((160\times126)-(126\times135))}{\sqrt[2]{(126\times34)\times(135\times25)}}\right)$$

$$\chi^2_{\text{Calculated}} = 160\times0.82841687\times0.82841687$$

$$\chi^2_{\text{Calculated}} = 109.8039216$$

The chi-square statistic, uncorrected for continuity, is calculated as X^2 = 109.8039216 and thus far equivalent to a P value of 0.000000000000000000000000108179. The calculated chi-square statistic exceeds the critical chi-square value of 3.841458821 (Table 12). 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 = +0.82841687, p Value = 0.000000000000000000000000108179). The result is significant at $p < 0.001$.

Q.e.d.

3.4. Epstein-Bar Virus Is a *Conditio sine qua* Non of Hodgkin's Lymphoma

Claims.

Null hypothesis:

An infection of human lymph nodes by Epstein-Bar virus is a conditio sine qua non of Hodgkin's lymphoma.

Alternative hypothesis:

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

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 2). The X^2 Goodness-of-Fit Test *with continuity correction* of a necessary condition (conditio sine qua non) known to be defined as p (Epstein-Bar virus DNA ← Hodgkin's lymphoma) [22]-[35] is calculated as

$$\chi^2(\text{SINE}) \equiv \left(\frac{\left(|-c| - \left(\frac{1}{2} \right) \right)^2}{(c+d)} \right) + 0 = \left(\frac{\left(|-11| - \left(\frac{1}{2} \right) \right)^2}{(11+70)} \right) = 1.361111111 \qquad (22)$$

Under these circumstances, the degree of freedom is d.f. = $N-1=2-1=1$. The critical X^2 (significance level alpha = 0.05) is known to be 3.841458821 (Table 12). The calculated X^2 value is 1.361111111and is less than the critical X^2 = 3.841458821. Hence, our calculated X^2 value is 1.361111111 and is not significant and we accept the null hypothesis. Due to this evidence, we do not reject the null hypothesis in favor of the alternative hypotheses. In other words, the sample distribution agrees with the hypothetical (theoretical) distribution. Our hypothetical distribution was the distribution of the necessary condition. Thus far, the data as published by Dinand *et al.* [21] do support our null hypothesis that an infection of human lymph nodes by Epstein-Bar virus is a conditio sine qua non of Hodgkin's lymphoma. In other words, *without* an infection of human lymph nodes by Epstein-Bar virus *no* Hodgkin's lymphoma.

Q.e.d.

3.5. 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.

($p_0 > p_{Crit}$).

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 2**). The proportion of successes in the sample of a conditio per quam relationship p(Epstein-Bar virus DNA → Hodgkin's lymphoma) is calculated [22]-[35] as

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

The critical value p_{Crit} (significance level alpha = 0.05) is calculated [39]-[44] 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.* [21] 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.

3.6. Epstein-Bar Virus Is the 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 2). The causal relationship k (EBV DNA, Hodgkin's lymphoma) is calculated [22]-[35] as

$$k\left(\text{EBV DNA, Hodgkin's lymphoma}\right) = \frac{\left(\left(100\times 19\right)-\left(30\times 19\right)\right)}{\sqrt[2]{\left(30\times 70\right)\times\left(19\times 81\right)}} = +0.739814235$$

The value of the test statistic $k = +0.739814235$ is equivalent to a calculated [22]-[35] chi-square value of

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

$$\chi^2_{\text{Calculated}} = 100\times 0.739814235\times 0.739814235$$

$$\chi^2_{\text{Calculated}} = 54.7325102881$$

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 12). 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 $p < 0.001$.

Q.e.d.

4. Discussion

A case-control study or a retrospective study is a type of an observational study where investigators compare a set of people with a certain disease (*the cases*) and a set of people with all but this certain disease (*the controls*) with regard to a special condition, cause or factor. Case-control studies usually require a smaller sample sizes than equivalent cohort studies and are cheap and quick. As a consequence, many factors, conditions or causes can be studied simultaneously. Still, etiological questions are ideally studied not through the case-control approach. A cohort study is a better type of an observational study to investigate etiological hypothesis, especially when a study population, which is free of a disease, is used at the outset. By contrast to a case-control study, in a cohort study, it is investigated whether a disease develops or not. In particular, a case-control study may provide data which are inaccurate under certain circumstances and is very likely to suffer from bias error. Among many source of bias, the problems arise especially from the way how controls are sampled with the consequence that the data as collected in a case-control study may not be appropriate to perform some causal investigations of interest. To be persuasive, case-control studies need to be conducted very carefully. Further details about case control studies are given

by secondary literature [50]. Despite of the view disadvantages of case-control studies discussed above, Dinand *et al.* [17] [21] detected EBV DNA by ***in situ* hybridization (ISH)**, which cannot be ignored. The in situ hybridization, like any method, is not completely free of bias and is labeled with some severe limitations. Still, the in situ hybridization provides the opportunity to distinguish EBV DNA in tumor cells from EBV DNA in non-tumor cells. In general, it is known that a great proportion of HL tissues is able to harbor EBV within tumor cells. Emerging evidence suggests that EBV is causality related to Hodgkin's lymphoma. According to the data as published by Dinand *et al.* [17], *without* an infection of human lymph nodes by Epstein-Bar virus *no* Hodgkin's lymphoma. In the same context, there is a highly significant causal relationship between an infection of human lymph nodes by Epstein-Bar virus and Hodgkin's lymphoma (k = +0.82841687, p-value = 0.00000000000000000000000108179). Thus far, Epstein-Bar virus is not only a cause, but the cause of Hodgkin's lymphoma. 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. Even if EBV is the cause of HL, many times a specific therapy is not indicated for most patients with an EBV infection. In point of fact, some drugs, i. e. acyclovir, are able to inhibit EBV replication and to reduce viral shedding. Sometimes, corticosteroid therapy is considered for patients with severe complications of an EBV infection and is able to shorten the duration of some symptoms and fever associated with an EBV infection. In point of fact, vaccination against EBV might be useful especially for people who are seronegative for EBV. In this context, preliminary studies in which EBV-seronegative children were vaccinated with vaccinia virus expressing gp350 where to some extent effective [51] [52].

5. Conclusion

Epstein-Bar virus is the cause of Hodgkin's lymphoma (k = +0.82841687, p Value = 0.00000000000000000000000108179).

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