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Predictability of the Test Methods Used in the Detection for Hepatitis B Virus Infection in a Defined Population[1]

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= Abstract = Predictability for positive (or negative) of the three test protocols (RPHA only; RPHA-PHA; RPHA-PHA-CORAB), which were commonly used in assessing hepatitis B virus (HBV) infection, were evaluated with the reference method of RIA for HBsAg, anti-HBc and anti-HBs in 123 adults in Seoul. RPHA-PHA-CORAB and RPHA-PHA protocols were of great value in estimating prevalence, especially when observed value is obtained from a general population with known sensitivity and specificity indices. Meanwhile, for the purpose of early detection of infection or to select the candidate of vaccination or to select the interviewee to elucidate risk factors of HBV infection, RPHA-PHA-CORAB protocol was the most recommended in detecting HBV infection, because of its high predictability of both positive and negative test results.

Key words: ***Screening test, Sensitivity, Specificity, Predictability, Hepatitis B virus, Radioimmunoassay, Reversed passive hemagglutination, Passive hemagglutination***

INTRODUCTION

In the situation of introducing a detection method with low accuracy, problems of false positive and false negative results are not avoidable in many circumstances. To get an unbiased value of a specific disease from a population, it is very important to adjust the test results from the data oberved, especially in case of observing large population. For the reason of its unstability over prevalence of the disease in different populations, predictability, not sensitivity and specificity, for a positive (or negative) test result should be used in the analysis of any screening tests (Fleiss 1981; Ko 1982).

There are many studies on hepatitis B virus (HBV) infection to observe the level of HBV infection (Hong and Kim 1982; Ahn *et al.* 1983; Choi 1986; Park *et al.* 1987; Lee *et al.* 1987; Yoo *et al.* 1988); to select candidates for vaccination or to test vaccination efficacy (Kim *et al.* 1985; Ahn *et al.* 1987); to elucidate the risk factors of HBV infection (Ahn and Yoo 1983; Oh and Kim 1985; Park *et al.* 1986; Ahn *et al.* 1987). It is a well known fact, as many authors indicated, that reversed passive hemagglutination (RPHA) method for HBsAg and/or passive hemagglutination (PHA) method for anti-HBs have relatively low accuracy-sensitivity and specificity-than radioimmunoassay (RIA) method in detecting HBV infection (Kim *et al.* 1984; Park 1987). Nevertheless, most of the study results were from those without using such methods as of high accuracy and high cost.

Predictabilities for positive (or negative) of the three detection protocols (RPHA only; RPHA-PHA; RPHA-PHA-CORAB), which were commonly used in assessing HBV infection, were evaluated in simulation with the reference method of RIA for HBsAg, anti-HBc and anti-HBs in a general adult population in Seoul.

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MATERIALS AND METHODS

1. Study population and data collection

Study population was drawn from the insured population of Korean Medical Insurance Corporation, living in Seoul. Among those target population of being checked for two-year-period health examination done by KMIC, 123 adults were randomly selected and entered into the study population. Age and sex distribution was as shown in Table 1.

Every study population checked her/his health status including liver function at the designated medical facilities nearest their office. Sera collected were refrigerated below −20°C. All samples were tested for HBsAg (AUSRIA®, Abbott Lab.), anti-HBc (CORAB®, Abbott Lab.) and anti-HBs (AUSAB®, Abbott Lab.) by radioimmunoassay method as a reference method in assessing validity. At the same time every sera were also tested for HBsAg by reversed passive hemagglutination method (Hepa S-Ag Test®, Green Cross) and for anti-HBs by passive hemagglutination (Hepa S-Ab Test®, Green Cross) with its titer. All tests were done by a well-trained medical technician at The Institute of Liver Diseases in College of Medicine, Seoul National University.

Table 1. Age and sex distribution of study population

Age	Male	Female	Both
20 — 29	9	11	20
30 — 39	35	10	45
40 — 49	38	2	40
50 — 59	16	2	18
Total	98	25	123

2. Data analysis

HBV infection was defined as positive for at least one seromarker by any test methods (Mushahwar *et al.* 1974). Susceptibles to HBV were accordingly defined as negative for all seromarkers. With the reference methods of RIA (AUSRIA, CORAB, AUSAB), predictabilities of three protocols were measured in simulation; 1) RPHA only; 2) combined protocol of RPHA and PHA; 3) RPHA and PHA with CORAB (only if both RPHA and PHA were negative).

Fixing the cut-off value for positive of PHA at 2^2 (Park 1987), every analysis was done when the cut-off value for positive of RPHA was set at 2^1 or 2^2, separately. Statistical significance test of every 2×2 contingency table were done by χ^2-test and Fisher's exact method.

3. Comparison of estimated value with true prevalence

Letting D and T represent the events 'person has disease' and 'person response positive', respectively, the following quantities can be defined (Yerushalmy 1947);

$$\alpha = \Pr(T \mid D) = \text{sensitivity}$$
$$\beta = \Pr(\bar{T} \mid \bar{D}) = \text{specificity}.$$

When test results are introduced in a large population, the predictive value of a positive (or negative) test result may be defined as a proportion of diseased (or nondiseased) persons with positive (or negative) test. This may be calculated as follows (Vecchio 1966; Fleiss 1981);

$$p^+ = \Pr(D \mid T) = \text{positive predictive value}$$
$$p^- = \Pr(\bar{D} \mid \bar{T}) = \text{negative predictive value}.$$

And also,

$$t = \Pr(T) = \text{test positivity}$$
$$pp = \Pr(D) = \text{point prevalence concerned}.$$

The latter four quantities can be expressed as follows;

$$t = (\alpha \times pp) + (1 - \beta)(1 - pp)$$
$$p^+ = (\alpha \times pp) / t$$
$$= \frac{(\alpha \times pp)}{(\alpha \times pp) + (1 - \beta)(1 - pp)}$$
$$pp = (t + \beta - 1) / (\alpha + \beta - 1).$$

Thus, if α and β are known, an estimate of point prevalence can be estimted as follows (Rogan and Gladen 1978);

$$\hat{pp} = (\hat{t} + \beta - 1) / (\alpha + \beta - 1).$$

This is an unbiased estimate with variance;

$$\mathrm{Var}(\hat{pp}) = \frac{t \times (1 - t)}{N \times (\alpha + \beta - 1)^2}$$

Using these equations, estimated prevalence, $\hat{pp}$ was calculated with its 95% confidence interval, which was compared to true point prevalence, pp.

Table 2. Relative frequency of HBV serologic markers tested by radioimmunoassay method

Serologic profile			Male		Female		Both	
HBsAg	anti-HBc	anti-HBs	No.	%	No.	%	No.	%
(+)	–	–	1	1.0	1	4.0	2	1.6
(+)	(+)	–	8	8.2	1	4.0	9	7.3
(+)	(+)	(+)	2	2.0	1	4.0	3	2.4
–	–	(+)	3	3.1	4	16.0	7	5.7
–	(+)	–	9	9.2	2	8.0	11	8.9
–	(+)	(+)	60	61.2	9	36.0	69	56.1
–	–	–	15	15.3	7	28.0	22	17.9
Total			98	100.0	25	100.0	123	100.0

RESULTS

1. Profiles of HBV serologic markers by RIA

As is shown in Table 2, serologic profiles of HBV markers tested by RIA were diverse. Overall positive rate of HBsAg was about 11%, anti-HBc about 74%, and anti-HBs about 64%. These values were not so different from those of actual population (Yoo *et al.* 1988).

2. Predictive values of various protocols for the detection of HBV infection

In the first protocol of introducing RPHA only, positive predictability was 83.6% and 88.5%, respectively, when cut-off value of RPHA test results for positive was at 2^1 and 2^2; in general, cut-off value of 2^1 was commonly used for positive (Table 3). In terms of false positivity, these findings referred to that the proportion of people, among those responding positive and who were actually free of disease, was 16.4% and 11.5%, respectively. It appeared to be of value to use in mass screening for the detection of HBV infection. However, such a low negative predictability as 30.3% and 19.6%; that is, false negative rate of 69.2% and 80.4% prevent this protocol from applying for the detection of HBV infection.

In contrast, RPHA and PHA protocol has better predictive value in the detection of HBV infection. As is shown in Table 3, ability of RPHA-PHA protocol to predict the true (reference) positivity was as high as 88.9%, with its cut-off value at 2^1, and moving up near to perfect level in predicting HBV infection status by RIA, with cut-off value at ^{2}. But the higher rate of false negative than the next protocol, 20.0% and 41.7%, respectively, would still act as a constraint in applying this protocol on mass or any other screening, which appeared to be due to omission of anti-HBc detection method.

Results of the third protocol, RPHA-PHA-CORAB, were also shown in Table 3. This was designed

Table 3. Comparison of predictability of various test methods for the detection of HBV infection[1)]

Screening methods	Cut-off value for positive of RPHA							
	2^1				2^2			
	Positive P.V.[2)](%)	Negative P.V.[3)](%)	χ^2	p-value	Positive P.V.[2)](%)	Negative P.V.[3)](%)	χ^2	p-value
RPHA only	83.6	30.8	1.7	N.S.	88.5	19.6	0.8	N.S.
RPHA -PHA	88.9	80.0	18.9	$p<0.01$	99.8	58.3	48.9	$p<0.01$
RPHA-PHA -CORAB[4)]	89.0	100.0	24.9	$p<0.01$	99.8	93.3	90.3	$p<0.01$

[1)] HBV infection was defined as positive for at least one marker.

[2)] Positive predictive value is the proportion of people, among those responding positive, who are actually responding positive by reference method (RIA).

[3)] Negative predictive value is the proportion of people, among those responding negative, who are actually responding negative by reference method (RIA).

[4)] Tet result in case of introducing RPHA for HBsAg, PHA for anti-HBs and CORAB for anti-HBc, which were introduced only to those negative for both RPHA and PHA.

Table 4. Comparison of test positivity, estimated prevalence and true prevalence of HBV infection[1] by various test methods

Screening methods	Sensitivity α (%)	Specificity β (%)	Test positivity $\hat{t}$(%)	Estimated $\hat{pp}$[2](%)	True pp[3](%)
⟨cut-off value for positive of RPHA=2^1⟩					
RPHA only	91.1	18.2	89.4 (83.6-95.2)	81.7 (23.2-100.0)	82.1 (74.9-89.3)
RPHA -PHA	99.0	25.0	95.6 (91.4-99.8)	85.8 (70.0-100.0)	85.8 (78.9-92.7)
RPHA-PHA -CORAB[4]	100.0	25.0	96.5 (92.7-100.0)	86.0 (72.4-99.6)	85.8 (78.9-92.7)
⟨cut-off value for positive of RPHA=2^2⟩					
RPHA only	22.8	86.4	22.1 (14.1-29.8)	92.4 (12.7-100.0)	82.1 (74.9-89.3)
RPHA -PHA	89.7	87.5	78.8 (70.8-86.8)	85.9 (76.1-95.7)	85.8 (78.9-92.7)
RPHA-PHA -CORAB[4]	99.0	87.5	86.7 (80.0-93.4)	85.8 (78.5-93.1)	85.8 (78.9-92.7)

[1] HBV infection was defined as positive for at least one marker.

[2] Estimated prevalence was calculated from the following equation;

$$\hat{pp} = (\hat{t} + \beta - 1)/(\alpha + \beta - 1).$$

[3] True prevalence of HBV infection measured directly by three RIA methods.

[4] Test result in case of introducing RPHA for HBsAg, PHA for anti-HBs and CORAB for anti-HBc, which were introduced only to those negative for both RPHA and PHA.

to compensate for the disadvantage of RPHA-PHA application. Positive and negative predictability were 89.0% and 100.0%, respectively, being almost perfect in predicting the true value of HBV infection, with the cut-off value of RPHA for positive at 2^1. With the cut-off value of 2^2, 99.8% of positive predictability and 93.3% of negative predictability were resulted, which told us that this protocol was the most recommended in detecting HBV infection.

3. Comparison of estimability of various protocols in estimating true prevalence

Table 4 shows simulated estimabilities of various protocols in estimating the true prevalence in terms of test positivity based on predetermined sensitivity and specificity; estimated prevalence from the equation of test positivity, sensitivity and specificity; and true prevalence from the results of HBV infection by RIA. As a whole, test positivity of each protocol was always higher than true prevalence, when cut-off value of RPHA for positive was at 2^1, which was due to relatively low sensitivity and/or specificity; conversely, when raising up to 2^2, test positivity underestimated the true value, except RPHA-PHA-CORAB

Using the equation presented above, estimated prevalence of RPHA only slightly underestimated the true prevalence, when cut-off value was at 2^1, and overestimated when at 2^2, which indicates that the test method is invalid in mass screening. While, the results of prevalence estimated by RPHA-PHA and RPHA-PHA-CORAB were nearly identical to the true values, regardless of its cut-off value.

DISCUSSION

The validity of a test in distinguishing diseased from nondiseased persons can be defined by its sensitivity and specificity. The advantage of these two characteristics is that they are constant across the different populations with different prevalences (Yerushalmy 1947). When such a test is to be used in a large population, however, it is important to know what its predictive value will be. This cannot be estimated directly from sensitivity and specificity, which used to be obtained in the preliminary test evaluation, even in laboratory setting (Vecchio 1966; Rogan and Gladen 1973). It is, therefore,

important to consider and adjust the measurements whenever the overall prevalence of HBs antigenemia or HBV infection rate in a defined population is to be measured through the screening method of imperfect validity. Results will apt to be overestimating or underestimating the true parameters, in the long run.

RPHA has only been once applied to observe HBV infection level or to determine whether a person be infected or not, i.e. to select adequate blood donors. This seromarker, however, as is indicated in the results, was too invalid to estimate the parameter (HBV infection rate) in a population. It should not be used in general purpose, any way, except for a special circumstance such as follow-up of chronic carrier, which need only the detection ability of high positive predictability, regardless of its negative predictability.

Still in recent years, RPHA and PHA protocol was the choice of mass screening method in estimating HBV infection or in the study of risk factor approach, because of its relatively high validity with its low cost. But most of the studies using RPHA and PHA have failed to correct their HBV infection rate. They did not need to adjust positive rate to the form of estimated prevalence since most of the study population was not so randomly selected, thus those values could not be transformed.

Fortunately, the results of this study can solve the problem because our study population is from the general, not from the hospital population, and because these results were from the simultaneous observation of five different detection methods (AUSRIA and RPHA for HBsAg, AUSAB and PHA for anti-HBs, CORAB for anti-HBc).

In conclusion, RPHA-PHA-CORAB and RPHA-PHA protocols are of great value in estimating prevalence, especially when the observed value is obtained from a general population with known sensitivity and specificity indices. Meanwhile, for the purpose of early detection of infection or to select candidates of vaccination or to select the interviewee to elucidate risk factors of HBV infection, RPHA-PHA-CORAB is the best recommendable protocol, because of its high predictability of both positive and negative test results.

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= 국문초록 =

B형간염 바이러스 감염의 판정에 사용되는 검사법의 예측도

서울대학교 의과대학 예방의학교실

유근영

인구집단을 대상으로 B형간염 바이러스 (HBV)의 감염수준을 측정하는 경우나, 개개인을 대상으로하여 예방접종 대상을 선정하는 경우, 헌혈혈액을 검색하는 경우, 위험요인 규명을 위한 감염자 선별의 경우, 그 결과를 해석함에 있어 주의하여야 할 사항은 '적용검사법이 진정한 의미의 양성 (혹은 음성)을 얼마나 반영하는 주는가?'의 예측도를 측정하는 것이다. 왜냐하면 검사법의 민감도와 특이도로 대변되는 정확도는 인구집단의 유병률에 의해 병동되지 않는 반면에, 예측도는 민감도 및 특이도와 더불어 대상집단의 유병률에 의해 크게 영향을 받기 때문이다.

본 연구는 HBV 감염여부를 판정하는데 흔히 이용되고 있는 몇가지 유형의 검사법 (RPHA 단독, RPHA-PHA, RPHA-PHA-CORAB)의 예측도를 일정 유병수준을 지닌 일반인구집단에서 평가하고자 성인 일반인구 123명으로부터 혈청을 수집하여 5가지의 검사법, 즉 RPHA (Reversed Passive Hemagglutination for HBsAg); PHA(Passive hemagglutination for anti-HBs); AUSRIA(Radioimmunoassay for HBsAg); CORAB(Radioimmunoassay for anti-HBc); AUSAB(Radioimmunoassay for anti-HBs)을 동일 시료에 대해 동시에 적용한 후, 그 결과를 상기한 세가지의 가상적 유형에 따라 분석하였다. 그 결과, RPHA 단독적용은 낮은 음성예측도 때문에 만성보균상태의 추적조사등 특수한 경우 이외에는 감염 측정법으로서의 가치가 없다고 평가되었다. 반면에 인구집단의 HBV 감염수준을 평가하는 경우에는 RPHA-PHA-CORAB 뿐만아니라 RPHA-PHA방법의 적용도 진정한 모수를 매우 근접적으로 추정할 수 있는 검사법이라 판단되었으나, RPHA-PHA 경우는 비교적 낮은 음성예측도를 가지고 있기 때문에 개개인의 감염상태를 보다 정확히 판정하기 위하여는 RPHA 및 PHA법과 더불어 이들 모두에 음성인 경우에 한하여 CORAB을 추가하는 검사법이 권장된다.