นิพนธ์ต้นฉบับ

The "e" determinant in chronic HBV carriers

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The prevalence of hepatitis B surface antigen and hepatitis "e" antigen was studied among voluntary new and regular blood donors in 1990-1991. The study revealed that 6.9% of 57,186 new donors in 1990 and 6.5% of 74,355 new donors in 1991 were found to have had hepatitis surface antigen in their serum for more than six months. Among the regular donors, the hepatitis "s" antigen was found in only 1.53% a nd 1.49% of donors in 1990 and 1991, respectively.

The presence of hepatitis "e" antigen among those asymptomatic long-term carriers of HBsAg was about 40%. There was no difference in sex observed between HBsAg carriers with and without the "e" antigen. The characteristics of HBeAg carriers included a higher percentage of younger, single, lower income and student compared with the HBV carriers without the "e" antigen. Liver function tests in hepatitis "e" antigen carriers showed a higher reverse A/G ratio, elevated levels of aspartate (AST) and alanine aminotransferase (ALT) as well as alkaline phosphatase. The difference in all of the tests between the two groups was statistically significant, which suggested that the "e" antigen might be a marker for persisting hepatitis dysfunction and a higher level of HBV infectivity.

The statistically significant differences in age and LFT between HBV carriers with and without the "e" determinant, together with the "e" antigen, were found more frequently in HBV carriers in the younger age group (about 20 years of age), which may explain the prevalence of hepatoma in the next two to three decades.

Key words : *HBsAg, HBeAg, Chronic HBV carrier, Blood donors, Prevalence.*

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ผลการตรวจคัดกรองหา HBsAg ในผู้บริจาคโลหิตให้แก่ศูนย์บริการโลหิตแห่งชาติสภากาชาดไทย จำนวน 139,208 ราย ในปี 1990 และ 167,221 ราย ในปี 1991 พบความชุกของพาหะไวรัสตับอักเสบบี ในชายมากกว่าในหญิง ทั้งในผู้บริจาครายใหม่ และผู้บริจาคประจำของทั้งสองปี อัตราการตรวจพบพาหะไวรัสตับอักเสบรวม (ทั้ง 2 เพศ) ในผู้บริจาครายใหม่ของทั้ง 2 ปี ใกล้เคียงกัน เช่นเดียวกับอัตราการติดเชื้อใหม่ที่พบในผู้บริจาคโลหิตประจำของทั้ง 2 ปี ไม่แสดงการเปลี่ยนแปลงมากนัก (1.5%) เปอร์เซ็นต์ของการตรวจพบเชื้อ HBsAg ในผู้บริจาคเลือดรายใหม่สูงกว่าผู้บริจาคเลือดประจำประมาณ 4-5 เท่า

ในผู้บริจาคโลหิตที่ตรวจพบเชื้อไวรัส "บี" ที่ได้จดหมายแนะนำให้มาพบแพทย์ที่หน่วยปรึกษาโรคตับอักเสบ ศูนย์บริการโลหิตแห่งชาติทุกรายจะได้รับการตรวจเช็คแอนติเจนต่าง ๆ ของไวรัสบีในเลือด (Seromarkers) พร้อมกับการตรวจเลือดดูหน้าที่ของตับ (LFT) ในครั้งแรกที่มา, 6 เดือนต่อมา และต่อไปทุกปี ผู้เป็นพาหะของโรคไวรัสตับอักเสบบี มากกว่า 6 เดือน คือตัวอย่างของการศึกษานี้ ซึ่งจะถูกแยกออกเป็น 2 กลุ่ม คือ กลุ่มที่ ตรวจพบ 'e" แอนติเจนและไม่พบ "e" แอนติเจน ผลของการศึกษาเลือดดูหน้าที่ตับใน ครั้งแรกจะถูกนำมาเปรียบเทียบกันใน 2 กลุ่มดังกล่าวนี้

ความชุกของการตรวจ "e" แอนติเจนในพาหะไวรัสตับอับเสบบี พบได้ร้อยละ 39.5 จากการตรวจเลือดดูหน้าที่ของตับทั้ง A/G ratio, SGOT, SGPT และ Alkaline Phosphatase พบว่าในกลุ่มพาหะที่ตรวจพบ "e" แอนติเจน มีเปอร์เซ็นต์ ของผู้ที่ตรวจ พบทั้งค่า SGOT, SGPT สูงกว่าปกติ (>38 u), สูงกว่าปกติสองเท่าขึ้นไป (≥ 76 u) และสูงกว่าปกติสามเท่าขึ้นไป (≥ 114 u) มากกว่าอีกกลุ่มหนึ่งอย่างมีนัยสำคัญทางสถิติ นอกจากนั้นยังพบว่าในกลุ่มพาหะที่มี "e" แอนติเจนด้วยมีเปอร์เซ็นต์ของ reverse A/G Ratio มากกว่าอีกกลุ่มหนึ่ง และมีค่าเฉลี่ยของค่า Alkaline Phosphatase สูงกว่าอีกกลุ่มอย่างมีนัยสำคัญ ส่วนค่า ∞ - feto protein นั้นมีความแปรปรวนมากในทั้ง 2 กลุ่ม โดยเฉพาะในกลุ่มพาหะที่ไม่มี "e" แอนติเจน จึงไม่ได้ทำการประเมินความแตกต่างของค่านี้ในทั้ง 2 กลุ่มในการศึกษานี้

Thailand is a high endemic area of HBV infection : the average prevalence of HBV infection reported in 1970-1980 was 7.35%.[1]

Although many studies on HBV carriers in Thailand have been carried out, only a few studies on hepatitis "e" antigen have been reported. It is well known that some of the hepatitis B surface antigen carriers may also have the "e" antigen which is a soluble, non-particulate antigen; it is immunologically and biochemically distinct from the hepatitis "s" and "c" antigens. It is generally agreed that the hepatitis "e" antigen is associated with the HBV virion and it indicates infectivity which is shown by vertical transmission of HBeAg from carrier mothers to their offspring.[2]

Four years ago, a counselling unit was established at the National Blood Centre, Thai Red Cross Society, for general counselling and to study the risk factors for HBV carriers[3] and for follow-up study on HBV carriers related to various aspects such as changes in liver function test in HBV carriers with and without the "e" antigen, disappearance of the "e" antigen, "s" antigen etc.

About 6% of all blood donors in whom HBsAg could be detected in their blood visited the clinic on referral. (HBsAg positive donors are given appointments to the clinic every six months in the first year with yearly appointments thereafter). The objective of this study is to determine the prevalence of the "e" determinant in HBV carriers identified from among voluntary non-remunerated blood donors. These carriers would have a great variety of characteristics such as differences in age, marital status, occupation, etc., which would form a large pool representing the general population, and enable the study of liver function tests in hepatitis "e" antigen carriers in comparison with the HBsAg carriers not having the "e" antigen.

Patients and methods

All voluntary non-remunerated blood donors in 1990-1991 whose sera were detected as having hepatitis B surface antigen received a letter urging them to attend the counselling clinic at the National Blood Centre, Thai Red Cross Society. At the clinic, blood was drawn initially to identify the seromarkers of HBV infection and for liver function tests (A/G ratio, SGOT, SGPT, alkaline phosphatase), as well as ∞ - fetoprotein. All these laboratory tests would be done routinely on the next visit six months later in the first year and yearly thereafter. The LFT data presented in this report were compiled from the subjects' first visit to the clinic.

A carrier of HBsAg was defined as any individual from whom two HBsAg positive in serum samples were obtained six or more months apart. In this study, there were altogether 663 chronic HBV carriers, all of whom were asymptomatic.

All sera were tested for HBsAg, anti-HBs, "e" antigen and antibody "e" by using the EIA method from Abbotts Laboratories.

Results

Table 1 shows that the total prevalence rates of HBsAg among new donors and regular donors were 6.94% and 1.55% in 1990 and 6.47% and 1.49% in 1991, respectively. The rate of HBsAg found in new donors of both sexes was about 4-5 times higher than that found in regular donors of both sexes in both years. The prevalence of HBV carriers among new male (8.1%) and female (4.3%) donors in 1990 was higher than in 1991 (7.65% in males and 4.07% in females).

Table 1. Prevalence of HBsAg in non-remunerated blood donors in 1990 and 1991 by type and by sex of donors.

	1990			1991		
	Males	Females	Total	Males	Females	Total
	No. tested (% Pos)	No. tested (% Pos)	No. tested (% Pos)	No. tested (% Pos)	No. tested (% Pos)	No. tested (% Pos)
New donors	39,602 (8.10)	17,584 (4.33)	57,186 (6.94)	49,873 (7.65)	24,482 (4.07)	74,355 (6.47)
Regular donors	63,433 (1.74)	18,589 (0.79)	82,022 (1.53)	69,816 (1.72)	23,050 (0.78)	92,866 (1.49)
Total	**103,035 (4.19)**	**36,173 (2.51)**	**139,208 (3.75)**	**119,689 (4.20)**	**47,532 (2.48)**	**167,221 (3.71)**

Hepatitis B "e" antigen (HBeAg) was found in 262 (39.5%) of 663 carriers of hepatitis surface antigen who were tested (table 2). The "e" determinant was detected in 30% of HBV carriers < 20 years of age and is 3.8% of those > 40 years, whereas only 15% of the HBV carriers without "e" antigen were < 20 years and 16.7% were > 40 years. The average age of HBV carriers with the "e" determinant was 23.76± 6.75 was younger than those without the "e" determinant, (29.6 ± 9.73), the difference was statistically significant (P<.05). 75.8% of the HBeAg positive and 73% of the HBeAg negative carriers were male, the difference in sex showing no statistical significance (P>.05). In addition, the HBV carriers with the "e" determinant were found more frequently in the younger age groups : those still single (78.4%) and students (38.2%) (Table 2).

Table 2. Prevalence of hepatitis "e" antigen among 663 HBV carriers and some characteristics of HBeAg carriers in comparison with HBV carriers without "e" antigen.

Characteristics	HBeAg		Test statistics
	Positive (n=262)	Negative (n=401)	
1. Prevalence of "e" determinant in HBV carriers (%)	39.5	60.5	
2. Age			
$\bar{X}$±SD (years)	23.76±6.75	29.6±9.73	unpaired t-test = 8.48*
<20 (%)	29.4	15.0	
≥40 (%)	3.8	16.7	
3. Sex : males (%)	75.8	73.0	X^2-test = 0.69
4. Marital status (%)			
Single	78.4	53.8	
Married	20.1	40.9	
Other	1.5	5.3	
5. Income : < 5,000 baht/month (%)	73.6	63.6	
6. Occupation (%)			
Officials and-State enterprise	11.0	20.2	
Students	38.2	24.6	
Others	50.8	55.2	

Table 3 shows that 43% of HBV carriers with the "e" determinant had higher SGPT values than normal (>38 u.), whereas in HBV carriers without the "e" determinant, only 28% had high SGPT levels. The difference was statistically significant (P<.05). SGPT levels were more than twice the normal levels in 17% and 4.6% of HBV carriers with and without the "e" antigen, respectively. In addition, this study showed that SGPT levels were more than three times the normal levels in 8.3% of the HBeAg carriers, compared with 1.9% of the HBV carriers without the "e" antigen; the differences in SGPT levels were statistically significant (P<.01).

* Statistical significance at ∞ = .05

Table 3. LFT and AFP in HBV carriers with and without hepatitis "e" antigen.

	HBeAag		
	Positive (%) N=264	Negative (%) n=411	test statistics
1. A/G ratio			$X^2 = 10.19^{**}$
<1.09	34.1	25.4	
1.10-1.49	64.0	68.8	
>1.50	1.9	5.6	
2. SGOT (AST)			t-test proportion
< 38	70.1	88.8	-
39-75	20.5	9.0	6.3^{*}
76-113	5.7	1.2	2.59^{*}
>114	3.8	1.0	2.3^{*}
3. SGOT (ALT)			t-test proportion
< 38	56.8	82.0	-
39-75	26.1	13.4	6.94^{*}
76-113	8.7	2.7	4.8^{*}
>114	8.3	1.9	178.1^{**}
4. Alkaline phosphatase			unpaired t-test
$\overline{X}$+SD	190.83±83.02	176.21±76.78	2.3^{*}
5. Alpha feto-protein (ng/dl)			
$\overline{X}$+SD	2.86±3.37	3.57±11.61	

* Statistical significance at $\alpha = .05$
** Statistical significance at $\alpha = .01$

The same pattern was shown for SGOT, as well as SGPT : 30%, 9.5% and 3.8% of the HBeAg positive group had SGOT levels higher than normal (>38 u), twice normal (≥ 76 u) and thrice normal (114 and up), respectively, whereas the levels were 11%, 2.2% and 1% , respectively, in the other group. The differences in the SGOT levels between the two groups were statistically significant (p<.05).

Only 1.9% of the HBeAg positive group had an A/G ratio of >1.5, whereas 5.6% of those in the HBeAg negative group had such an A/G ratio. The most highly reversed A/G ratio (<1.09) was found more frequently in HBV carriers with the "e" antigen (34%) than in those without the "e" antigen (25.4%); the difference in A/G ratio was statistically significant (P<.01).

The mean ± standard deviation of alkaline phosphatase found in HBeAg carriers was 190.83 ± 83.02, whereas in those without the "e" antigen, it was 176.21 ± 76.78, which was statistically significant (p<.05).

α–fifetoprotein fluctuated in both groups, but more so in the HBeAg negative group. For this reason, no evaluation of α fetoprotein was done in this study.

Discussion

Infection with HBV leads to a variety of outcomes, the most common being transient subclinical infection followed by the clearance of virus and the production of antibody and immunity. A small proportion of cases, however, become asymptomatic HBsAg carriers. Studies in the past decade have shown the average prevalence of HBsAg carriers among various groups of the Thai population to be 7.35%.(4) The present study showed the prevalence of HBV carries among new blood donors to be about 6.9% in 1990 and 6.47% in 1991, which suggests that HBV infection in the Thai population is mose or less stable. The prevalence of hepatitis surface antigen among regular blood donors was 1.53% in 1990 and 1.49% in 1991 which suggests that new HBV infection during the studies period was also unchanged.

It is well known that hepatitis "e" antigen develops transiently, early in the course of acute HBV infection and it usually disappears before the disappearance of HBsAg. In Thailand, the HBeAg positive rate among HBsAg carriers has varied from 20% to 85%.[1] In Taiwan, HBeAg was detected in 32.4% of 222 asymptomatic carrier.[5] This study showed the prevalence of HBeAg to be 39.5% of asymptomatic HBV carriers, with no "e" antibody being observed in their sera six month later, although both hepatitis "s" antigen and "e" antigen still persisted at that time. This finding suggests that the antigen had been present before the antibody.[6] The disappearance of antigen and the development of antibody shold be a favourable sign. Nevertheless, six months of evaluation in this study is too short to show any change in seromarkers.

The findings of Wallace's study[7] that HBeAg carriers frequently found in the younger age group and that the presence of HBeAg was not affected by the patients' sex was also observed in this study.

In most studies, the seromarkers were identified with gel diffusion methods, but in our study they were identified by ELISA together with a parallel liver function study. SGOT and SGPT levels in HBV carriers in this study were about 20-30% abnormal, whereas the series of Koshi[8] showed that 33% were abnormal with regard to SGOT and alkaline phosphatase levels. It was shown in this study that there were statistically significant differences in SGOT and SGPT, with regard to levels higher than normal (>38 u), twice normal (≥76 u) and thrice normal (≥114 u) between the two groups of HBV carriers (those with and without the "e" determinant). Also a higher frequency of HBsAg carriers with the "e" antigen on reverse A/G ratio and elevated serum alkaline phosphatase levels were found in this study. These findings suggest on-going viral replication and infectivity in HBeAg carriers. According to Simon and Patel,[9]all asymtomatic carriers had histologic abnormalities of the liver and a large majority of them had laboratory abnormalities. ALT seems to be most useful parameter among all liver function tests since at the levels of thrice normal (>114 u), the difference in SGPT can still be shown to be statistically significant ($p<.01$). It was shown in the series of Feinman et al. that serum glutamic pyruvic transaminase (SGPT) closely reflected histologic abnormalities; thus, only SGPT can be used alone or with SGOT in interpreting liver function and the increasing elevation of SGPT should be used as a criterion in the selection of asymatomatic HBeAg carriers for liver biopsy and treatment, in spite of the suggestion of some investigators that biopy is not warranted in asymptomatic carriers.

Conclusion

The rate of HBsAg carriers in the Thai population, as represented by voluntary non-remunerated blood donors, is still high (6.5%). The prevalence of the "e" determinant among HBV carriers is also as high as 39.5% by the ELISA method. Abnormal liver function, particularly ALT, was shown to be higher in HBeAg positive carriers than in HBeAg negative carriers, with the difference being statistically significant. The value of α fetoprotein could not be evaluated in this study.

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