VALIDITY OF A RISK ASSESSMENT FORM FOR SCREENING HEPATITIS C VIRUS INFECTION AND HBsAg POSITIVE CARRIER IN BLOOD DONORS

NANTAPORN THAMMATA

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Miss Nantaporn Thammata

Candidate

Assoc.Prof. Pipat Luksamijarulkul ,

B.Sc.,M.Sc.

Major-advisor

Assistant Prof. Dusit Sujirarat ,

B.Sc., M.Sc.

Co-advisor

Lect.Mujarin Tiloklurs , B.Sc

Co-advisor

Prof. Liangchai Limlomwongse,

Ph.D.

Dean

Faculty of Graduate Studies

Assoc.Prof.Amornrath Podhipak,Ph.D.

Chairman

Master of Science (Public Health)

Major in Infectious Diseases

Faculty of Public Health

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on

May 12, 2000

.....

Miss Nantaporn Thammata

Candidate

.....

Assoc.Prof. Pipat Luksamijarulkul ,B.Sc.,M.Sc.

Chairman

.....

Assoc.Prof.Tharadol Kengganpanich,B.Sc.,M.A.

Member

.....

Assist.Prof.Oranut Pacheun,B.Sc,M.Sc.,Dr.P.H

Member

.....

Prof. Liangchai Limlomwongse, Ph.D.

Dean

Faculty of Graduate Studies

Mahidol University

.....

Assoc.Prof. Kanda Vathanophas

M.D.,M.Sc. in Hygiene(P.H.Microbiology)

Dean

Faculty of Public Health

Mahidol University

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A cross-sectional analytic study of 2,167 blood donors in Phitsanulok Province was conducted during April to September 1999 in order to assess seroprevalence, risk factors and the validity of a risk assessment form for screening HCV infection and HBsAg positive carrier. Subjects were interviewed by a using risk assessment form which was adapted from the form of the National Blood Center,Thai Red Cross Society and blood samples were collected for determining anti-HCV and HBsAg by ELISA techniques. The results revealed that the prevalence of anti-HCV was 2.90% and HBsAg was 4.61%. The risk factors of HCV infection in blood donors determined by multivariate analysis were (1)residence in rural areas (OR = 2.14, 95% CI = 1.19-3.85), (2) education - primary school/illiteracy (OR = 5.64, 95% CI = 2.10 –15.15), (3) history of receiving blood or blood products (OR = 4.13, 95% CI = 1.87-9.16) and (4) history of IDUs (OR = 2.32, 95%CI = 1.41-3.82). The risk factors of HBsAg positive carriers were (1) age - equal to or more than 30 years(OR = 1.86, 95%CI = 1.70-2.02), (2) gender - male (OR = 3.04, 95% CI = 1.45-6.35), (3) history of IDUs (OR = 1.41, 95%CI = 1.07-1.86), (4) history of hepatitis or jaundice (OR = 5.39, 95% CI = 2.86-10.15), (5) history of hemophilia (OR = 18.85, 95% CI = 1.61-220.77) and (6) history of extramarital sex relations (OR = 1.18, 95% CI = 1.08-1.29).

The results from Multiple Logistic Regression were used to formulate the risk scale model by applying the Receiver Operating Characteristic (ROC) Curve. The risk scale of HCV infection had the appropriate cut-off point at a score of 7 or greater with the sensitivity of 55.2% and specificity of 85.7%, and the risk scale of HBsAg positive carriers had the appropriate cut-off point at a score of 6 or greater with the sensitivity of 45.1 % and specificity of 77.7%.This risk assessment form may be used as a screening tool. Individuals who have a score over the cut-off point should be undergo laboratory testing for these two infections.

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นันทพร ทำมาตา : ความตรงของแบบประเมินความเสี่ยงในการตรวจกรองการติดเชื้อไวรัสตับอักเสบ ซี และพาหะของการติดเชื้อไวรัสตับอักเสบ บี ในผู้บริจาคโลหิต (VALIDITY OF RISK ASSESSMENT FORM FOR SCREENING HEPATITIS C VIRUS INFECTION AND HBsAg POSITIVE CARRIER IN BLOOD DONORS) คณะกรรมการควบคุมวิทยานิพนธ์ :พิพัฒน์ ลักษมีจรัลกุล วท.ม., ดุสิต สุจิรารัตน์ วท.ม., มุจริน ติลกเลิศ วท.บ. 125 หน้า. ISBN 974-664-036-4

การศึกษานี้เป็นการศึกษาภาคตัดขวางเชิงวิเคราะห์ เพื่อประเมินความชุกทางน้ำเหลืองปัจจัยเสี่ยงและความตรงของแบบประเมินความเสี่ยงในการตรวจกรองการติดเชื้อไวรัสตับอักเสบ ซี และพาหะของการติดเชื้อไวรัสตับอักเสบ บี ในผู้บริจาคโลหิต จังหวัดพิษณุโลก จำนวน 2,167 ราย ทำการเก็บข้อมูลระหว่างเดือนเมษายน 2542 ถึง กันยายน 2542 โดยใช้แบบประเมินความเสี่ยงที่ดัดแปลงมาจากแบบประเมินของสภากาชาดไทย และเก็บเลือดเพื่อตรวจหาการติดเชื้อไวรัสตับอักเสบ ซี และพาหะของการติดเชื้อไวรัสตับอักเสบ บี ด้วยวิธีอีไลซ่า ผลการศึกษาพบความชุกของการติดเชื้อไวรัสตับอักเสบ ซี (Anti-HCV) ร้อยละ 2.90 ความชุกของพาหะไวรัสตับอักเสบ บี (HBsAg) ร้อยละ 4.61 ปัจจัยเสี่ยงของการติดเชื้อไวรัสตับอักเสบซีในผู้บริจาคโลหิตโดยการวิเคราะห์ความถดถอยเชิงซ้อน ได้แก่ (1) อาศัยอยู่ในชนบท (OR = 2.14, 95% CI = 1.19-3.85), (2) การศึกษาอยู่ในระดับประถมศึกษาหรือไม่ได้เรียนหนังสือ (OR = 5.64, 95% CI = 2.10-15.15), (3) มีประวัติได้รับเลือดหรือผลิตภัณฑ์จากเลือด (OR = 4.13, 95% CI = 1.87-9.16), (4) มีประวัติใช้ยาเสพติดชนิดฉีด (OR = 2.32, 95% CI = 1.41-3.82). ปัจจัยเสี่ยงของการเป็นพาหะของไวรัสตับอักเสบ บี ได้แก่ (1) อายุมากกว่าหรือเท่ากับ 30 ปี (OR = 1.86, 95% CI = 1.70-2.02), (2) เพศชาย (OR = 3.04, 95% CI = 1.45-6.35), (3) มีประวัติการใช้ยาเสพติดชนิดฉีด (OR = 1.41, 95% CI =1.07-1.86) และ (4) มีประวัติตัวเหลืองตาเหลืองหรือตับอักเสบ (OR = 5.39, 95% CI = 2.86-10.15), (5) มีประวัติเป็นโรคฮีโมฟีเลีย (OR = 18.85, 95%CI = 1.16-220.77) และ (6) มีประวัติมีเพศสัมพันธ์นอกคู่สมรส (OR = 1.18, 95% CI = 1.08-1.29). ผลที่ได้นำมาประเมินความตรงของแบบประเมินความเสี่ยง โดยใช้สถิติวิเคราะห์ The Receiver Operating Characteristic (ROC) Curve ซึ่งความเสี่ยงของการติดเชื้อไวรัสตับอักเสบซี มีจุดตัดคะแนนตั้งแต่ 7 ขึ้นไป มีความไวร้อยละ 55.2 มีความจำเพาะร้อยละ 85.7 และความเสี่ยงของการเป็นพาหะของไวรัสตับอักเสบ บี มีจุดตัดที่คะแนนตั้งแต่ 6 ขึ้นไป มีความไวร้อยละ 45.1 และความจำเพาะร้อยละ 77.7 แบบฟอร์มนี้สามารถนำใช้ในการตรวจคัดกรองบุคคลที่เสี่ยงต่อการติดเชื้อไวรัสตับอักเสบซี และเสี่ยงต่อการเป็นพาหะของไวรัสตับอักเสบ บี กรณีมีคะแนนสูงกว่าจุดตัดควรได้รับการตรวจเลือดต่อไป

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CHAPTER I

INTRODUCTION

Hepatitis C virus (HCV) was differentiated in 1989 (1). It is the predominant cause of post-transfusion Non-A Non-B hepatitis patients (PT- NANBH) worldwide (2-7). There is no special vaccine for prevention and no effective drugs for treatment. Over 50% of patients with HCV infection will develop chronic infection and gradual progression to liver cirrhosis or hepatocellular carcinoma (HCC)(8).

The report of National Blood Center, Thai Red Cross Society revealed that the prevalence among blood donors in Northeastern Thailand was 6.5% in male and 0.9% in female (9). The prevalence in males was higher than that in females and increased with age(9-10). There was a report showing a 2.4% prevalence rate of HCV infection in blood donors in Northern Thailand (11) and one study reported a high prevalence (4.1%) in Bangkok blood donors (12).The risk groups of HCV infection included injecting drug users (IDUs,35.7%), heterosexual individuals (30%), homosexual men (25%), transfused patients (5.9%), occupational infection (0.7%) and patients with unknown source infection (2.6%)(13). Luksamijarulkul P. and Pluktaweesak S.(14) in 1996 showed that 95.33% of males IDUs were anti-HCV positive and the anti-HCV prevalence increased by risk factors for example the history of imprisonment, tattooing, ears piercing, extramarital sex relation without condom and duration of needle sharing. The other report found that the factors determined to be significantly associated with anti-HCV seropositive included low social class, being unmarried, a history of abortion, wounds which were sutured, sharing toiletlies with the partner and sexual contact outside the partnership without condom use (15).The report of Saltoglu,*et al.*(16) showed that the anti-HCV prevalence in the families of index patients was significantly higher than general population (4.3% and 0.85%). From a study in 1997, Guadagino,*et al.*(10) showed that the prevalence of HCV infection among general population was 12.6% and the risk factors included age, gender, syringe sharing, dental therapy and transfused patients.

The major mode of transmission of HCV was parenteral route and sexual transmission seems to be the minor importance in the spread of HCV (17). Both sexual and parenteral route were the predominant modes of hepatitis B virus (HBV) transmission (18). Both HBV and HCV are major health concerns in several countries, including Taiwan(19), Japan and Thailand(9,11,12,14). The study of Wang CS,*et al.* showed that frequent medical injections might be an important risk factor for HBV and HCV infection. The report of Welman, *et al.*(20) showed HBV and HCV coinfection associated with more severe liver disease than HCV alone. The factors associated with HBV infection included two or more sexual partners in the previous year, at least one tattooing in the previous year, injecting drug use and homosexual activity (21,22). A history of syring sharing of IDUs with imprisonment was significantly associated with HBV,HCV and Human Immunodeficiency Virus(HIV) infections (23). Risk factors for HBV and HCV infections were similar in blood transfusion, hemodialysis, IDUs and sexual contact with IDUs. For HBV infection, sexual relation with multiple partners, increasing age and birth in South East Asia or Africa were additional risk factors(24).

In the present time, sexual active individuals who got married, most of them were not screened their blood. Moreover, the community hospital was lack of the budget for screening HCV infection among blood donors. This study attempted to develop and analyse the validity of a risk assessment form for screening the HCV infection and HBV carrier state. The findings may be valuable for premarital screening and/or blood donors screening of both infections and may be applied for HIV infection.

Objectives

General objective : To analyse the validity of a risk assessment form for screening HCV infection and HBsAg carrier in blood donors.

Specific objectives : 1. To find out the prevalence of anti-HCV and HBsAg positive in blood donors.

2. To identify risk factors of HCV infection and HBsAg positive in blood donors.
3. To analyze the validity of risk assessment form for screening HCV infection and HBsAg positive carrier in blood donors.

Hypotheses

1. There were some studied factors associated with HCV infection and HBsAg carrier in studied blood donors.

2. The risk assessment form has a high specificity (85%-95%) and moderate sensitivity (50%-75%) for screening HCV infection and HBsAg carrier among blood donors.

Definition

Blood donors : Blood donors who filled in the risk assessment form at the Blood Center in Phitsanulok Province

HCV infected blood donor
: A blood donor who was positive for anti-HCV by ELISA

HBsAg carrier blood donor
: A blood donor who was positive for HBsAg by ELISA

The prevalence rate of anti-HCV

$$: \frac{\text{Number of anti-HCV positive persons}}{\text{Number of studied persons}} \times 100\ \%$$

The prevalence of HBsAg

$$: \frac{\text{Number of HBsAg positive persons}}{\text{Number of studied persons}} \times 100\ \%$$

Risk assessment form
: The form including socio - economic factors and risk behaviors for HCV and HBV infection

Validity of risk assessment form

: The sensitivity and specificity of risk assessment form were assessed by the Receiving Operating Characteristic (ROC) Curve

Limitation of research

This study was carried out in blood donors at Phitsanulok Province. The validity of a risk assessment form was analysed in this target group and might not be representative general population due to the voluntary subjects.

CHAPTER II

LITERATURE REVIEW

Hepatitis C Virus

History of Hepatitis C Virus

It is well known that an acute non-A, non-B hepatitis (NANBH) is characterized by a high incidence of chronicity and lead to chronic liver disease such as cirrhosis of the liver and eventually, hepatocellular carcinoma (HCC). Recent advances in technology have made it possible to identify hepatitis C virus (HCV) infection by assaying antibodies to HCV (anti-HCV) and by a detection of HCV-RNA(23).

Although acute hepatitis type C is often anicteric and asymptomatic, it may progress to chronicity in a significant percentage of patients. Thus, the chronicity is the most characteristic clinical feature of hepatitis C. A study of chronic hepatitis established, the rate of spontaneous cure of the liver disease is very rare (below 2%). The duration from the onset of acute hepatitis until the time of diagnosis of cirrhosis and of HCC was about 20 and 30 years, respectively. The long term clinical course of hepatitis C is devided into the three phases of acute, silent and reactivated. The acute phase lasts from the onset of disease until 2-3 years and the silent phase lasts for 10-15 years. In the silent phase, the serum transferase level remains relatively low, below 100 IU/L, and is somtimes within the normal range. In the reactivated phase, the level of serum aminotransferase increases and remains at high or moderate level until HCC develops. The mechanism of the chronicity of hepatitis C is unknown. However, recent advances in molecular analysis may soon eludicate this(23).

Structure of HCV

Hepatitis C virus is considered in *Flaviviridae* family. It is a positive stranded RNA virus and enveloped virus. The size is less than 80 nm., and consisted of approximately 9,400 nucleotides (24-27). Such a physical structure is consistent with early studies examining the size and sensitivity of infectious HCV particles to lipid solvents (28). However, the HCV particles have never been visualized by electron microscope, and thus its exact physical appearance is not known. There is low level of nucleotide sequence related between HCV and the flavivirus, and even similarities in the hydrophathy profiles of the putative viral structural proteins. However, there is greater genetic relatedness with the pestiviruses, important veterinary pathogens that are also classified within the *Flaviviridae* family and that include bovine viral diarrhea virus, classic swine fever virus (previously known as hog cholera virus) and border disease virus (26,29). Based on similarities in the structure and organization of the genomes of HCV, flaviviruses and pestiviruses, HCV have been tentatively classified within a third, independent genus of the *Flaviviridae* family (which also includes flavivirus and pestivirus genera).

The HCV genome contains a single large open-reading frame (ORF) that follows a relatively lengthy 5′ nontranslated region of approximately 342 bases (Figure 1). The 5′ nontranslated RNA appears to play a critical role in controlling virus translation and may be important in the pathogenesis of HCV infection (30,31). The 3′ nontranslated region of the genome appears to contain a unique polyuridylic acid tract, although the existence of a more typical polyadenylic acid tract has been reported as well (32). The large ORF encodes a polyprotein that undergoes post-translational cleavage mediated by both cellular and virus specified proteases (33,34). The amino third of the polyprotein contains a series of structural proteins that include a 21-kD core (nucleocapsid) protein (C) and two envelope glycoproteins, E1 and E2 (Figure 1). These proteins are cleaved from the polyprotein by a signal peptidase present within the endoplasmic, and appears to be an internal viral structural protein somewhat resembling the core protein of hepatitis B. Preliminary evidences suggest that it may undergo additional proteolytic processing during virus replication. E1 and

E2 are translocated into the endoplasmic reticulum and subsequently become heavily glycosylated during transport through the Golgiapparatus (33). These glycoproteins are thought to be presented in the lipid envelope of the HCV particle. The flaviviruses possess only a single major envelope protein, but E2 may be analogous to the NS1 protein of flaviviruses. In contrast, the pestiviruses have three major envelope proteins(34).

The remainder of the viral polyprotein contains a series of nonstructural viral proteins involved in the replication of the virus. The processing of this region of the polyprotein is complex, and the functions of several of the individual proteins remain obscure. These functions include, however, at least two viral proteases (NS2 and NS3) (35), a putative helicase (NS4) (36), and an RNA polymerase (NS5).

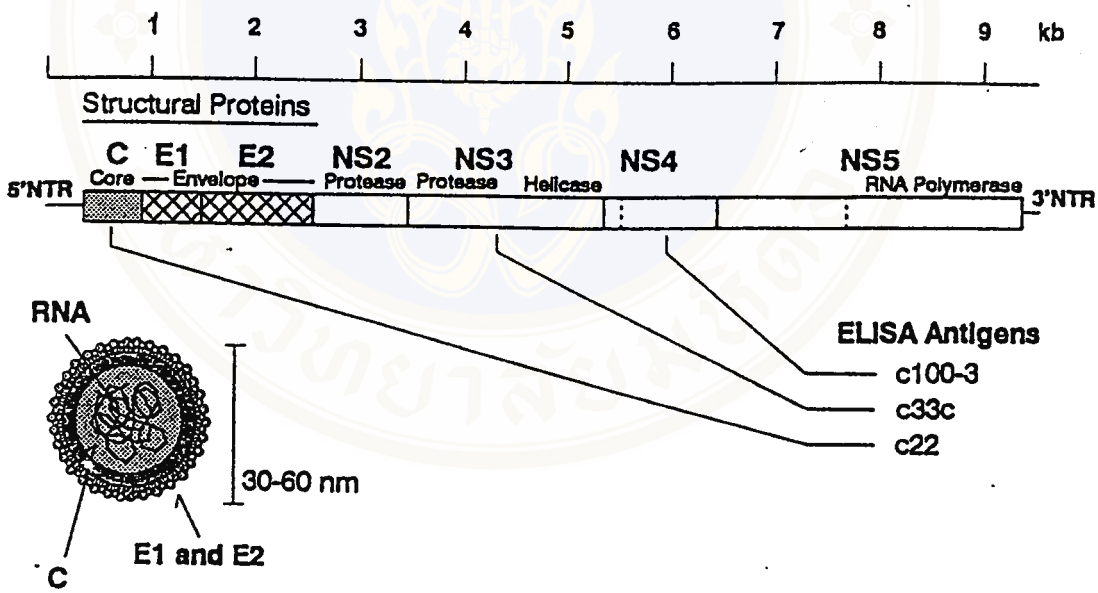


Figure 1 Organization of the RNA genome of HCV. The probable structure of the HCV particle is depicted on the left. NTR, nontranslated regions.

From : Lemon SM and Brown EA. Hepatitis C virus. In : Mandell GL, Bennett JE, Dolin R,editors. *Principles and Practice of Infectious Diseases.* USA : 1995 :1474-86.

Virus Persistence and Immunity

Infection with HCV tends to become persistent in most infected individuals, apparently reflecting an inability of the immune system to mount an effective antiviral response. Current estimates are that at least 50%, and perhaps as many as 70-90%, of infected individuals fail to clear the virus during the acute phase of the illness (15-18). The reason for the failure of the immune system to clear the virus after infection is unknown.

The role of cell-mediated immunity in chronic HCV infection is being explored by several research groups (41-44). CD_8^+ cytotoxic T lymphocytes have been identified in the liver of chronic infected patients and chimpanzees, and several T-cell epitopes have been defined. The relative contributions of this T-cells response to immunity and to disease pathogenesis, however, remain uncertain. Because these virus specific, cytotoxic T-cell were identified in chronically infected individuals, it is apparent that they are not capable of eliminating the infection.

Immunity has also been assessed by rechallenge of previously HCV infected chimpanzees with heterologous and homologous HCV strains (45-46). These studies indicate that reinfection (including reappearance of hepatocellular pathology and/or HCV viremia) is common after rechallenge of previously infected animals. Nevertheless, manifestations of HCV infection were generally significantly reduced in secondary infection compared with primary infection. Reinfection could be demonstrated with homologus as well as heterologous virus rechallenges, indicating that poor immunity is not related simply to antigenic variation among different strains of HCV. These data collectively suggest that immunity develops following primary HCV infection, but that it is incomplete. Such data have important implications for vaccine development efforts, as they show that the absence of solid immunity against hepatitis C is common after primary infection. However, it is possible that a more completeness from immunity might develop in the small proportion of individuals who appear to completely clear the infection.

Clinical manifestations of HCV infection

There is no clinical feature of HCV infection that can distinguish any individual case from type A or B or other hepatitis. Each clinical form of viral hepatitis that occurs with HBV also occurs with HCV (47,48). The spectrum of illness caused by HCV ranges from asymptomatic infection to acute fulminant hepatitis and a large proportion of HCV cases develops chronic hepatitis, which can range from mild disease to severe chronic active hepatitis (CAH) and may lead to cirrhosis and hepatocellular carcinoma (HCC) (47,49).

Acute Hepatitis C

Acute hepatitis C is clinically indistinguishable from other types of acute viral hepatitis in the individual patient. However, the incubation period for acute hepatitis C averages about 6 weeks, based on studies of transfusion recipients (39), and in thus intermidiate between type A and B hepatitis. Symptoms are usually gradual in their onset, and often milder than in either hepatitis A or B. Biochemical indices of hepatocellular dysfunction, including elevations of serum alanine aminotransferase (ALT) activities and bilirubin, are also generally less pronounced in hepatitis C. Prospective studies of transfusion recipients show that over 75% of acute hepatitis C infection are anicteric, and suggest that 50% or more would be missed without careful screening for ALT elevations (39). Sporadic cases of acute hepatitis C presenting without antecedent history of blood transfusion tend to be more severe (about 75% of cases are jaundice) (40). Undoubtedly reflecting a reporting artifact, extrahepatic manifestations of acute hepatitis C infection have not been recognized.

Chronic Hepatitis C

Chronic hepatitis C is often but certainly not always found in individuals with prior risk factors involving parenteral exposure to blood (i.e., blood transfusion, injecting drug use, or occupational exposures). The typical picture is that of a

relapsing, remitting infection with recurrent bout of hepatitis marked by periodic fluctuations in serum ALT activities. Specific symptoms and signs related to liver dysfunction, such as jaundice, ascitis, or gastrointestinal bleeding, are present only in far advanced disease. During quiescent periods, even the serum ALT may be normal or near normal. Thus, the absence of ALT abnormalities does not preclude chronic HCV infection (50). In fact, clinical studies evaluating the utility of ALT as a surrogate marker for NANB hepatitis in blood donors suggested that over half of all viremic patients have normal ALT levels (51). Serum ALT activities are also very poor indicators of the severity of chronic liver disease associated with HCV infection, as ALT levels are often normal or near normal even in patients with biopsy proven, advanced liver disease (52). The measurement of serum albumin concentrations and/or prothrombin time are much better laboratory measurement of the severity of liver disease, but become abnormal only very late in the cause of the disease. Liver biopsy provides the only certain means of evaluating the extent and activity of liver disease in patient with chronic HCV infection. This invasive procedure is probably warranted in patients with serologic evidence of HCV infection who demonstrate elevated levels of serum ALT over periods of 6-12 months, and may be helpful in selecting patients for treatment with recombinant α interferon.

Hepatocellular Carcinoma (HCC)

Primary HCC is a late complication of chronic hepatitis C (53-56), usually occurring in patients with cirrhosis. Liver cancer occurring in association with chronic hepatitis C has been particularly evidence in Japan, and often follows two or more decades of presumed HCV infection. Like liver cancer in general, hepatitis C-related HCC appears to develop much more frequently in male than in female. Clinical findings usually include a sudden worsening of previously evident symptoms and signs of cirrhosis (fatigue, ascites, jaundice), often in association with right upper quadrant pain. Serum alkaline phosphatase levels are often very high. Ultrasonography or computerized tomography reveals an intrahepatic mass but specific diagnosis requires liver biopsy.

Diagnosis of HCV infection

As our knowledge of the structure of HCV has increased, serum tests for the detection of HCV infection have become more sensitive. At present, there are no direct tests for HCV antigens. Rather, subjects are tested for the presence of anti-HCV antibodies. This usually involves an initial screening test with an enzyme-linked immunosorbent assay (ELISA), described below. Positive results are then cheeked with the more sensitive recombinant immunoblot assays (RIBAs). If avaliable, tests for HCV-RNA (using PCR or the newer, branched DNA signal amplification method) may also be done to determine the presence or absence of virus in serum.

1. Diagnosis by using ALT level

For many years, a diagnosis of NANB hepatitis was necessity tentative. Such a diagnosis could only be based on exclusion of other etiological agents of hepatitis such as the viruses for hepatitis A and B, cytomegalovirus and Eptein-Barr virus. In 1981, the transfusion-transmitted virus study showed that ALT level in blood donors predicted the risk of NANB hepatitis in transfusion recipients (57,58). ALT screening was introduced as a surrogate for NANB hepatitis.

In 1986, blood bank throughout the United States was problematic for several reasons. The test was neither specific nor reproducible overtimes. There was no obsvious cut-off that separated infection, blood donor from noninfection, and the advice given to donor was often confused and not medically useful. The implementation of ALT testing led to the discarding of approximately 2% of blood donation in the United States (58,59).

2. Diagnosis by using Enzyme-Linked Immunosorbent Assay (ELISA)

In 1989, an incision and extensive attack of this problem is now possible because of the elegant (and arduous) pioneering work of Chiron Laboratories who actived milestone success by expressing in a recombinant system, an antigen after cloning part

of the genome of what we know as hepatitis C virus. The first of such assay incorporated the C-100-3 polypeptide express in yeast. This protein was used to coat the solid phase of an antiglobulin-based assay, initially as a radioimmunoassay for antibody to HCV. Commercially, the assay is marketed worldwide in a microplate ELISA formatted by Ortho Diagnotics and is now also available as a bead-based ELISA from Abbott Diagnostics(60).

In 1992, on the basis of first-generation test was inadequately sensitive (61) in patients with a prolonged window period and in patients who had lost HCV antibody. The development of more sensitive HCV antibody have contains NS5 in addition to core, NS3, and NS4 antigens (Matrix HCV 2.0, Abbott Laboratories) (62). On the basis of second-generation test was implemented for donor screening in the United States.

After cloning of hepatitis C virus has led to the development of several serologic assays for the detection of HCV infection. The successive addition of expressed antigen resulted in anti-HCV test of increasing sensitivity (63). Extensive use of these assays in screening blood donation has substantially reduced the occurrence of PTH (64,65). In early 1996, a more study reported that with third-generation screening, only 1 in 103,000 donors would be in the seronegative window period of HCV infection (66).

Third-generation ELISA now widely used in blood donor screening is even more sensitive and specific than the earlier ELISA, and is practically 100% effective in preventing transmission of HCV to recipients. However, HCV may still remain undetected in individuals who were infected less than 6 months previously and in immunosuppressed patients. False-positive result also remain common among blood donors. All samples which were positive on ELISA should therefore be confirmed using supplementary tests(66).

3. Supplementary test : Recombinant Immunoblot Assay (RIBA)

A recombinant immunoblot assay (RIBA) for anti-HCV has proven to be a valuable research tool for elucidating the validity of the majority of ELISA- positives.

First generation supplementary RIBAs have been succeeded by tests that detect antibodies to two viral antigens (RIBA-I,Table1). The second-generation RIBA (RIBA-II) has proven very useful in excluding false-positive ELISA results and also in identifying infectious patients. A close correlation has been reported between RIBA-II positive and viremia (HCV-RNA) based on polymerase chain reaction (PCR). The third generation RIBA (RIBA-III,Table1) has also been developed, with enhanced sensitivity and specificity compared to RIBA-II (67).

Table 1. Supplementary tests : RIBAs (67)

Supplementary assays :	
1st generation RIBA	Anti-c100/NS4, anti-5-1-1 (Proteins from NS3/NS4 regions)
2nd generation RIBA-II	Anti-c100/NS4, anti-5-1-1 anti-c33/NS3, anti-c22/C
3rd generation RIBA-III	Anti-c22/C, anti-c100/NS3/NS4,NS5

The RIBA is the HCV confirmatory test that uses to determine the specificity of the HCV ELISA (68,69). The full RIBA positively is associated with HCV infectivity, whereas an ELISA positive, RIBA intermediate result reflects a non-infectious anti-HCV positive (70).

4. HCV RNA detection by Polymerase Chain Reaction (PCR)

Amplification of complementary DNA sequence of HCV RNA synthesized with reverse transcriptase has been described by Weiner,*et al.*(71). However, Kanoko,*et al.* (72) pointed out that HCV was very heterogeneous in terms of its RNA nucleotide sequence. The following sequence of testing may be necessary for a full

understanding of a sample that is positive for HCV antibodies by ELISA . However, until the PCR has been optimised and its limitations clearly defined, the question of "re-entry" of RIBA-positive (or interminate). Data obtained by using PCR assays have indicated that viremia could be detected within only a few days of exposure to the virus, many weeks before elevation of circulating and viral antibody level (73-75). Such PCR assay, also provide valuable information concerning the viremic states when anti-HCV is present but liver function is normal (74). The PCR assay can be used to diagnose HCV infection in chronic NANB hepatitis who may be seronegative (44). Also, PCR assays have enabled the detection of vertical transmission of HCV from chronically infected mother. It is interesting to know that many of these infected babies show evidence of persistent viremia in the absence of seroconversion (76).

Epidemiology of HCV infection

The epidemiology of hepatitis C resembles the epidemiology of hepatitis B both as a transfusion associated disease and also an infection transmitted by apparently nonparenteral exposure (47).This disease is predominantly spread between individuals by the parenteral route (77). Except for human, there is no known animal reservoir in nature (77). From the transfusion studies, it has become clear that a carrier state of hepatitis C exists and that these carriers frequently have no overt signs of the disease (47).

Prevalence of HCV infection

- **HCV infection in posttransfusion hepatitis (PTH)**

HCV is the cause of PTH. PTH patients include the patients that receive blood or blood components during operation and hematologic diseases who receive multiple transfusion. The prevalence of anti-HCV in PT-NANBH was 40-76% in USA (78-80), 47-85% in German and Spain (81,82),61-69% in Taiwan or Japan or China (83-86). In another study, in Thailand NANBH was reported as being the etiology of

62.5% of HCV (87). Punyagupta concluded that NANBH was more common than hepatitis B virus in the etiology of PTH in Thailand (88).

- **HCV infection in blood donors**

The prevalence of anti-HCV in blood donors varies from approximately 0.2 to1.5% around the world. Generally the values range from 0.2-1% in Europe and North America and about 1.5% in Japan and much higher in Africa, whereas prevalence data in African sera are as yet unreliable. Previous studies on the prevalence of HCV infection in Asian countries reported average prevalence of less than 1.5% (88). The anti-HCV prevalence in Thai blood donors was 1.5-2.6% (89,90). At the National blood Center, Thai Red Cross Society was found that new male donor showed the highest prevalence(88). In 1995, Anti-HCV antibodies were detected in 6.5% of male blood donors and 0.9% of female blood donors in Northestern Thailand (91).The commercial blood donors are higher risk of NANBH carriers than volunteer blood donors (47,70). There was an increased hepatitis risk from blood donated by military volunteers compared to community volunteer blood donors. It indicated that the high risk group of NANBH occurred in low socioeconomic population (47,77).

- **HCV infection in injecting drug users (IDUs)**

The prevalence of anti-HCV in IDUs varied between 42-86%. The anti-HCV prevalence in Asian IDUs was 43-81% (83,90,93,94), in USA was 42-85% (79,95,96), in Europe was 48-80% (97-101) and in developed countries was 48%(102,81). In Thailand, the reported prevalence of anti-HCV in this group was 95%(103).

- **HCV infection in hemophiliacs**

The prevalence of anti-HCV in hemophiliacs was quit high, in Sweden and German were 54-78% (99,105), in Taiwan was 90-100% (83,93,106), in Australia and

UK were 61-75% (100,107). The study in Thai hemophiliacs found that the anti-HCV prevalence was 32.6% (109).

- **HCV infection in hemodialysis patients**

The prevalence of anti-HCV in hemodialysis patients varied from one dialysis unit to another (77), in Japan was 20-39% (94,108,109), the other countries were 5-20% (98-100) and they found that anti-HCV positive was associated with the duration of hemodialysis (94,109). The underlying reason why HCV infection occurred among patients without episodes of blood transfusion is unclear (109).

- **HCV infection in liver disease patients**

It is now known that approximately 80% of NANBH progress to chronicity and that the chronic carrier state lead in 25-50% of cases to the development of chronic hepatitis, cirrhosis, and eventually, HCC (110).

The highest prevalence of HCV in HCC is found in Italian, Japanese and Spanish patients; the prevalence is lower in Chinese and African patients, in whom the disease is more commonly associated with chronic hepatitis B (111).

- **HCV infection in health care workers**

The prevalence of anti-HCV in health care workers was 0.7-2% (108,112,113). The persons having direct contact with the patients, such as doctors and nurses,, seem to be at a higher risk of infection than those with only indirect contact, such as laboratory technicians and cleaning personnel (113). However, the prevalence rates in this group didn't differ significantly from prevalence rates found among volunteer blood donors or general population (112,114).

HCV transmission

Although the mechanism of transmission of HCV is likely to be similar to that of HBV, it is clear that there are some significant differences.

1. Transmission by blood and blood products

It is clear that transfusion of infected blood products is a particularly efficient route of HCV transmission. There is now clear evidence that approximately 80-90% of cases of PT-NANBH are due to HCV infection. Although blood transfusion is an efficient route of transmission, it can not account for the prevalence and maintenance of HCV in the population, most individuals do not receive a transfusion during their lifetime. However, transfusion can be a significant source of infection. Hemophiliacs, who are exposed to large amounts of single donor product or to product derived from large pool of plasma are a high risk group (40,115,116).

2. Transmission by injecting drug users (IDUs)

Individuals sharing syringes or needles for injecting drug use are at high risk of infection by parenterally transmitted blood-borne infectious agents consequenly, the prevalence of anti-HCV in IDUs is high (104,117).

3. Transmission by organ and tissue transplantation

HCV infection can be transmitted through organ and tissue transplantation. Post-transplantation liver disease is still an important cause of morbidity and mortality, especially in renal transplant recipients (118). Present data on HCV transmission by donated organ and tissue are limited but it is clear that the screening of potential organ donors for anti-HCV would be beneficial in minimizing any risks of post-transplantation HCV infection.

4. HCV Transmission by a history of imprisonment

High risk behaviors for transmission of HIV and HBV (ie, IDUs and unprotected sex) appear to be widespread in prisons and young often institutions. It is not surprising, therefore, that the risk of HCV infection is also increased among prisoners. In a study of intravenous drug abusers in Wales, there was a significantly compared to those without such a history (46% versus 29%,respectively; $p<0.05$)(21).

5. HCV Transmission by hemodialysis (129)

Transmission of HCV between hemodialysis patients may also occur, although the risk has been reduced by more stringent environmental control measures and screening of patients for anti-HCV antibodies.

6. Mother-infant transmission

By comparison with HBV, maternal transmission would also seem likely. However, transmission from HCV carrier mother to their offsprings has been reported only few studies(130,131). A study of Giovannini M, *et al.*(132) documented that transmission to children increased when simultaneously infected with maternal HIV.

7. Intrafamilial transmission

The role of intrafamilial transmission requires classification. Although household transmission appears to be relatively infrequent, the true attack rate may be underestimated, since the prevalence of HCV infection in family members is based on the current diagnostic test, which usually reflect chronic infection rather than resolved disease. In Japan, up to 8% of family members of an index patient with HCV infection were found to be anti-HCV positive, but no specific relation could be linked to HCV positive, making it was difficult to identify the route of infection (133).

8. Transmission through non-human vectors

Transmission through non-human vectors, not ably arthropods, is a well-defind route for some infectious agents. Yellow fever virus, the prototype flavivirus, is transmitted by this route. Currently, however, there are no confirmed reports of non-human transmission of HCV (134).

9. Sexual transmission

At first sight, sexual transmission might appear to be one of the most likely cause of spreading HCV. The studies in homosexual males found that the HCV prevalence ranged from 1.3 to 7.8% (119-121), 10% of hepatitis patients with the only identifiable source of infection is heterosexual activity with multifiable partners or household or sexual exposures to a contact with hepatitis (122) and in female sex workers were 3 to 12%(123-127). Anti-HCV has been found in 11% of sexual partners of anti-HCV positive IDUs and correlates with the presence of anti-HIV (128).

Treatment of HCV infection

Therapy should not be started until a firm diagnosis of chronic hepatitis C is made. Chronic hepatitis C can be diagnosed on the basis of finding persistant elevations of ALT, anti-HCV in serum and liver histology compatible with chronic hepatitis. Patients should have elivations of serum ALT for at least 6 months, or for a shorter period if it clears from the history that the disease has longstanding. A liver biopsy is necessary to document that chronic hepatitis is present, to assess the severity of hepatocellular injury and fibrosis and to help rule out other diagnoses such as alcoholic liver disease, hemochromatosis and sclerosing cholangitis. Patients should have firmed serological or epidemiological evidence of hepatitis C. Alpha interferon is currently licensed for use in chronic hepatitis C. The recommended regiment of alpha interferon is 3 MU given either subcutaneously or intramuscularly three times

weekly for 6 months (24 weeks). Actually, the optimal dose and duration of therapy are controversial, with some investigators recommending higher dose (5 MU three times weekly) for more prolonged periods (12 months or 48 weeks) (134). Alpha interferon has many side-effects, but must are self-limiting and tolerable, and almost all are rapidly and fully reversible when the drug is stopped (135). The fear of interferon's side-effect should not cause avoidance of therapy. In randomized trial of alpha interferon, only 3-10% of patients were not able to tolerate the full 6 months of treatment (136,137).

Prevention and control of HCV infection

To interrupt transmission and reduce the incidence of hepatitis C, the prevention and control measures should be included :

- **Proper selection and screening of blood donors for anti-HCV**

Because parenteral exposure to blood is the predominant mechanism of transmission, so screening of donors for anti-HCV with a sensitive test now is possible to identify most carriers (138) and it should greatly reduce the risk of transfusion associated NANBH about 60-85% (138-143). However, this test can not identify all serum samples infected with HCV. The lack of antibodies in donors may still transmit the disease. Thus, the further improvement of specificity and sensitivity of the current method is required not only for improvement of prevention of PT-NANBH but also for diagnosis of HCV infection (80,138,141). Besides these, the elimination of commercial blood and the person at risk donors is the major factor that results in the decrease in the rate of post-transfusion hepatitis (138,140,144). Data from the Nation Blood Center, Bangkok and blood donors in Northeastern Thailand showed high anti-HCV prevalence in donate blood (91). The study suggested that preventive measures against post-transfusion HCV should be further continued advocating routine blood screening of donors for the anti-HCV.

• Health education about hepatitis C in hospital and community

General measures used to prevent hepatitis in hospital settings including non-reuse or sterilization of needles and sharp instruments, blood precautions when dealing with patients or their body secretions and basic procedures to minimize the potential of percutaneous or mucosal blood contact in all settings (138) are important in preventing NANBH transmission. In hepatitis C patients, the physician should inform their infections and give the knowledge about hepatitis C to them in order to control their diseases and delay becoming chronic hepatitis; moreover, they may be monitored for the development of chronic hepatitis and treated early in their course with hope for improvement in quality of life and survival, avoid exposure to hepatotoxins and change their lifestyle to decrease the likelihood of infecting other (145).

In the community prevention will depend on measuring the risk associated with sexual lifestyle, sharing private wares, percutaneous doing, and person to person contact with hepatitis patients (146). The recent study of HCV-RNA in saliva suggested that the saliva of patients infected with HCV should be a source of HCV infection (147). It wants further investigation to improve the strategy of HCV prevention.

• Specific prevention

There is no specific prevention for PT-NANBH (119,140,148), but the expression of antigens associated with HCV will allow for the possible preparation of vaccine in the future (140). The efficacy of passive immunoprophylaxis by immune serum globulin (ISG) for prevention of transfusion associated hepatitis give mixed results, however at this time using of ISG is not recommended and can not be adequately ascertained (138,140,144).

Hepatitis B Virus (HBV) Infection

History

Although acute hepatitis following percutaneous exposure to human serum or blood was recognized in human more than 100 years ago (149), but the modern history of hepatitis B began in 1960s. The modern era in viral hepatitis research began after the description by Dr. Blumberg and colleagues of an isoprecipitant in the serum of Australian Aborigines (150-152). It was called an Australia antigen or a serum antigen, now termed as hepatitis B surface antigen (HBsAg). In the 10 years following the discovery of HBsAg, the virus was fully characterized, virions, antigen and antibody systems were described, and sensitive immunoassays for the detection of these virion components were developed. The availability of serological tests for HBsAg provided the mean for expanding knowledge of the epidemiology and consequence of infection with HBV and, through screening of the blood supply, rapidly eliminated a major cause of posttranfusion hepatitis (153). Globally, chronic infection with hepatitis B virus is one of the leading cause of death among adults because of chronic liver disease and primary hepatocellular carcinoma (PHC) (154).

Physical and chemical characteristics

The abundance of viral structures in blood has made physical and chemical analyses possible despite the inability to propagate the virus in cell cultures.

Virions (Dane particles) and isolated 28 nm. Cores (nucleocapsids) contain the viral genome, a circular, partially double-stranded DNA molecule, which consists of a long (L) strand of constant length with a free 3′ terminus and a 5′ terminus that is covalently linked to a small protein and a short (S) strand that varies from 15-16% of the cycle length (Figure 3). The position of the 5′ termini of the L(-) and S(+) strands are fixed, whereas the 3′ termini of the S(+) strand is variable. The 5′ ends of both strands are base-paired, which assures the circular form of the double-stranded DNA.

In addition, at both sides of the cohesive 5′ ends there is an 11-base-pair direct repeat (5′TTCACCTCTGC); similar direct repeats (DR1 and DR2) are found in all the hepadnaviruses, which suggests that they play an important role. This DNA is smaller than that of any known animal virus containing double-stranded DNA. The nucleocapsid consists of the core Ag (HBcAg), a DNA polymerase, and a protein kinase. The DNA polymerase of the virion is dependent on the four deoxynucleoside triposphates and Mg^{+2} ; it has complete the single-stranded regions without any added DNA or RNA primer, producing covalently closed, double-stranded circular DNA. The polymerase also serves as a reverse transcriptase.

The viral envelope contains three glycoproteins termed the major, middle, and large protein (Figure 3). The hepatitis B surface Ag (HBsAg), which is the prodominant component of the 22 nm. Particles found in the blood of patient with active disease and carriers, is a disulfide-bonded dimer of two molecules of the major protein. In the middle protein a 55 amino acid sequence, which is the region distinct from the major protein, probably contains the receptor for polymerized human serum albumin that appears to mediate the attachment of the virus to hepatocytes. The large protein consists of the middle protein plus about 128 amino acids added to its N terminus; this exposed additional sequence appears also to be important for attachment to hepatocytes (155).

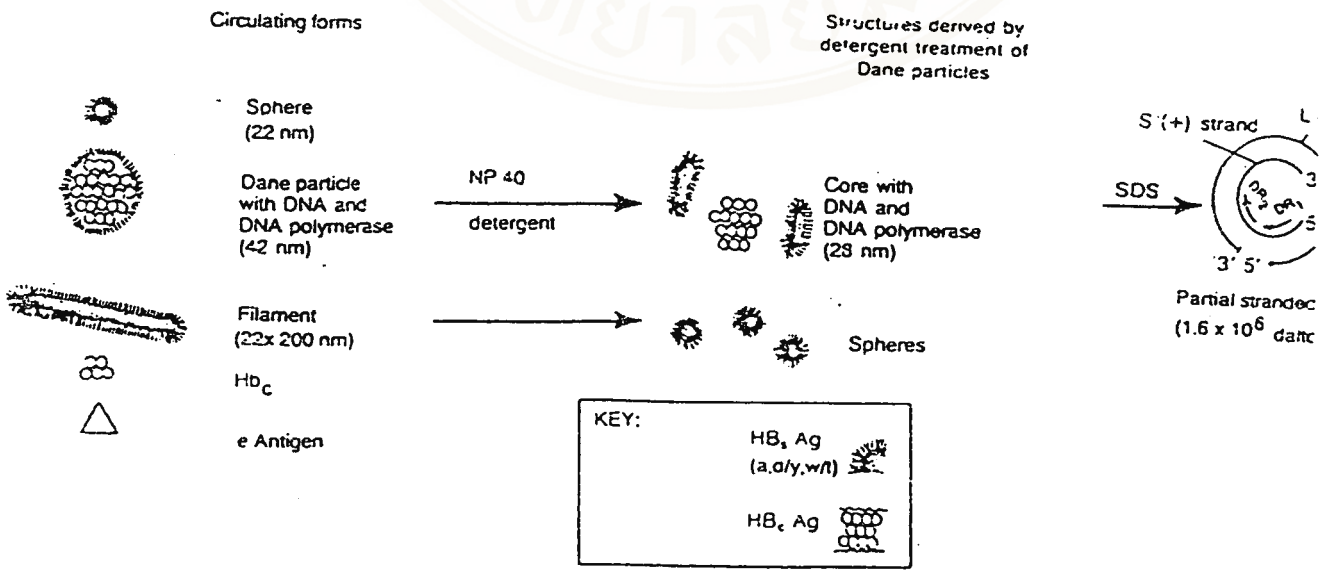


Figure 2. Physical and Chemical Characteristics of Hepatitis B Virus (Harold SG,1988)

Pathogenesis and pathology

The mechanism of liver cell injury in hepatitis B has not been established, but there has been much conjectured about the role of the immune response (156). The existence of asymptomatic hepatitis B carriers with normal liver histology and function indicates that the virus is not directly cytopathic. The facts that lymphoid cells are juxtaposed with necrotic hepatocytes in the livers of patients with liver injury and that patients with defects in cellular immune competence are more likely to remain chronically infected rather that to clear the virus are cited to support the role or cellular immune responses in the pathogenesis of hepatitis B-related liver injury. Laboratory observations suggest that HBcAg, present on the hepatocyte cell, is the viral target for cytotoxic T-cell to destroy HBV-infected hepatocytes (157). At the same time, the kupffer cells and endothelial cells proliferate, and kupffer cells often contain excess lipofusion pigment (158). In the icteric phase of hepatitis, the walls of hepatic vein tributaries may be thickened and are frequently infiltrated, with proliferation of the lining cells in the terminal hepatic veins. Cholestasis may be found in the bile canaculi (159). As the edema extended and obstruction of the bile canaculi increases, the jaundice deepens, the liver becomes tender and palpable, the urine darkness, and the feces become pale and fatty (160). This pathologic picture is a recognized precursor to cirrhosis and possibly to primary hepatocellular carcinoma (161).

Antigenic structure of hepatitis B virus

Hepatitis B virus is a new virus family as Hepadnaviridae (162). These agents share common morphologic and biochemical properties, and all exhibit prominent liver tropism with a tendency toward chronic infection (163-165). HBV genome is a double stranded circular DNA. Electron microscopy of HBV shows three types of particles (166,169).

1. Dane Particle is a complete virus , large, double shelled spherical particle

with a diameter of 42 nm. (168). Its outer shell is composed of the hepatitis B surface antigen (HBsAg),within its core contains HBV DNA, the hepatitis core antigen (HBcAg) and a non structural antigen called the hepatitis e antigen (HBeAg).The latter circulated in the blood of patients with active replication of HBV (168).

2. Spherical particles with an average diameter of about 22 nm (167), which may be somewhat variable in size (14-38 nm.)(169)(Figure 3).

3. Filamentous form of 20 nm. Diameter and variable length (average 50-230 nm.)

The most numerous particles are spherical and filamentous forms, they are antigenically identical with the outer surface of hepatitis B and they are though to represent exess viral protein(159).

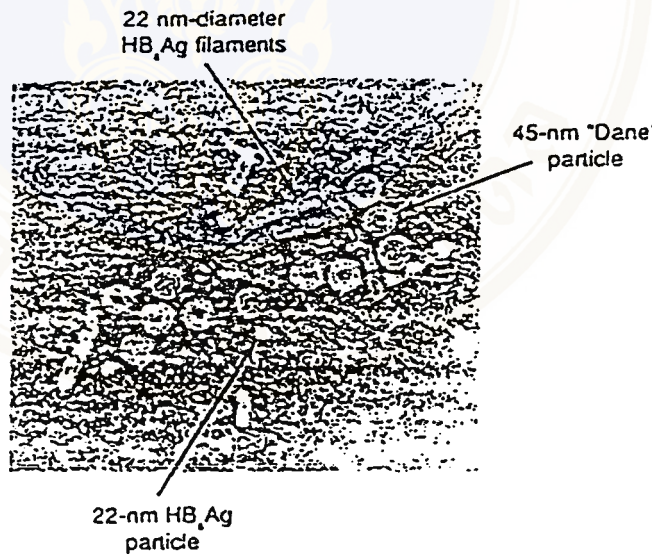


Figure 3. Electro-micrograph of 22 nm. Small, spherical aggregates of hepatitis B surface antigen (HBsAg) particle, 22 nm. Wide filamentous aggregates of the antigen, and 42-45 nm. Hepatitis B virus (" Dane") particle(Zukerman AJ, 1990)(170).

Epidemiology

The World Health Organization estimated that worldwide carrier rate of HBV has risen to 350 million people and about 434,000 cases a year of liver cancer (171). These carriers will die from cirrhosis of the liver and primary hepatocellular carcinoma(PHC)(172). There is a wide global variation in the prevalence of carrier. Among those infected, 5-10 % become carriers(173). Host factors predisposing to chronic HBV include infection in the neonatal period, a subclinical acute infection and the presence of immunologic defects in the host. Chronic infection occurs in 90% of infants infected at birth, 25-50% of children infected at 1-5 years of age, and about 5-10% infected as older children or adults(173).

Geographical Distribution : The patterns of the endemicity of hepatitis B infection various parts of the world vary greatly (174-176). The endemicity of hepatitis B infection can be considered high in those areas of the world where the prevalence of HBsAg is 8-15% and when 70-95% of the population have serological evidence of previous infection. Associated with the high rate of chronic HBV infection in these parts of the world are high rates of PHC. Within these populations, groups at high risk of infection can be identified (177), and these include homosexual men, intravenous drug abusers, health care workers, resident of institution for the developmentally disabled, chronically transfused patients, and heterosexual contacts of cases or carriers; for most such groups infection occurs in adulthood. The remaining part of the world fall into intermediate endemicity levels, with HBsAg prevalence 2-7% and overall prevalence of infection 20-60%. In these areas, transmission in infancy, childhood, and adulthood are important.

HBV infection in Thailand

Thailand is a intermediate endemicity area of HBV infection with the prevalence rate for carrier 2.5%-4.3% (178). In recent years , The National Blood Center screened for HBsAg in volunteer's blood during the three-years period. HBsAg prevalence in the total number of donors slightly decreased from 4% in 1989 to 3.5%

and 3.7% in 1990 and 1991. HBsAg prevalence rates by sex and years revealed similar percentage in male donors (4.2-4.3%) during the three-years period, whereas in female the rates were 2.9% in 1989 and 2.5% in 1990 and 1991, the prevalence in male donors was higher than in female donors(178). Data from Division of Epidemiology, Minitry of Public Health surveillance in 1997 were shown in Table3 The decade of morbility rates, the peak was slightly decreased in 1991 until in 1997.

Table 3. Reported cases of hepatitis B by morbility rate, mortality rate, Thailand,1988-1997(179)

Years	No. of cases	Morbidity rate (100,000 pop.)	No. of deaths	Mortality rate (100,000 pop.)	Mortality rate (%)
1988	1,781	3.27	8	0.01	0.45
1989	1,831	3.30	10	0.02	0.55
1990	3,162	5.62	9	0.02	0.28
1991	3,391	5.98	3	0.01	0.09
1992	3,093	5.35	3	0.01	0.10
1993	2,574	4.41	0	0.00	0.00
1994	2,653	4.49	5	0.01	0.19
1995	1,854	3.12	5	0.01	0.27
1996	1,314	2.21	5	0.01	0.38
1997	1,328	2.23	2	0.00	0.15

Patterns of host response

Hepatitis B infection can cause a broad spectrum of disease ranging from asymptomatic infection to fulminant hepatitis. Those individuals with persistant infection can remain asymptomatic or progress to chronic liver disease and primary hepatocellular carcinoma. A prodomal phase of disease usually occurs, with malaise, weakness, and anorexia being the most common findings. Myagia and arthralgia without clinical signs have been described in 10-30% of patients with acute hepatitis

B (180,181). Jaundice develops in 20-30% of infected individuals and may persist for several weeks to several months.

The patterns of expression of the various antibodies to HBV have been described in the previous section. Briefly, HBsAg becomes detectable 1 to 2 month prior to the onset of clinical symptoms and is followed by the appearance of IgM anti-HBc, and anti-HBc by onset of illness. In the late convalescent phase of those infections that resolve, the levels of HBsAg begin to decrease and eventually disappear; however, IgM anti-HBc remains detectable for at least another 6-10 weeks. Anti-HBs becomes detectable during the late convalescent phase, although a window phase may occur during which neither HBsAg nor anti-HBs is detectable by immunoassay (IgM anti-HBc will remain positive during this time) (182). Hepatitis B e antigen (HBeAg) will be present during the course of acute infection, and as the infection resolves, it will also disappear.

Among individuals in whom HBV infection persists, both HBsAg and anti-HBc remain detectable indefinitely. HBeAg will initially be detectable in all individuals; however, the loss of HBsAg occurs in about 10% of chronic carriers per beginning at a variable time period after the initial infection and appears to factors such as age of initial infection and possibly to race or other genetic factors (183,184).

Immunology

- **Humoral immune response (HIR)**

The most frequent clinical response to HBV is subclinical infection, and anti-HBs develops during convalescent, it contains high levels a long time (185). Anti-HBc response in acute hepatitis B, to be detectable early infection, is initially primary of the IgM type. It persists for a short time and declines, then anti-IgG elevates(186). Most individuals have anti-HBc detectable thoughout their life time (186). Anti-HBs develops after anti-HBc in acute infection and correlates with loss of viral replication (187) (Figure 4). Anti-HBs arises during convalescence, usually after clearance of HBsAg (186). Window period, anti-HBc may represent serologic evidence of current

or recent HBV infection, and blood contains anti-HBc in the absence of HBsAg and anti-HBs. Anti-HBs is the protective antibody.

- **Cell - mediated immune response (CMIR)**

The study of CMI, hepatocyte injury is apparently caused by the activation of cytotoxic immune machanism (188). The T cells have been shown to be specific for HBsAg expressed on the hepatocyte membrane and additionally anti-HBc has been shown to block T-cell cytotoxicity (187). CMI to HBsAg appears near the end of the acute phase of hepatitis (Figure 4), and its increase is correlated with the appearance of the circulating antigen (166).

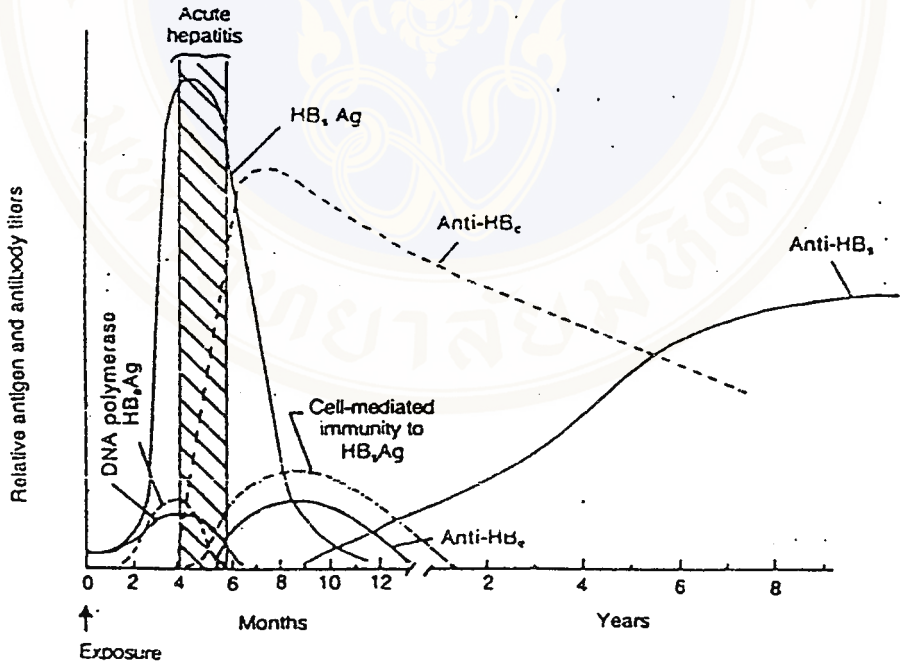


Figure 4. Diagram of the time course of circulating HBV Ags and the immune response. Note that HBcAg is not found free in the plasma. (Harold SG, 1988)(189)

Incubation period

The incubation period varies from 30-180 days, it is generally 60-90 days (190).

Serological and Immunologic Diagnostic Methods.

A variety of diagnostic methods are now at the disposal of the clinician, epidemiologist, and researcher in order to determine the consequences of HBV infection in both patients and population (182).

HBsAg is the glycoprotein coat of HBV and exists both associated with the complete virion and independently. The presence of HBsAg in the serum of an individual indicates acute or chronic HBV infection. Tests for HBsAg have evolved from the insensitive immunodiffusion methods to the most sensitive third generation tests configured either as reversed passive hemagglutination assay (RPHA), radioimmunoassay (RIA), or enzyme-linked immunoassay(EIA). Currently RIA and EIA are the most widely used methods to detect HBsAg (182). Recently monoclonal antibodies to HBsAg have been produced, and there have been reports of improved test sensitivity using these probes (191,192).

A serum sample positive for HBsAg indicates presence of HBV infection, but it does not fully determine the stage of the disease without additional clinical information such as the duration of test positivity, nor does it determine the potential infectivity of the patient. An individual is classified as a chronic carrier of HBV if HBsAg remains positive for more than 6 months. However, the relative degree of infectivity can be assessed by the presence of other markers such as HBeAg or HBV DNA. Several subtypes of HBsAg exist, and reagents for subtyping are available (193,194).

Anti-HBs is the specific antibody to HBsAg and appears after exposure to this antigen either following naturally occurring infection or through immunization. The

use of anti-HBs testing in routine evaluations is usually not indicated (195). This test is more commonly used in epidemiologic surveys to determine the immune status of an individual, in order to provide the appropriate prophylactic therapy following HBV exposure or in follow-up after hepatitis B vaccination. Both RIA and EIA tests exist for determining anti-HBs, and quantitative comparisons of anti-HBs must be made using a reference standard to determine millinternational units (mIU) of the antibody (182). The RIA for anti-HBs is considered positive at a signal-to-noise ratio of greater than 2.1; however, the biological significance of SRU ratios less than 10 is doubtful, as indicated by normal response to vaccine and lack of production against infection among persons with anti-HBs levels of less than 10 SRU (196). The use of EIA has eliminated this problem in most commercial tests through its slightly lower sensitivity.

Anti-HBc is rapidly induced soon after the establishment of HBV infection and is usually positive at the onset of illness. This antibody is initially of the IgM class and then awitches to predominantly IgG-class antibody, which persists along with anti-HBs for the lifetime of the previously infected individual. Anti-HBc may be detected alone (without detectable HBsAg or anti-HBs) during the early convalescent phase of acute infection (window phase) (197). In addition, this pattern may persist for several years and, in a small proportion of cases, may be accompainied by ongoing HBV replication with subdetectable levels of HBsAg. This may account for the low level infectivity of HBsAg-negative blood transfusions. Finally, this pattern may result many years after resolved infection because of the decay of anti-HBs and the persistence of only anti-HBc (196).

Hepatitis B Virus Transmission

More than 40% of patients are unaware of how they acquired the virus. However, modes of transmission are well established. Both sexual and parenteral routes were the predominant modes of HBV transmission (166).

1. Transmission by injecting drug users (IDUs)

The risk factors for transmission of HBV among IDUs are well documented(206). Between 15% to 36% of IDUs in England and Wales have evidence of prior or current infection with HBV(207). Duration of injecting drug use was also a strong predictor of HBV infection(208). A history of using shared syring in IDUs was significantly associated with HBV, HCV and Human Immunodeficiency Virus (HIV) infections (23).

2. Transmission by blood transfusion

Hepatitis B infection in transfusion recipients was investigated by the national blood services to identify if they were transmitted by transfusion. The reports in England and Wales during 1991-1997 were found that 24 of 4,188 transfusion recipients (0.6%) were associated with transfusion. Hepatitis B virus is bloodborne pathogen, it can be transmitted parenterally (2%) by blood or blood products and tissue or organ transplantation (212). These data show that transmission of HBV by transfusion frequently occurs(208).

3. Transmission by dialysis

The report in 1994-1997 found that the prevalence rates of HBsAg positive among patients on dialysis treatment were 5.3%, 5.3%, 4.5% and 4.2%(209).

4. Transmission by ear piercing or tattooing

The cross-sectional study of 705 drug addicts at Thanyarak Hospital in Thailand,1997 showed that ear piercing was associated with HBV markers (210). In any case, the study of first blood donors in Netherlands in 1993 found that there was no the association between ear piercing and HBV marker(211).

5. Sexual transmission

HBV is one of sexually transmitted micro-organisms in about 33% of cases because it is present in semen and vaginal secretions (212). The risk of HBV infection was to have two or more sexual partners (20,24) and the other factor was the history of homosexual activity(22). Furthermore, Regular sexual contacts of adults with acute HBV infection are at high risk of infection(approximately 30%)(215).

6. Transmission by contacting secretion or body fluids

Contact of nonintact skin or mucous membranes with patient's blood or body fluids is another mode of HBV transmission (213).

7. Transmission in family (Intrafamilial transmission)

The HBV is easily transmitted, as is demonstrated in one report where 10 members of an extended family of 29 (34%) became infected cases from a source in the family . Transmission occurred in a variety of ways : sexually in some, through shared toothbrushes and close contact in others (214). Previous studies have shown that the persons living in the same household as an HBV carrier have a 40% or higher likelihood of current or prior HBV infection(216,217).

8. Mother-Infant transmission

In Thailand, vertical transmission is a major source of the infection. About 5% of pregnant woman sera were positive for HBsAg and 40-50% of them were positive for HBeAg . Eighty to ninety per cent of infants born to HBsAg and HBeAg positive mothers had hepatitis B infection without having hepatitis vaccination (215).

Prevention and control

A number of control measures have been utilized to interrupt transmission of HBV and reduce the incidence of hepatitis B. These included:

1. The use of simple enviromental procedures to limit the risk of infection (197); for example, to change the gloves when contaminated with blood or when contact with potentially infective materials.
2. Vaccination of at-risk groups (197), such as infants born to carrier mothers, individuals who frequently change sexual partners, and intravenous drug abusers.
3. Administration of high-titered hepatitis B immune globulin when indicated (197); such as infants born to carrier mothers, health care workers who are accidentally pricked with needles used for patients with hepatitis B.
4. Proper selection and screening of blood donors for HBsAg and anti-HBc (197).
5. Education of health-care workers and other high-risk groups concerning patterns of transmission (197); such as personnel, including teaching and training staff, directly involved in patient care over a period of time, and the spouses and other sexual contacts of patients with acute hepatitis B or carriers of hepatitis B virus, and other family members in close contact.
6. Disinfection of hepatitis B, nondisposible medical instruments and equipment; such as heat sterilization, chemical disinfection (198).

CHAPTER III

MATERIALS AND METHODS

Study design

The study design was a cross-sectional analytic study by using a risk assessment form for anti-HCV and HBsAg screening at least 2,000 blood donors who attended the Regional Blood Center of Buddhachinaraj Hospital in Phitsanulok Province during April 1999 - September 1999.

Sample size calculation

The sample size in this study was calculated by using the following formula.

$$n = \frac{(15.4)\,P\,(1-P)}{(R-L)^2}$$

n = sample size

P = mean of R and L

R = high predictive value of test

L = low predictive value of test (220)

To calculate the sample size twice times, the first was to calculate the sensitivity and the second was to calculate the specificity. This study predicted the sensitivity of the risk assessment form ranging from 50% to 75% and the specificity from 85% to 95%.

Thus $n1 = \frac{(15.4)\,(.625)\,(.375)}{(.25)^2}$ n1 = number of positive cases

$= 57.75$

$$\approx 58 \text{ cases}$$

$$n2 = \frac{(15.4)(.9)(.1)}{(.1)^2} \qquad n2 = \text{number of negative cases}$$

$$= 138.6$$

$$\approx 139 \text{ cases}$$

Previous studies showed that the prevalence of HCV infection was 1.5-2% and HBV carrier rate was about 5%(11,178). Therefore, this study conducted at least 2,000 blood donors until the positive cases of anti-HCV or HBsAg were approximately 58 cases.

Research instruments

1. Risk assessment form

It included socio-economic factors and risk behaviors for HCV and HBV infections adapted from the form of the National Blood Center, Thai Red Cross Society. The content validity was evaluated by all thesis.

2. Laboratory methods

- Assay of anti-HCV by ELISA (ABBOTT HCV EIA 3.0)
- Assay of HBsAg by ELISA (Enzygnost® HBsAg 5.0)

Laboratory methods

Assay of anti-HCV

Sera were collected and kept at 2 °C to 8 °C for up to fourteen days. After throwing, the serum samples were thoroughly mixed and dispensed. Dispense 10 µl of each control or specimen into the bottom of an individual test tube, pre-dilution tray or equivalent. Dispense control in the sequence of 3 replicates of the negative control followed by 3 replicates of the positive control. Dispense specimens after control and dispense 400 µl of specimen diluent to each tube, pre-dilution well or equivalent mix thoughly by gently tapping and transfer 200 µl of each diluted control or specimen into appropriate well of reaction tray. Carefully add one bead to each well containing diluted control or specimen. Apply cover seal and tap tray gently. Incubate at 40 ± 2 °C for 1 hour ± 5 minutes in a commander dynamic incubator in the rotation mode later remove and discard cover seal and wash each bead. Pipette 200 µl of diluted conjugate into each well containing a bead and apply new cover seal. Tap tray gently incubate at 40 ± 2 °C for 30 ± 2 minutes in a commander dynamic incubator in the rotation mode. Remove and discard cover seal. Wash each bead and transfer bead immediately to properly identified assay tubes. Prime dispense immediately prior to dispensing OPD Substrate Solution. Pipette 300 µl of freshly prepared OPD Substrate Solution into two empty tube (substrate blanks) and then into each tube containing a bead. Cover and incubate at room temperature (15°C to 30°C) for 30 ± 2 minutes. Add 1 ml of 1 N Sulfuric Acid to each tube agitate to mix. The assay was read within two hours after acid addition and cut-off value for anti-HCV reactive followed the criteria of ABBOTT HCV EIA 3.0. The sensitivity for this assay which was determined using two different samples collective with clinically diagnosed acute NANBH was 100 % or chronic NANBH was 99% and the specificity was estimated to be 99.6%(204.).

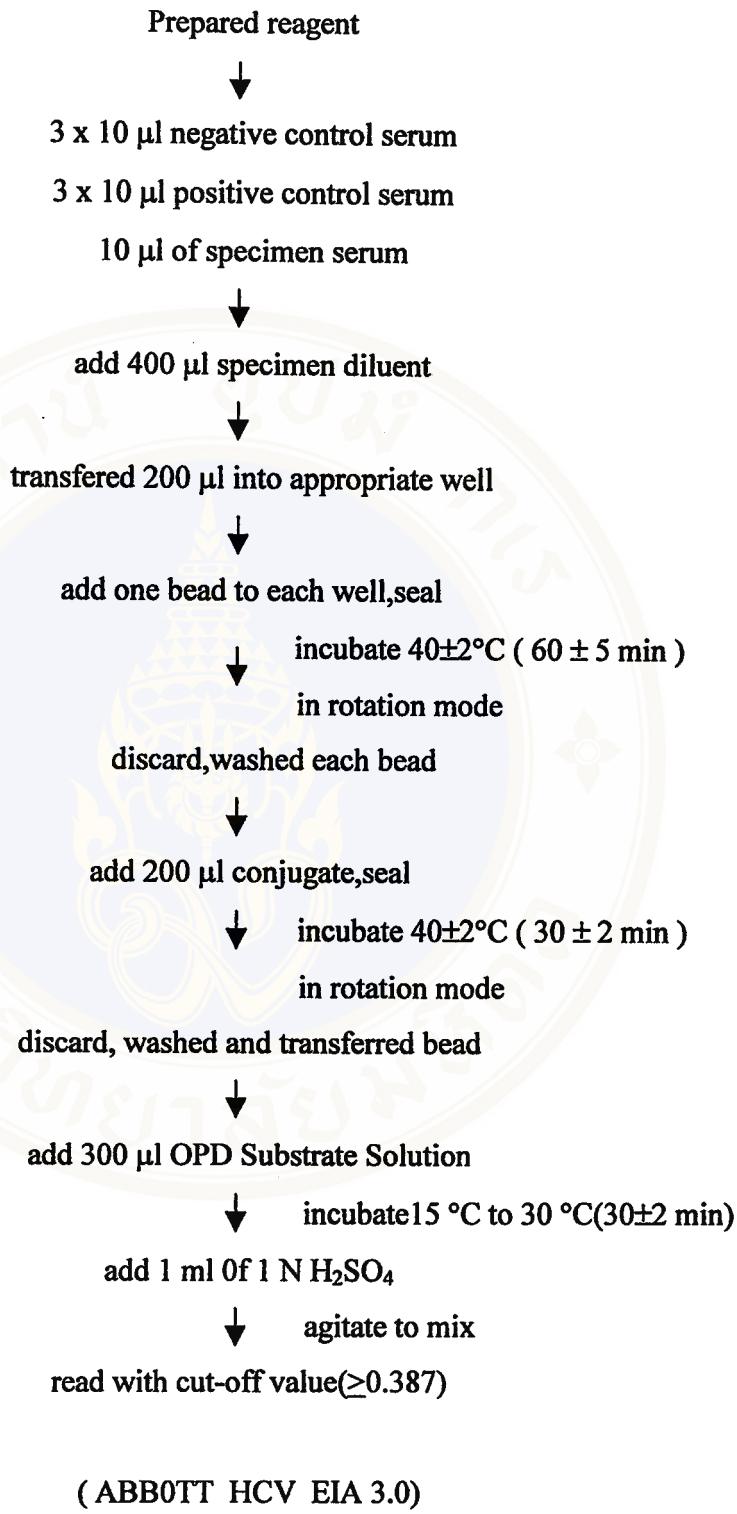
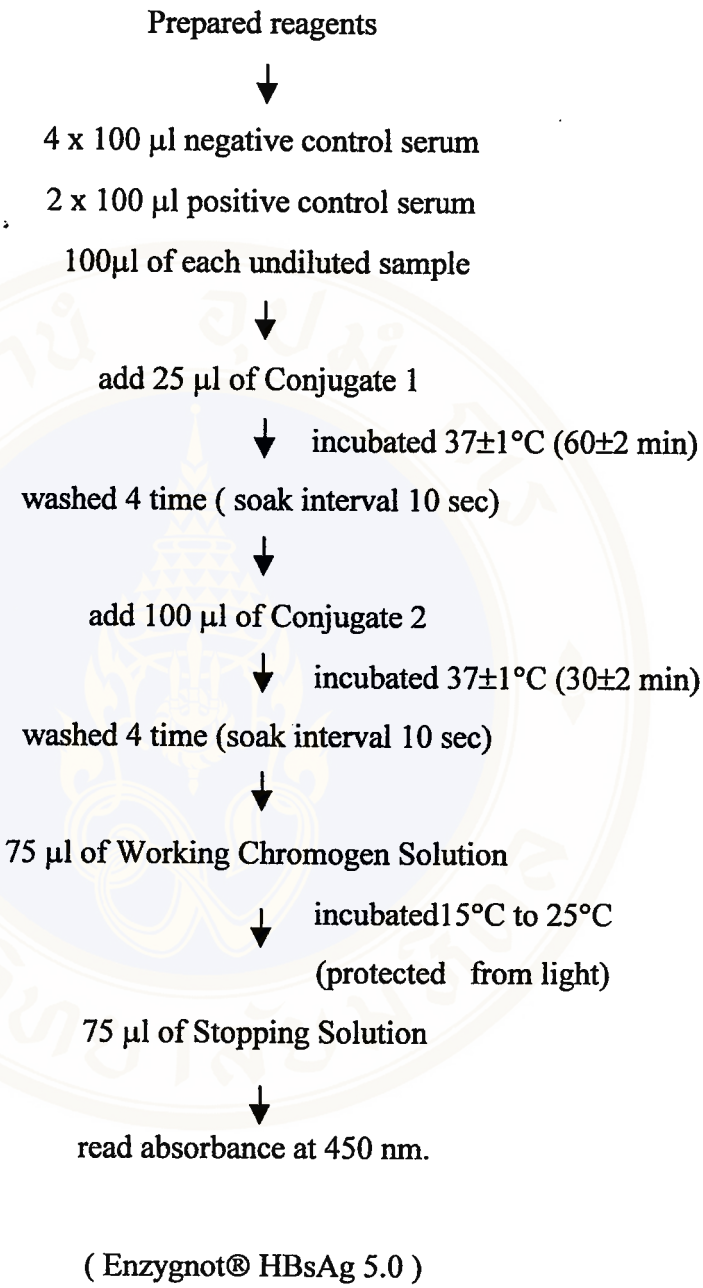


Figure 5. Diagram of assay for anti-HCV

Assay of HBsAg

Sera were collected and kept at 2°C to 8°C before beginning the test. After throwing, the serum samples were throughly mixed. Pipette 100 µl/well of the negative control into 4 wells and 100 µl/well of the positive control into 2 wells, then fill the following wells with 100 µl/well of undiluted sample. Fill each well with 25 µl of Conjugate 1 (Anti-HBs/Biotin) and seal with foil, place into the incubator and incubate at 37 ± 1 °C for 60 ± 2 minutes. Remove the foil and aspirate all the wells. Then wash 4 times with approximately 0.3 ml of washing solution each time, with a 10- sec soak interval per wash. Immediately after completing the washing cycles dispense Conjugate 2 the wells cannot dry out. Fill each well with 100 µl of Conjugate 2 (Streptavidin/POD) and seal with foil, place into the incubator and incubate at 37 ± 1 °C for 30 ± 2 minutes. Remove the foil and aspirate all the wells. Then wash 4 times with approximately 0.3 ml of washing solution each time, with a 10-sec soak interval per wash. Fill each well with 75 µl of the Working Chromogen Solution and seal the plate with fresh foil. Incubate at 15 to 25 °C for 30 ± 2 minutes protected from light. Remove the foil, add 75 µl of Stopping Solution POD to each well and read at 450 nm within 1 hour. The sensitivity of this test was 100% and the specificity was 99.83% for initial testing and 99.85% for retest results (205).



(Enzygnot® HBsAg 5.0)

Figure 6. Diagram of assay for HBsAg

Data analysis

The data were analyzed by the computer using the SPSS FOR WINDOW version 7.5 program and presented in following ;

1. Descriptive Analysis

The general characteristics of the studied subjects were shown by percentage, mean, and standard deviations.

2. Analytic Analysis

2.1 Univariate analysis

The risk factors of blood donors with or without anti-HCV and/or HBsAg positive were compared by using odds ratio and their statistical significance was tested by the Chi-square test.

2.2 Multivariate analysis

A multiple logistic regression analysis was performed in order to identify risk behaviors/factors associated with anti-HCV and/or HBsAg positive and to control for possible confounding factors. A p-value of 0.05 or less was considered to indicate statistical significance.

The odds ratio estimated of the multiple logistic regression model could be used for calculating any individual behaviors risk scores of HCV infection and HBsAg positive. An optimal cut-off point for total risk scores was determined between risk and no risk to HCV and HBV infections by the Receiver Operating Characteristic Curve (ROC) (219). The validity of the assessment form was calculated from ROC curve.

CHAPTER IV

RESULTS

The 2,167 blood donors in Phitsanulok Province were carried out to investigate the seroprevalence , risk factors and validity of risk assessment form for screening HCV infection and HBsAg positive carriers. The results were presented into four parts following :

1. General characteristics of studied blood donors in Phitsanulok Province.
2. Prevalence rates of anti-HCV and HBsAg positive in studied blood donors.
3. Risk factors/behaviors of HCV infection and HBsAg positive carrier.
4. Validity of the risk assessment form for screening HCV infection and HBsAg positive carriers.

1. General characteristics of studied blood donors in Phitsanulok Province

The ages of studied blood donors ranged from 17 to 60 years (The mean age was 32.07 years). Among 2,167 blood donors, 1,716(79.18%) were male and 451 (20.82%) were female. Most of them (53..49%) were married and 45.77% were single. A majority of them finished secondary school(35.77%). The occupations of them were police/military(33.74%), laborer(19.79%), agriculture (11.85%) and government officer (11.77%). Most of them(59.39%) lived in the urban and they were buddhist. The details are shown in Table 3.

Table 3. The general characteristics of 2,167 studied blood donors in Phitsanulok Province

General appearance	Number	Percentage
Age group (years)		
17-30	1,063	49.25
31-45	863	39.86
46-60	236	10.89
	median = 31.00 , min = 17 , max = 60	
	mean = 32.07 , SD = 10.14	
Sex		
Male	1,716	79.18
Female	451	20.82
Marital status		
Single	992	45.77
Married	1,159	53.49
Widowed/Divorced/Separated	16	0.74
Education		
Primary school	496	22.88
Secondary school	775	35.77
Vocational level	330	15.23
Undergraduate level	566	26.12
Occupation		
Government officer	255	11.77
Police and military	731	33.74
Student	233	10.75
Theologian	75	3.46
Commerce/business	77	3.56
Laborer	429	19.79
Agriculture	257	11.85
State enterprise	67	3.09
Unemployed	43	1.99
Residence		
Urban area	1,287	59.39
Rural area	880	40.61

Table 3,(Continued)

General appearance	Number	Percentage(%)
Religion		
Buddhism	2,131	98.33
Christian	35	1.62
Other	1	0.05
Blood group		
A	433	19.98
B	787	36.32
AB	156	7.19
O	791	36.51
Total	**2,167**	**100.00**

2. Prevalence rates of anti-HCV and HBsAg positive in studied blood donors

Out of 2,167 blood donors, 63(2.90%) appeared anti-HCV positive and 100 (4.61%) were HBsAg positive (Table4).

The highest prevalence of anti-HCV (3.36%) was found in donors with age 31 to 45 years, whereas the highest prevalence of HBsAg (5.89%) was found in age group 15-30 years(Table 4). The prevalence in male was significantly higher than female (3.20% & 1.77% for anti-HCV and 5.36% & 1.77% for HBsAg)(Table 5). The single has higher anti-HCV prevalence and HBsAg positive rate than the married group(3.12% & 2.76% for anti-HCV and 5.95% & 3.45% for HBsAg)(Table 6). The prevalence in donors with primary school was higher than the other class(7.05% for anti-HCV and 5.24 for HBsAg positive)(Table 7). When the prevalence was classified by occupation, 8.95% was higher positive for anti-HCV in agriculture group and 15% was higher positive for HBsAg in police/military(Table 8). The prevalence in rural area was significantly higher than in urban area(4.77% & 1.63% for anti-HCV and 5.23% & 4.19% for HBsAg positive)(Table9). The prevalence in buddhism was higher than the other group(2.91% for anti-HCV and 4.65% for HBsAg positive) (Table 10). When the prevalence was classified by blood group,4.62% was higher

positive for anti-HCV in blood group A and 5.81% was higher positive for HBsAg in blood group O (Table 11).

Table 4. Prevalence rates of anti-HCV and HBsAg in blood donors classified by age

Age group (years)	No. of tested	No. of positive cases (%)	
		Anti-HCV	HBsAg
≤30	1,068	32 (2.99)	63 (5.89)
31-45	863	29 (3.36)	29 (3.36)
≥46	236	2 (0.85)	8 (3.39)
Total	2,167	63 (2.90)	100 (4.61)

Table 5. Prevalence rates of anti-HCV and HBsAg in studied blood donors classified by sex

Sex (years)	No. of tested	No. of positive case (%)	
		Anti-HCV	HBsAg
Male	1,716	55 (3.20)[a]	92 (5.36)[b]
Female	451	8 (1.77)[a]	8 (1.77)[b]
Total	2,167	63 (2.90)	100 (4.61)

[a] Statistically significant difference by proportional Z test ($p < 0.001$)
[b] Statistically significant difference by proportional Z test ($p < 0.001$)

Table 6. Prevalence rates of anti-HCV and HBsAg in studied blood donors classified by marital status

Marital status	No. of tested	No. of positive cases (%)	
		Anti-HCV	HBsAg
Single	992	31 (3.12)[a]	59 (5.95)[b]
Married	1,159	32 (2.76)[a]	40 (3.45)[b]
Widowed/Divorced/ Separated	16	0	1 (6.25)
Total	2,167	63 (2.90)	100 (4.61)

[a] Statistically significant difference by proportional Z test ($p < 0.001$)
[b] Statistically significant difference by proportional Z test ($p < 0.001$)

Table 7. Prevalence rates of anti-HCV and HBsAg in studied blood donors classified by education

Education	No. of tested	No. of positive cases (%)	
		Anti-HCV	HBsAg
Primary school	496	35 (7.05)	26 (5.24)
Secondary school	775	14 (1.80)	41 (5.29)
Vocational	330	9 (2.73)	12 (3.64)
Undergraduate level	566	5 (0.88)	21 (3.71)
Total	2,167	63 (2.90)	100 (4.61)

Table 8. Prevalence rate of anti-HCV and HBsAg in studied blood donors classified by occupation

Occupation	No. of tested	No. of positive cases (%)	
		Anti-HCV	HBsAg
Government	255	3 (1.17)	5 (1.96)
Police/military	731	21 (6.90)	48 (15.00)
Student	233	4 (1.72)	5 (2.14)
Theologian	75	1 (1.33)	5 (6.67)
Commerce/ Business	77	1 (1.29)	2 (2.59)
Laborer	429	7 (1.63)	21 (4.89)
Agriculture	257	23 (8.95)	11 (4.28)
State enterprise	67	2 (2.98)	3 (4.48)
Unemployed	43	1 (3.45)	0 (0.00)
Total	2,167	63 (2.90)	100 (4.61)

Table 9. Prevalence rates of anti-HCV and HBsAg in studied blood donors classified by address.

Address	No. of tested	No. of positive cases (%)	
		Anti-HCV	HBsAg
Rural	880	42 (4.77)[a]	46 (5.23)[b]
Urban	1,287	21 (1.63)[a]	54 (4.19)[b]
Total	2,167	63 (2.90)	100 (4.61)

[a] Statistically significant difference by proportional Z test ($p < 0.001$)

[b] Statistically significant difference by proportional Z test ($p < 0.001$)

Table 10. Prevalence rates of anti - HCV and HBsAg in studied blood donors classified by religion

Religion	No. of tested	No. of positive cases (%)	
		Anti-HCV	HBsAg
Buddhism	2,131	62 (2.91)	99 (4.65)
Christianty	35	1 (2.86)	1 (2.86)
Other	1	0	0
Total	2,167	63 (2.90)	100 (4.61)

Table 11. Prevalence rates of anti – HCV and HBsAg in studied blood donors classified by blood group

Blood group	No. of tested	No. of positive cases (%)	
		Anti-HCV	HBsAg
A	433	20 (4.62)	18 (4.16)
B	787	23 (2.92)	30 (3.81)
AB	156	5 (3.20)	6 (3.20)
O	791	15 (1.89)	46 (5.81)
Total	2,167	63 (2.90)	100 (4.61)

3. Risk factors/behaviors of HCV infection and HBsAg positive carriers

3.1 Univariate analysis

HCV infection : After analysis, the significant association between risk factors or behaviors and HCV infection among studied blood donors included (1) gender - male (OR = 1.94), (2) education - primary school or illiteracy (OR = 4.15), (3) residence in rural (OR = 3.09), (4) history of receiving blood or blood products (OR = 5.21), (5) history of tattoing (OR = 1.70), (6) history of IDUs (OR = 41.43), (7) history of sexual service (OR = 4.24) and (8) history STDs (OR = 3.87),Table12.

HBsAg positive : After analysis, the significant association between risk factors or behaviors and HBsAg positive among studied blood donors included (1) ages as equal or more than 30 years (OR = 1.81), (2) gender - male (OR = 3.09), (3) marital status as single (OR = 1.76), (4) history of IDUs (OR= 1.95), (5) history of extramarital sex relation (OR = 3.01) and (6) history of liver disease or jaundice (OR = 4.04), Table13.

HCV infection and HBsAg positive : After analysis, the significant association between risk factors or behaviors and HCV infection and HBsAg positive among studied blood donors included (1) ages as equal or more than 30 years (OR = 1.46), (2) gender - male (OR = 2.48), (3) marital status - single (OR = 1.49), (4) education as primary school or illiteracy (OR = 2.04), (5) residence in rural (OR =1.84), (6) history of IDUs (OR = 20.24), (7) history of receiving blood or blood products (OR = 2.89), (8) history of liver disease or jaundice (OR = 2.88),(9) alcohol consumption (OR = 1.47) and (10) history of extramarital sex relation (OR = 3.19), Table14.

Table12. Summary of risk factors to HCV infection by univariate analysis

Risk factors	Crude OR	95% CI	p-value from X^2 test
Ages (years)			
≥ 30	0.89	0.53-1.48	0.711
< 30	1.00		
Gender			
Male	1.94	1.01-4.08	0 .042
Female	1.00		
Education			
Primary school/Illiteracy	4.15	2.59-6.65	< 0.001
≥ Secondary school	1.00		
Marital status			
Single	1.23	0.74-2.05	0.449
Others	1.00		
Residence			
Rural	3.09	1.84-5.18	< 0.001
Urban	1.00		
Religion			
Buddhism	1.12	0.16-22.29	0.981
Others	1.00		
History of receiving blood or blood products			
Yes	5.21	2.26-12.01	< 0.001
No	1.00		
History of operating or delivery			
Yes	1.32	0.70-2.45	0.907
No	1.00		
History of transplantation			
Yes	0.38	0.75-29.15	0.535
No	1.00		
History of contacting with blood or secretion			
Yes	1.03	0.41-2.61	0.586
No	1.00		

Table 12. (Continued)

Risk factors	Crude OR	95% CI	p-value from X^2 test
History of tattooing			
Yes	1.70	1.02-2.86	0.043
No	1.00		
History of IDUs			
Yes	41.43	17.52-97.99	< 0.001
No	1.00		
History of liver disease or jaundice			
Yes	1.20	0.29-4.07	0.470
No	1.00		
Hemophilia			
Yes	1.85	0.00-71.32	0.174
No	1.00		
Alcohol consumption			
Yes	1.52	0.91-2.55	0.113
No	1.00		
History of STDs			
Yes	3.87	1.49-10.04	0.021
No	1.00		
History of sexual service			
Yes	4.24	1.23-13.10	0.017
No	1.00		
History of extramarital sex relation			
Yes	0.71	0.41-1.23	0.247
No	1.00		
History of homosexual activity			
Yes	1.05	0.26-4.17	0.956
No	1.00		

Table 13. Summary of risk factors to HBsAg positive carriers by univariate analysis

Risk factors	Crude OR	95% CI	p-value from X^2
Ages (years)			
≥ 30	1.81	1.22-2.68	0.002
< 30	1.00		
Gender			
Male	3.09	1.51-6.31	< 0.001
Female	1.00		
Education			
Primary school/illiteracy	1.19	0.74-1.89	0.401
≥ Secondary school	1.00		
Marital status			
Single	1.76	1.19-2.59	0.002
Others	1.00		
Residence			
Rural	1.31	0.88-1.96	0.105
Urban	1.00		
Religion			
Buddhism	1.74	0.25-34.53	0.852
Others	1.00		
History of receiving blood or blood products			
Yes	1.33	0.32-4.54	0.811
No	1.00		
History of operation or delivery			
Yes	0.67	0.35-1.25	0.543
No	1.00		
History of transplantation or dialysis			
Yes	3.40	0.49-18.94	0.760
No	1.00		
History of contacting with blood or secretion			
Yes	0.67	0.35-1.29	0.157
No	1.00		

Table 13. (Continued)

Risk factors	Crude OR	95% CI	p-value from X^2 test
History of tattooing			
Yes	0.86	0.55-1.34	0.828
No	1.00		
History of IDUs			
Yes	1.95	1.45-8.42	0.002
No	1.00		
History of liver disease or jaundice			
Yes	4.04	2.41-6.79	< 0.001
No	1.00		
Hemophilia			
Yes	0.09	0.00-1.08	0.135
No	1.00		
Alcohol consumption			
Yes	0.71	0.47-1.08	0.119
No	1.00		
History of STDs			
Yes	1.23	0.31-4.74	0.905
No	1.00		
History sexual service			
Yes	0.95	0.94-0.96	0.824
No	1.00		
History extramarital sex relation			
Yes	3.01	1.93-4.67	< 0.001
No	1.00		
History homosexual activity			
Yes	2.24	0.85-5.58	0.116
No	1.00		

Table 14. Summary of risk factors to HCV infection and HBsAg positive carriers by univariate

Risk factors	Crude OR	95% CI	p-value from X^2 test
Ages (years)			
≥ 30	1.46	1.08-1.96	0.007
< 30	1.00		
Gender			
Male	2.48	1.49-4.11	< 0.001
Female	1.00		
Education			
Primary school/Illiteracy	2.04	1.52-2.74	< 0.001
≥ Secondary school	1.00		
Marital status			
Single	1.49	1.11-1.99	0.005
Others	1.00		
Residence			
Rural	1.84	1.34-2.52	< 0.001
Urban	1.00		
Religion			
Buddhism	1.43	0.33-8.66	0.868
Others	1.00		
History of receiving blood or blood products			
Yes	2.89	1.43-5.88	< 0.001
No	1.00		
History of operation or delivery			
Yes	0.93	0.60-1.45	0.830
No	1.00		
History of transplantation or dialysis			
Yes	4.84	0.65-28.23	0.172
No	1.00		
History of contacting with blood or blood products			
Yes	0.77	0.45-1.32	0.209
No	1.00		

Table 14. (Continued)

Risk factors	Crude OR	95% CI	p-value from X^2 test
History of tattooing			
Yes	1.21	0.86-1.70	0.301
No	1.00		
History of IDUs			
Yes	20.24	8.62-47.52	< 0.001
No	1.00		
History of liver disease or jaundice			
Yes	2.88	1.84-4.52	< 0.001
No	1.00		
Hemophilia			
Yes	0.16	0.01-1.84	0.214
No	1.00		
Alcohol consumption			
Yes	1.47	1.06-2.04	0.020
No	1.00		
History of STDs			
Yes	4.01	0.415-38.76	0.275
No	1.00		
History of sexual service			
Yes	0.30	0.05-1.68	0.275
No	1.00		
History of extramarital sex relation			
Yes	3.19	2.24-4.56	< 0.001
No	1.00		
History of homosexual activity			
Yes	1.53	0.67-3.38	0.373
No	1.00		

3.2 Multivariate analysis

Multiple logistic regression was applied for controlling confounders and for evaluating the effect of risk variables on HCV infection , HBsAg positive and HCV infection and HBsAg positive. The order variables which were significant ($p < 0.05$) and the corresponding p-values ($p \leq 0.1$) were entered into the logistic regression model to be as following :

HCV infection : After analysis, four variables directly related to HCV infection included (1) residence-rural (OR = 2.14, p = 0.0092), (2)education - primary school (OR = 5.64, p = 0.0006),(3) history of receiving blood or blood products (OR = 4.13, p = 0.0029) and (4)history of IDUs (OR = 2.32, p = < 0.0001), Table 15.

HBsAg positive : After analysis, six variables directly related to HBsAg positive included (1) age - equal or more than 30 years (OR = 1.86, p = 0.0038), (2) gender - male (OR = 3.04, p = 0.0007), (3)history of IDUs (OR = 1.41,p = 0.0184) , (4)history of liver disease or jaundice (OR = 5.39, p = < 0.0001), (5)history of hemophilia (OR = 18.85, p = 0.0193) and (6)history of having extramarital sex relation (OR = 1.18, p = 0.0013), Table 16.

HCV and HBsAg positive : After analysis, eight variables directly related to HCV infection and HBsAg positive included (1)age as equal or more than 30 years (OR = 1.46, p = 0.0025), (2)gender as male (OR = 2.48, p < 0.0001), (3)education as primary school (OR = 2.34, p = 0.0015), (4) residence in rural(OR = 1.60, p = 0.0083), (5)history of receiving blood or blood products (OR = 4.46, p = 0.0409), (6) history of IDUs(OR = 1.97, p = < 0.0001), (7)history of liver disease or jaundice(OR = 4.46, p = < 0.0001) and (8)history of having extramarital sex relation (OR = 1.17, p = 0.0006), Table 17.

Table 15. Risk factors/behaviors of HCV infection in studied blood donors after analysis by logistic regression model (Multivariate Analysis)

Risk factors/behaviors	Adjusted Odd Ratio	95% CI	p-value
Address			
Rural	2.14	1.19-3.85	0.0092
Urban	1.00		
Education			
Primary school	5.64	2.10-15.15	0.0006
Secondary school	1.71	0.60-4.91	0.3135
Vocational level	2.84	0.91-8.86	0.0718
Undergraduate level	1.00		
History of receiving blood or blood product			
Yes	4.13	1.87-9.16	0.0029
No	1.00		
IDUs			
Yes	2.32	1.41-3.82	<0.0001
No	1.00		

Table 16. Risk factors / behaviors of HBsAg positive in studied blood donors after analysis by logistic regression model (Multivariate Analysis).

Risk factors/behaviors	Adjusted Odd Ratio	95% CI	p-value
Age (years)			
≥ 30	1.86	1.70-2.02	0.0038
< 30	1.00		
Gender			
Male	3.04	1.45-6.35	0.0007
Female	1.00		
IDUs			
Yes	1.41	1.07-1.86	0.0184
No	1.00		
History of liver disease /jaundice			
Yes	5.39	2.86-10.15	< 0.0001
No	1.00		
History of hemophilia			
Yes	18.85	1.61-220.77	0.0193
No	1.00		
Extramarital sex relation			
Yes	1.18	1.08-1.29	0.0013
No	1.00		

Table17. Risk factors/behaviors of HCV infection and HBsAg positive in Studied blood donors after analysis by logistic regression model (Multivariate Analysis).

Risk factors/behaviors	Adjusted Odd Ratio	95% CI	p-value
Age (years)			
≥ 30	1.46	1.08-1.96	0.0025
< 30	1.00		
Gender			
Male	2.48	1.49-4.11	< 0.0001
Female	1.00		
Education			
Primary school	2.34	1.41-3.87	0.0015
Secondary school	1.00		
Vocational			
Undergraduate level			
Residence			
Rural	1.60	1.13-2.27	0.0083
Urban	1.00		
History of receiving blood/blood product			
Yes	4.46	2.48-8.03	0.0409
No	1.00		
IDUs			
Yes	1.97	1.30-2.99	< 0.0001
No	1.00		
History of liver disease /jaundice			
Yes	4.46	2.48-8.03	< 0.0001
No	1.00		
Extramarital sex relation			
Yes	1.17	1.08-1.27	0.0006
No	1.00		

4. Validity of the risk assessment form for screening HCV infection and/or HBsAg positive

The risk assessment was analyzed by risk scores calculation with weight by odds ratio value and probability for prediction and the ROC curve decided the cut-off point for risk scores to be risk or no risk to HCV infection and/or HBsAg positive. The validity of the risk assessment form was analysed.

- **Risk scores of HCV infection**

The risk assessment of HCV infection in studied blood donors was carried out by the risk scores followed :

1. Formula of risk scores model was calculated by SPSS FOR WINDOW version 7.5 and cut-off point was calculated by SPSS FOR WINDOW version 9.0 program. The adjusted odds ratio of each variable in Table 15 was used to calculate risk scores by weight as following :

Risk scores = Residence + Education + History of receiving blood or blood products + History of IDUs

when ;

Scores of residence = 2 for rural area
= 0 for urban area

Scores of education = 6 primary school/illiteracy
= 3 for vocational education
= 2 for secondary school education
= 0 for academic degree

Scores of history of receiving blood or blood products
= 4 when history of receiving blood or blood products
= 0 when never

Scores of IDUs = 2 when history of IDUs
= 0 when never

2. The calculation of a risk scores from the model was analyzed and shown in Table15. The diagnotic prognosis by risk scores was convinced for screening HCV infection(95%CI = 1.24-1.43, $p < 0.001$). The validity of this model used for a predictor of the risk of HCV infection was shown in Table18 and Figure7 (sensitivity of this model = 55.2% and specificity = 85.7%). The risk assessment form of HCV infection was shown in Figure8. The blood donors who had scores 0-6 was no risk to HCV infection, whereas another one who had scores equal to or more than 7 has risk to HCV infection. Risk scores of individual blood donors has weight by adjusted odds ratio and calculated by logistic model.

Table 18. Validity of risk assessment form by risk scores model as a predictor of HCV infection in blood donors

Risk score positive if greater than or equal to :	Sensitivity (%)	Specificity (%)	1-Specificity
0	100	0	1
1	98.5	19.8	0.802
3	86.6	46.3	0.532
4	73.1	56.8	0.432
5	61.2	71.8	0.282
6	61.2	77	0.23
7	55.2	85.7	0.143
8	55.2	85.8	0.142
9	17.9	98.9	0.011
11	17.9	99.2	0.008
13	10.4	99.8	0.002
15	7.5	99.9	0.001
17	6	99.9	0.001
22	3	99.9	0.001
26	3	100	0
27	0	100	0
29	0	100	0

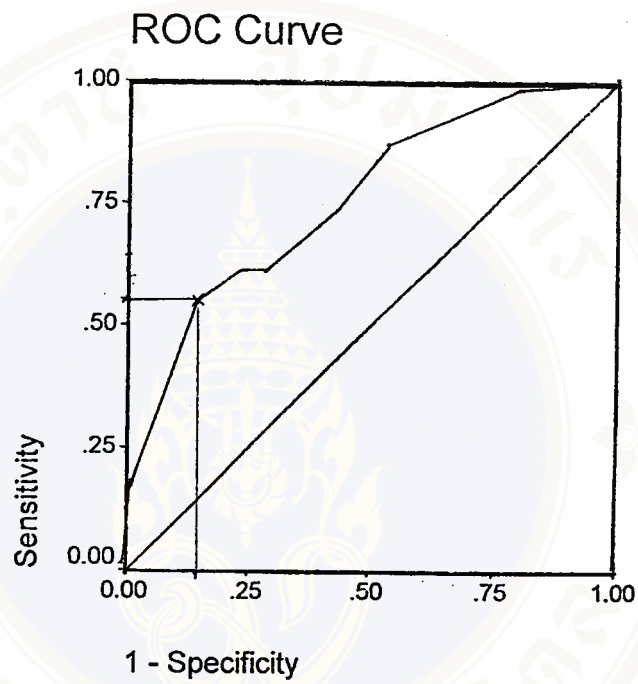


Figure 7. ROC Curve in the prediction of HCV infection .

- Cut-off point at risk score of 7
- Sensitivity was 55.2% , Specificity was 85.7%
- Area under curve was 75.9%

Figure 8. The Risk Assessment form of HCV infection in blood donors

Risk assessment form of HCV infection in blood donors

Name.....Marital Status.....

Address.....Telephone.....

Date of Birth.....Age....years....months Gender.....Race.....Religion.....

Education.....Occupation.....

Risk factors/behaviors		Scores	Checklist Scores
Residence	: Rural	2	
	Urban	0	
Education	: Primary school	6	
	Secondary school	2	
	Vocational level	3	
	Undergraduate level	0	
History of receiving blood or blood products			
	: Yes	4	
	No	0	
History of IDUs	: Yes	2	
	No	0	
Total scores			

Results : Score 0-6 means you have no risk to HCV infection

: Score ≥ 7 means you have risk to HCV infection increasing with scores

Figure 9. An example of blood donors who had risk for HCV infection

Risk assessment form of HCV infection in blood donors

Name…**Ann**……Marital Status…**Single**……..

Address…**Chartrakran,Phitsanulok**…TelEphone…000000……..

Date of Birth..-.Age..-..years..-..months Gender..male..Race..Thai..Religion..Budhist..

Education……**Prathom 6**……...Occupation……Agriculture……

Risk factors/behaviors		**Scores**	**Checklist Scores**
Residence	: Rural	2	2
	Urban	0	-
Education	: Primary school	6	6
	Secondary school	2	-
	Vocational level	3	-
	Undergraduate level	0	-
History of receiving blood or blood product	: Yes	4	-
	No	0	0
IDUs	: Yes	2	2
	No	0	-
Total scores			**10**

Results : Score = 10 means she was at risk to HCV infection and she should check her blood.

• **Risk scores of HBsAg positive**

The risk assessment of HBsAg positive in studies blood donors was carried out by the risk scores followed :

1. Formula of risk scores model was calculated by SPSS FOR WINDOW version 7.5 and cut-off point was calculated by SPSS FOR WINDOW version 9.0 program. The adjusted odds ratio of each variable in Table 17 was used to calculate a risk scores by weight as following :

Risk scores = Age + Gender + History of IDUs + History of hemophilia + history of extramarital sex relation + History of liver disease or jaundice

When ;

Score of age = 1 when age was ≥30 years
= 0 when age was <30 years

Score of IDUs = 1 having history of IDUs
= 0 when never

Score of history of hemophilia
= 19 for having history of hemophilia
= 0 when never

Score of history of extramarital sex relation
= 1 for having history of extramarital sex relation
= 0 when never

Score of history of liver disease or jaundice
= 5 for having history of hepatitis or jaundice
= 0 when never

2. The calculation of risk scores from the model was analyzed and shown in Table 16. The diagnotic prognosis by risk scores was convinced for screening HBsAg positive carriers. The validity of this model used for a predictor of the risk of HBsAg positive was shown in Table19 and Figure10 (sensitivity of this model = 45.1% and specificity = 77.7%). The risk assessment form of HBsAg positive was shown in

Figure 11. The blood donors who had scores 0-5 have no risk to HBsAg positive, whereas another one who had scores equal to or more than 6 have risk to HBsAg positive. Risk score of each individual blood donor was weighted by adjusted odds ratio and calculated by logistic model.

Table 19. Validity of risk assessment form by risk scores model as a predictors of HBsAg positive in blood donors.

Risk score positive if greater than or equal to :	Sensitivity (%)	Specificity (%)	1-Specificity
0	100	0	1
2	95.1	11.9	0.881
3	92.2	19.2	0.808
4	92.2	20.6	0.794
5	62.7	48.4	0.516
6	45.1	77.7	0.223
7	29.4	90.1	0.099
8	25.5	93.5	0.065
9	23.5	95.1	0.049
10	16.7	96.7	0.033
11	9.8	98.5	0.015
12	5.9	99.1	0.009
13	3.9	99.3	0.007
14	2.9	99.4	0.006
15	2	99.5	0.005
16	2	99.6	0.004
17	2	99.7	0.003
18	2	99.8	0.002
19	2	99.8	0.002
20	2	99.9	0.001
22	1	99.9	0.001
25	1	100	0
26	0	100	0

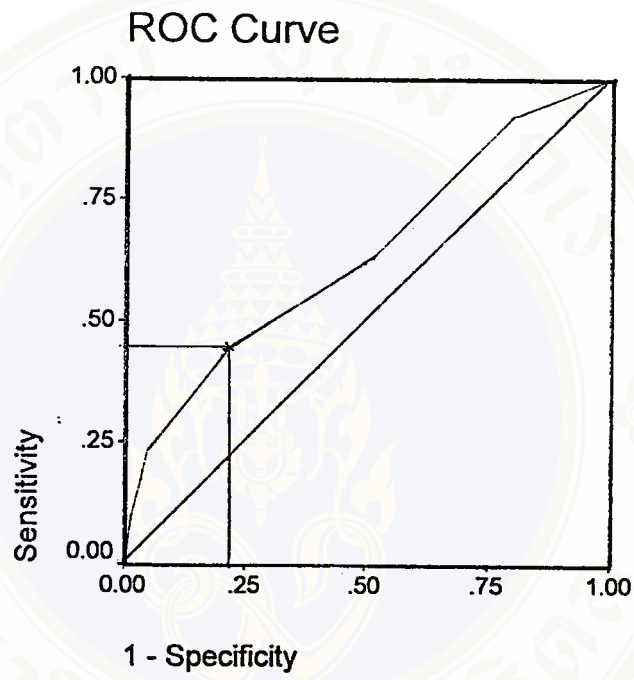


Figure 10. ROC Curve in the prediction of HBsAg positive carriers

- Cut-off point at risk score of 6
- Sensitivity was 45.1% , Specificity was 77.7%
- Area under curve was 63.6%

Figure 11. The Risk Assessment Form of HBsAg positive.

Risk assessment form of HBsAg positive in blood donors

Name.....Marital Status.....

Address.....Telephone.....

Date of Birth.....Age....years....months Gender.....Race.....Religion.....

Education.....Occupation.....

Risk factors/behaviors		Scores	Checklist Score
Age (years)	: ≥ 30	1	
	< 30	0	
Gender	: Male	3	
	Female	0	
History of IDUs	: Yes	1	
	No	0	
History of hemophilia	: Yes	19	
	No	0	
Extramarital sex relation	: Yes	1	
	No	0	
History of liver disease or jaundice	: Yes	5	
	No	0	
Total Score			

Results : Score 0-5 means you have no risk to HBsAg positive

: Score ≥ 6 means you have risk to HBsAg positive increasing with scores.

Figure 12. An example of a blood donor who had risk for HBsAg positive.

Risk assessment form of HBsAg positive in blood donors

Name……..**Bee**……Marital Status…**Married**……..

Address……**Muang, Phitsanulok**……Telephone……**111111**……..

Date of Birth .-. Age.**25.**years..-..months Gender.**Male**..Race.**Thai**.Religion..**Budhist**..

Education……..**Bachelor**……..……Occupation...**Government** ……

Risk factors/behaviors		Scores	Checklist Score
Age (years)	: ≥ 30	1	1
	< 30	0	-
Gender	: Male	3	3
	Female	0	-
History of IDUs	: Yes	1	-
	No	0	0
History of hemophilia	: Yes	5	-
	No	0	0
Extramarital sex relation	: Yes	1	1
	No	0	-
History of liver disease or jaundice	: Yes	19	19
	No	0	0
Total Score			**24**

Results : Score = 24 means he was at risk to HBsAg positive and he should check his blood.

- **Risk scores of HCV infection and HBsAg positive carriers in blood donors.**

The risk assessment of HCV infection and HBsAg positive in blood donors was carried out by the risk scores as followed :

1. Formula of risk scores model was calculated by SPSS FOR WINDOW version 7.5 and cut-off point was calculated by SPSS FOR WINDOW version 9.0 program. The adjusted odds ratio of each variable in Table 18 was calculated risk scores by weight as following :

Risk scores = Age + Gender + Education + Residence + History receiving blood or blood products + IDUs + History of liver disease or Jaundice + history of extramarital sex relation

When ;

Score of age = 1 when age was ≥ 30 years
= 0 when age was < 30 years

Score of gender = 2 for male
= 0 for female

Score of education = 2 for primary school/illiteracy
= 1 for secondary school or vocational level
= 0 for undergraduate level

Score of residence = 2 when address was rural
= 0 when address was urban

Score of receiving blood or blood products
= 2 for having history of receiving blood or blood products
= 0 when never

Score of IDUs = 2 for having history of IDUs
= 0 when never

Score of history of liver disease or jaundice
= 4 for having history of hepatitis or jaundice
= 0 when never

Score of history of extramarital sex relation
= 1 for having history of extramarital sex relation

= 0 when never

2. The calculation of risk scores from the model was analyzed. The diagnotic prognosis by risk scores was convinced for screening HCV infection and HbsAg positive carriers(95%CI = 1.15-1.27, $p < 0.001$). The validity of this model used for a predictor of the risk of HCV infection and HBsAg positive carrier was shown in Table 20 and Figure 13 (sensitivity of this model = 50.3% and specificity = 67.1%). The risk assessment form of HCV infection and HBsAg positive carrier was shown in Figure 14. The blood donors who had scores 0-6 was no risk to HCV infection and HBsAg positive carrier, whereas another one who had scores equal to or more than 7 was risk to HCV infection and HBsAg positive carrier. Risk score of each individual blood donor was weighted by adjusted odds ratio and calculated by logistic model.

Table 20. Validity of risk assessment form by risk scores model as a predictor of HCV infection and HBsAg positive carrier in blood donors

Risk score positive if greater than or equal to :	Sensitivity (%)	Specificity (%)	1-Specificity
0	100	0	1
2	99.4	0.6	0.994
3	97.6	4	0.96
4	95.2	10.1	0.899
5	83.8	27.2	0.728
6	72.5	42.7	0.573
7	50.3	67.1	0.329
8	38.9	82.6	0.174
9	26.9	91.2	0.088
10	19.2	95.3	0.047
11	15	97.5	0.025
12	10.2	98.8	0.012
13	7.2	99.1	0.009
14	4.8	99.3	0.007
15	4.8	99.6	0.004
17	2.4	99.7	0.003
19	2.4	99.7	0.003
20	2.4	99.8	0.002
21	2.4	99.8	0.002
22	2.4	99.9	0.001
23	1.8	99.9	0.001
25	1.8	100	0
27	1.2	100	0
28	0	100	0

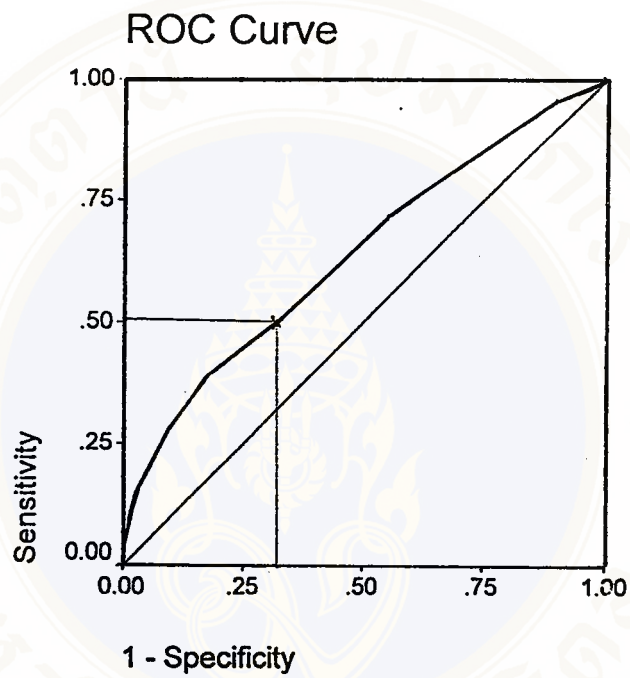


Figure 13. ROC Curve in the prediction of HCV infection and HBsAg positive carrier.

- Cut-off point at risk score of 7
- Sensitivity was 50.3% , Specificity was 67.1%
- Area under curve was 63.5%

Figure 14. The Risk Assessment Form of HCV infection and HBsAg positive carrier in blood donors

Risk assessment form of HCV infection and HBsAg positive carrier in blood donors

Name.....Marital Status.....
Address.....Telephone.....
Date of Birth.....Age....years....months Gender.....Race.....Religion.....
Education.....Occupation.....

Risk factors/behaviors		Scores	Checklist Scores
Age (years)	: ≥30	1	
	< 30	0	
Gender	: male	2	
	female	0	
Education	: Primary school/illiteracy	2	
	Secondary school	1	
	Vocational level	1	
	Undergraduate level	0	
Residence	: Rural	2	
	Urban	0	
History of receiving blood/blood products:	Yes	2	
	No	0	
History of IDUs	: Yes	2	
	No	0	
History of liver disease or jaundice	: Yes	4	
	No	0	
History of extramarital sex relation	: Yes	1	
	No	0	
Total scores			

Results : Score 0-6 means you have no risk to HCV infection and HBsAg positive carrier.
: Score ≥7 means you have risk to HCV infection and HBsAg positive carrier increasing with scores.

Figure 15. An example of blood donors who had risk for HCV infection and HBsAg positive carrier in blood donors.

Risk Assessment form of HCV infection AND HBsAg positive carrier in blood donors

Name……**C**……Marital Status…**Married**……
Address…**Chartrakran, Phitsanulok**...Telephone……**222222**……..
Date of Birth..-..Age.**45**.years..-..months Gender.**Male**.Race.**Thai**..Religion..**Buddist**
Education……**Prathom 4**……...Occupation……**Employee**……

Risk factors/behaviors		Scores	Checklist Scores
Age (years)	: ≥30	1	1
	<30	0	0
Gender	: male	2	2
	Female	0	0
Education	: Primary school/uneducated	2	2
	Secondary school	1	-
	Vocational level	1	-
	Undergraduate level	0	-
Residence	: Rural	2	2
	Urban	0	0
History of receiving blood/blood products	: Yes	2	2
	No	0	-
History of IDUs	: Yes	2	0
	No	0	-
History of liver disease/jaundice	: Yes	4	-
	No	0	0
History of extramarital sex relation	: Yes	1	-
	No	0	0
Total scores			**8**

Results : Score = 9 means he was at to HCV infection and HBsAg positive carrier and he should check his blood.

CHAPTER V

DISCUSSION

1. The prevalence of anti-HCV and HBsAg positive in studied blood donors

- **The prevalence of anti-HCV positive**

In this study, the prevalence of anti-HCV positive was 2.90% (63/2,167 blood donors). It was cohered with the previous studies which ranged from 2.4% - 4.1% (11,12) and it was higher than other studies which ranged from 1.5% to 1.6% (1,2). The prevalence of anti-HCV positive in males was higher than that in female and increased with ages. It was similar to other reports (9,10,3,4) with age peaking in the 25 to 34 years of age group (3). Our findings showed that HCV infection rate was high in blood donors whose education was primary school or illiterate (7.05%), in donors' occupation with agriculture (8.95%) and in donors living in rural area (4.77%). It indirectly indicated some correlation of the infection with low socioeconomic conditions, the use of unsterilized needles,injecting drug use, the sexual relation and ear piercing or tattooing setups. Donors with blood group A (4.62%) has higher prevalence than the other groups(B = 2.92%, AB = 3.20%, O = 1.89%). There was no previous study of association between HCV infection and blood group. The reason might be associated with the immunity characteristic of blood group A, the future study should be done.

- **The prevalence of HBsAg positive.**

In this study, the prevalence of HBsAg positive was 4.61% (100/2,167 blood donors). It was cohered with the study of Thai Red Cross Society which was 3.70% to 4% of HBsAg positive(178). The high prevalence in male was similar to previous studies(33). Sometime, it was due to the behavior of male i.e, outside socialization, habitual extramarital sex relation, alcohol consumption and more prevalence of hepatoma.

Findings showed that HBsAg positive carriers were high in donors whose education was primary school or illiterate (5.24%) or secondary school (5.29%). It indicated indirectly some correlation of the disease with low socioeconomic status and individual hygiene likely HCV infection.

The high prevalence in 15-30 years old (5.89%) was cohered with the studies in Egyptian tourism workers. The prevalence of a seromarker for HBV infection was relatively constant for subjects 20-29 years old (1).

2. Risk factors

- **Risk factors of HCV infection in studied blood donors.**

Education : Among respondents whose education was primary school or illiterate had significantly higher risk of HCV infection (OR = 5.64; 95 % CI = 2.10-15.15). This study was compatible with some studies(199) It referred that, blood donors from illiterate population should be selected with extra-care by ensuring that they clearly understand what high-risk behavior and blood-transmitted infection mean before they are asked about their high-risk behavior and past history of illness.

Residence : Who lived in rural had higher risk than who lived in urban (OR = 2.14; 95% CI = 1.19-3.85). It was compatible with the reports in Malawian and other sub-Sahara countries. It showed that HCV and HBV infections were highly prevalence in rural area. Blood donors living in rural area had compatible with the primary school or illiterate education. It suggested that we should give valuable information and education for preventing, controlling and reducing the HCV transmission to them.

History of receiving blood or blood products : Donors with history of receiving blood or blood products had significant risk of HCV infection (OR= 4.13; 95 % CI = 1.87-9.16). It was compatible with the other reports that individuals who had previous blood transfusions had risk for HCV infection(5,39,51,58). It was suggested that the possibility of infection might be due to the lack of HCV screening or the low sensitivity of HCV screening test in the previous donation. So, we have a further consideration of the costs and benefits of testing for antibodies to HCV

infection in blood donors. The serious hazards of transfusion scheme and collaborative work between the national blood services and Public Health Laboratory Service should be continued to monitor post-transfusion infections .

History of IDUs : The result revealed that those respondents who had a history of drug injection got more risk of HCV infection (OR = 2.32; 95 % CI = 1.41-3.82). It was supported by the other studies(7,13,14,21,23,82,95,104). Duration of injecting drug use was the strongest predictor of HCV infection . Hepatitis C is highly prevalence among drug users and likely to prove difficult of control because of rapid acquisition early in the injecting career. Preventive programs for HCV infection in IDUs or general population need to significantly reduce the prevalence of injecting drug use, in addition to encouraging safe injecting practices through needle-exchange schems, in order to reduce HCV transmission among drug users. Individual who had history of injecting drug use had risk for HCV, HBV and HIV infections. Such a change in risk behavior of injecting drug use or general population should be emphasized.

- **Risk factors of HBsAg positive carriers in studied blood donors**

Age : Among respondents, those who were ages over than 30 years had significant risk of HBsAg positive (OR = 1.81; 95 % CI = 1.22-2.68). This study was compatible with some studies in Thailand (25,26,28,32). It was concluded that the risk of HBsAg positive increased by age(64).

Gender : Those who were male had higher risk than female and with significant association (OR = 3.04, 95 5 CI = 1.45-6.35). It was compatible with other studied which they were associated with behaviors i.e, outside socialization, habitual of sex relation with the partner, alcohol consumption and more prevalence of hepatoma(10,17,199).

IDUs : The results revealed that those respondents who had a history of drug injection got risk of HBsAg positive(OR = 1.41, 95%CI = 1.07-1.86). It was cohered with the other studies that duration of injecting drug use was also a strong predictor of HBV infection. Such a change in risk behavior of injecting drug use would have important benefits for HBV prevention as well(179,200).

Hemophilia : Among respondents, those who had history of hemophilia and receiving blood or blood products had significantly higher risk of HBsAg positive(OR = 18.85, 95%CI = 1.61-220.77). Although, the risk of HBV infection from history of hemophilia and receiving blood or blood products has been reported from some studies(200,201). The study in England and Wales(201) showed that 0.6% of cases of acute hepatitis B infection were associated with transfusion. Probably, a donation is concluded as having been infectious if the donor was surface antigen negative but had evidence of acute infection or of carrying the virus (antibody to hepatitis B core antigen with no or low titers of antibody to surface antigen) or if the donor was surface antigen positive and blood was erroreously released or mutant strains of hepatitis B virus not detected by routine surface antigen tests also pose a risk of infectious donations being transfused.

Extramarital sex relation : Blood donors with history of extramarital sex relation had significant risk of HBsAg positive(OR = 1.18, 95%CI = 1.08-1.29). This study was compatible with the study in the United Kingdom It was found that in homosexual men(38.7%), in heterosexual men(3.5%) and in woman (3.7%) had HBsAg positivity. Homosexual men remained at high risk of infection. In this risk factor had refered to contacting of saliva, secretion, blood or sexual relation without condom use(202).

History of liver disease or jaundice : Blood donors with history of hepatitis or jaundice had significantly high risk of HBsAg positive(OR = 5.39, 95%CI = 2.86-10.15). The past exposure to hepatitis B virus or history of jaundice may be introduced to HBV infection but could not detect of infection because it was on the window period of HBV infection(203).

3. The validity of Risk Assessment Form and its application

• Risk assessment form for screening HCV infection

The risk assessment form for screening HCV infection among studied blood donors in Phitsanulok Province was evaluated by using the information of odds ratio and MLR to formulate the risk score. The risk score ranging 0-29, efficiency of

cut-off point at score ≥ 7 was conducted. The risk score also was tested for accuracy i.e, sensitivity (55.2%) and specificity (85.7%). The validity of risk assessment form supported the hypothesis that the risk assessment from has a high specificity(85%-95%) and moderate sensitivity(≈50-75%) for screening HCV infection among blood donors. HCV infection is a public health problem in many countries. It will develop to chronic active hepatitis and gradual progression to liver cirrhosis and hepatocellular carcinoma. At present, there is no effective vaccine or drug for HCV infection. We should be emphasized the risk assessment form for primary screening in blood donors, premarital persons in premarital counseling program and in general population for preventing and decreasing the risk of HCV infection.

- **Risk assessment form for screening HBsAg positive carriers**

The risk assessment form for screening HBsAg positive carrier among studied blood donors in Phitsanulok Province was evaluated by using the information of odds ratio and MLR to formulate the risk score. The risk score ranging 0-26, efficiency of cut- off point at score ≥ 6 was conducted. The risk score which was tested for this formula was the screening of those who had no risk of HBsAg positive carrier accurately as 77.7% (specificity = 77.7%) but for screening of actual positive case of those who had risk of HBsAg positive was only 45.1%(sensitivity = 45.1%). The validity of this risk assessment form was not supported the hypothesis. Sometime, it might be due to prior screening before donating in this time. If we used this form screen in the new blood donors or in general population it would get a moderate sensitivity and high specificity. We should be emphasized the risk assessment form for screening in premarital persons in premarital counseling program, blood donors and general population. Those who had risk for HBV infection should check their blood. If we found the HBV infection, it would receive the appropriate advise. If they had no risk to HBV infection, they should be vaccinated for prevention and control the HBV infection.

CHAPTER VI

CONCLUSION AND RECOMMENDATION

Conclusion

This study is to investigate the prevalence, risk factors/ risk behaviors of HCV infection and HBsAg positive carriers and to assess the validity of risk assessment form for screening HCV infection and HBsAg positive carrier among 2,167 blood donors. The prevalence rate of anti-HCV was 2.90%. The risk factors/risk behaviors associated with HCV infection in studied blood donors by univariate analysis were: (1) Gender as male, (2)Education - primary school/illiteracy, (3)Address - rural, (4) History of receiving blood or blood products, (5) History of IDUs and (6) History of STDs. After removing of confounders by Multivariate Logistic Regression, the risk factors were : (1) Residence in rural, (2) Education as primary school, (3) History of receiving blood or blood products and (4) History of IDUs. The HCV risk assessment form was improved by using predictors including address, education, history of receiving blood or blood products and history of IDUs. The validity of the form was 55.2% of sensitivity and 85.7% of specificity when used the cut-off score $\geq$ 7.. The blood donors who had risk score over or equal to 7 were considered as the risk of HCV infection and they should check their blood.

The prevalence of HBsAg positive was 2.61%. The risk factors/risk behaviors associated with HBsAg positive in studied blood donors by univariate analysis were: (1) Age as over or equal to 30 years, (2) Gender as male, (3) Marital status as single, (4) History of IDUs, (5) Extramarital sex relation and (6) History of liver disease or jaundice. After removing of confounders by Multiple Logistic Regression, the risk factors were : (1) age equal to or more than 30 years, (2) Gender - male, (3) History of IDUs, (4) History of liver disease/jaundice, (5) History of hemophilia,

(6) History of extramarital sex relation. The risk assessment form was improved by using predictors including age, gender, history of extramarital sex relation. The validity of the form was 45.1% of sensitivity and 77.7% of specificity when use the cut-off score ≥ 6. The blood donors who had risk score over or equal to 6 were considered as the risk of HBsAg positive and they should check their blood.

Recommendation

Based on the results of the study, the researcher recommends the following :

1. To apply the risk assessment form for screening HCV infection and HBsAg positive in general population or premarital persons.
2. To apply and promote the risk assessment form to the community hospitals which lack of blood screening tests, one has to risk of HCV infection or HBsAg positive carrier i.e, blood donors and premarital person.

Recommendation for further study :

1. To encourage and distribute the knowledge of blood-borne pathogens and mode of transmission particular to HCV, HBV and HIV infections .
2. To apply this risk assessment form for screening HIV infection because the mode of transmission or risk factors of HIV were similar in HCV and HBV infections.
3. To apply this study model in other study i.e, to assess the occupational risk from blood-borne pathogens, to assess the occupational risk from environment of working.

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APPENDIX

APPENDIX A

- **The assay of Anti-HCV**

Specimen : Sera were collected and kept at 2 to 8 °C for up to fourteen days. After throwing, the serum samples were thoroughly mixed, commercial kit by Abbott HCV EIA 3.0.

Procedure

Materials provided

- Reaction Trays
- Cover Seals
- Assay Tubes

Materials required

- Distilled or deionized water which is free from microbial contamination
- 1 *N* Sulfuric acid, No. 721(Most U.S. and International locations)
- Precision pipettes 10 µl, 200 µl,300 µl and pipette tips
- Dispenser for measuring OPD Diluent
- Metal free containers for the OPD Substrate Solution
- Qwik Wash® or equivalent device for washing beads to deliver 5±1 ml per well, with a vacuum source
- Commander Dynamic Incubator at 40±2 °C.
- Commander PPC, Quantum II or Quantumatic
- Bead Dispenser
- Nonmetalic forceps
- Membrane Seal Punture Tool for acid bottles
- OPD Tray Cover

- Reagent Blanking Beads (for COMMANDER testing)
- Protective gloves

Reagents

1. 100/1000 Hepatitis C Virus Encoded Antigen (Recombinant c100-3, HC-34, HCr43, NS5)
2. 3 vials(1 ml each)/ 3 vials(5ml) Conjugate Concentrate. Goat Antibody to Human IgG (GAMMA-CHAIN) : Peroxidase (Horseradish). Minimum concentration : 0.02 µl/ml in Tris Buffer with Animal Serum(Calf) and dye number 33.
3. 3 vial(19 ml each)/ 3 vial(95 ml each) Conjugate Diluent containing 20% Animal Sera(Goat, Calf) in TRIS Buffer.
4. 1 vial(2ml)/ 2 vial(2 ml each) Positive Control. Inactivated Human Plassma reactive for anti-HCV and nonreactive for HBsAg and anti-HIV-1/hiv-2. Minimum titer : 1:2.
5. 1 vial(2 ml)/ 2 vial(2 ml each) Negative Control. Human Plasma, nonreactive for anti-HCV, HBsAg and anti-HIV-1/HIV-2.
6. 2 vial(20 ml each)/4 vial(100 ml each) Specimen Diluent containing TRIS Buffer, 0.2% Trion® X-100, Protein Lysates and Animal Sera (Goat, Calf).
7. 1 Bottle(10 Tablets)/ 2 Bottles(40 Tablets each) Diluent for OPD(O-Phenylenediamine• 2 HCL) Tablets. OPD/Tablets : 12.8 mg.
8. 1 Bottle(55 ml)/ 2 Bottle(220 ml each) Diluent for OPD(O-Phenylenediamine• 2 HCL). Citrate-Phosphate Buffer containing 0.02% Hydrogen Peroxide.

Preparation of Conjugate Reagent

1. Bring Conjugate Concentrate and Conjugate Diluent to room temperature before mixing.
2. Carefully empty the contents of a Conjugate Concentrate vial(with red dry) into a vial of Conjugate Diluent. Slowly squeez the small vial

2 to 3 times while maintaining the nozzle within the opening of the large vial. Avoid foaming. One vial of diluted conjugate is sufficient for 100 tests kit/500 tests kit.

3. Reseal the large vial. Mix thoroughly by slowly inverting the vial several time. Do not vortex.
4. Write the date of dilution and expiration date in the space provided on the conjugate diluent label. Conjugate is stable for 14 days(though not to exceed the kit expiration date) after dilution when stored at 2 to 8 ° C.
5. Allow diluted conjugate to equilibrate at room temperature for approximately 60 minutes prior to use.
6. Store diluted conjugate at 2 to 8 °C. Bring to room temperature before use.
7. Do not mix vials of diluted conjugate. Separate Negative and Positive Controls must be run with each vial of diluted conjugate.

Assay procedure

1. Dilution of specimen
   - Dispense 10 µl of each control specimen into the bottom of an individual test tube, pre-dilution tray or equivalent.
   - Dispense control in the sequence of 3 replicates of the Negative Control followed by 3 replicates of the Positive Control Dispense specimens after controls.
   - Dispense 400 µl of Specimen diluent to each test tube, pre-dilution well or equivalent.
   - Mix Thoroughly by gently tapping.
   - Transfer 200 µl of each diluted control or specimen into appropriate well of reaction tray.
2. Carefully add one bead to each well containing diluted control or specimen.

3. Apply cover seal. Tap tray gently.
4. Incubate at 40±2 °C for 1 hour ± 5 minutes in a Commander Dynamic Incubator in the Rotation mode.
5. Remove and discard cover seal. Wash each bead.
6. Pipette 200 µl of diluted conjugate into each well containing a bead.
7. Apply new cover seal. Tap tray gently.
8. Incubate at 40±2 °C for 30±2 minutes in a Command Dynamic Incubator in the Rotation mode.
9. Remove and discard cover seal. Wash each bead.
10. Transfer beads immediately to properly identified assay tubes.
11. Prime dispenser immediately prior to dispensing OPD Substrate Solution.
12. Pipette 300 µl of freshly prepared OPD Substrate Solution into two empty tubes(substrate blanks) and then into each tube containing a bead.
13. Cover and incubate at room temperature(15 to 30°C) for 30±2 minutes.
14. Add 1 ml of 1 *N* Sulfuric Acid to each tube. Agitate to mix.
15. Pipette approximately 2 ml of distilled or deornized water into an empty tube.
16. Select mode for processing HCV EIA 3.0. Determine the absorbent of control and specimens and cut-off value for anti-HCV reactive followed the criteria of ABBOTT CV EIA 3.0.

The sensitivity for this assay determined by using two different sample collective with clinically diagnosed acute NANBH was 100% or chronic NANBH was 99% and the specificity was estimated to be 99.6%.

- **The assay of HBsAg 5.0**

Specimen : Sera were collected and kept at 2 to 8 °C before beginning the test. After throwing, the serum samples were thoroughly mixed, commercial kit by Enzynost® HBsAg 5.0.

Procedure

Enzygnost® HBsAg is an enzyme immunoassay based on a two-step method. In the first step, the HBsAg contained in the sample reacts simultaneously with the polyclonal anti-HBs antibodies attached to the wells of the microtitration plate and with Conjugate 1(Anti-HBs/Biotin,coloured blue). In the second step, after removing the unbound reactants, Conjugate 2(Streptavidin/POD, coloured yellow) then react with Conjugate 1. After removing the unbound reactants, the enzyme activity of the bound Conjugata 2 is determined(blue colour reaction). The enzymatic conversion of the chromogen is terminated by the addition of Stopping Solution POD(yellow colour reaction). The colour intensity is proportional to the concentration of antigen in the sample.

Material required

- Enzygnost® HBsAg 5.0 Test Plate
- Conjugate 1(Anti-HBs/Biotin) 6.5 ml
- Conjugate 2(Streptavidin/POD) 15 ml
- HBsAg Control Serum, positive 1.5 ml
- HBsAg Control Serum, negative 3 ml
- Precission Pipettes 100 µl and pipette tipe
- Cover seal
- Buffer/Substrate TMB
- Stopping Solution TMB

- Empty bottle for the Working Chromogen Solution
- Bechring ELISA Processor II (BEP II)

Composition

Enzygnost HBsAg 5.0 Test plate	:	Microtitation plate coated with sheep antibodies to HbsAg.
Conjugate 1(Anti-HBs/Biotin)	:	Mouse monoclonal anti - HBs, biotin - conjugated, coloured blue.
Conjugate 2(Streptavidin/POD)	:	Streptavidin , peroxidase (POD) - Conjugated, coloured yellow.
HBsAg Control Serum,negative	:	Human serum, nominal absorbance ≤ 0.150.
HBsAg Control serum, positive	:	Stabillized human serum, (0.4±0.2 U/ml) nominal absorbance : 0.7.

Procedure for the BEP II

1. **Assay scheme :** Ascertain the number of wells required (= number of samples to be tested plus 6 wells for the controls).
2. **Dispense samples :** Pipette 100 µl/well of the negative control into 4 wells(A1 to D1) and 100 µl/well of the positive control into one well (E1), then fill the following wells with 100 µl/well of undiluted sample and finally at the end of the series/test plate again fill one well with 100 µl of positive control.
3. **Dispense Conjugate :** Fill each well with 25 µl of Conjugate 1(Anti-HBs/Biotin)). Seal with foil and, immediately after completing the conjugate dispensing step, place into the incubator.
4. **Incubate :** Incubate at 37±1 °C for 60±2 min. Immediately afterwards proceed to the first washing step.

5. **Wash :** Remove the foil and aspirate all the wells. Then wash 4 times with approx. 0.3 ml of washing solution each time, with a 10-sec soak interval per wash. Immediately after completing the washing cycles dispense Conjugate 2 so that the wells cannot dry out.
6. **Dispense Conjugate 2 :** Fill each well with 100 µl of Conjugate 2 (Streptavidin/POD). Seal with foil and, immediately after dispensing the conjugate, place in to the incubator.
7. **Incubate :** At 37±1 °C for 30±2 min. Then immediately proceed to the next washing step.
8. **Wash :** Remove the foil and aspirate all the wells. Then wash 4 times with approx. 0.3 ml of washing solution each time, with a 10-sec soak interval per wash. Immediately after completing the washing cycles dispense the substrate so that the wells cannot dry out.
9. **Dispense substrate :** Fill each well with 75 µl of the Working Chromogen Solution and seal the plate with fresh foil.
10. **Incubate :** At +15 to +25 °C for 30±2 min protected from light.
11. **Stop reaction :** Remove the foil, add 75 µl of Stopping Solution POD to each well, keeping to the same timing as in step 9.
12. **Read :** Read at 450 nm within 1 hour. The wavelength recommended for the reference reading is 650 nm (if necessary, between 615 and 690 nm).

The sensitivity of this test was 100% and the specificity was 99.83% for initial testing and 99.85% for retest results.

APPENDIX B

Statistic Use in the Analysis

1. Percentage

$$\text{Percent} = \frac{100\ fi}{n}$$

When : fi = frequency of the i group

$n = \Sigma\ fi$

I = 1,2,3,...r, r = total groups

2. Chi-square test

Variable 1	Variable 2		Total
	1	2	
1	a	b	R 1
2	c	d	R 2
	C 1	C 2	

$$\chi^2 = \frac{N[\,|ad-bc|\,-N/2\,]^2}{R1\ R2\ C1\ C2},\ df = 1$$

3. Variable Association

3.1 Dicotomous Variable

Exposure	Disease		Total
	Disease	Non-disease	
Exposed	a	b	a+b
Non-exposed	c	d	c+d
	a+c	b+d	N

$$OR = \frac{ad}{bc}$$

3.2 In order categorial variables

$$ORi = \frac{ai\ b1}{a1\ bi}$$

Level of risk factor	Case	Control
1	a1	b1
2	a2	b2
3	a3	b3
.	.	.
.	.	.
k	ak	bk
Total	n1	n2

3.3 The 95% confidence interval of odds ratio was calculated by using Woolf 's method of confidence interval.

95% Confidence Interval :

$$= \ln(OR) \pm 1.96\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}$$

$$OR_L = OR \exp\left[-1.96\sqrt{\text{var}(\ln OR)}\right]$$

$$OR_U = OR \exp\left[+1.96\sqrt{\text{var}(\ln OR)}\right]$$

4. Validity

Exposure	Disease	Non-disease	Total
Exposure	a	b	a+b
Non-exposure	c	d	c+d
	a+c	b+d	N

Sensitivity = [a / (a+c)] 100

Specificity = [d / (b+d)] 100

5. Logistic Regression Mode

Prob (Event) = $P(X^i)$

Prob (d = 1 $|x^i$) = $\frac{1}{1+e^{-(\beta 0 + \beta 1X1 + \beta 2X2 + \ldots \beta pxp)}}$

$P(X^i)$ = Probability of disease

d = 1

Odds (X^i) = $P(X^i)$

X^i = Independent Variable (i = 1,2,3,...) such as risk factors, confounding variables

β_0 = Constant

β_1 = Beta coefficients or parameter of each risk factors

e = Exponential logarithm

Logistic coefficients

Px = $\frac{1}{1 + e^{-(a+bx)}}$

Px = The probability of the disease for a specific value of avariable represented as x

a = Constant

b = Beta coefficient or parameter of a variable

e = Exponential logarithm

6. Adjusted Odds Ratio

Odds Ratio = $\exp(\beta I)$

95% CI Adjusted OR = $\exp[(\beta_I) \pm Z_{\alpha/2} SE(\beta_I)]$

When : $Z_{\alpha/2}$ = 1.96

7. Receiver Operating Characteristic Curve (ROC)

The ROC curve shows how severe the trade-off between sensitivity and specificity is for a test and can be used to healp decide where the best cut-off point should be. Generally, the best cut-off point is at or near the shoulder of the ROC curve. The overall accuracy of a test can be described as area under the ROC curve ; the large area was the better test.

APPENDIX C

Risk Assessment Form for screening Hepatitis C Virus infection and HBsAg positive carrier in blood donors

Subject's code.....

Date.....

Name.....Marital Status.....

Address.....Telephone.....

Date of Birth.....Age.....years.....months Gender.....Race.....Religion.....

Education.....Occupation.....BW.....kgs High.....cms.

T.....°C P...../mine R...../mine BP.....mmHg

Lung.....Heart.....

.....

Doctor Signature

Blood group.....Rh.....Total blood.....cc. Unit No.....

Please writing a √ in □ with truly

	Yes	No
1. History of receiving blood transfusion.	□.....times/month	□
2. History of operation or delivery in hospital.	□.....times/month	□
3. History of transplantation or dialysis.	□.....times/month	□
4. History of direct contacting with blood or secretion other persons.	□	□
5. History of tattooing or ear piercing.	□.....times/month	□
6. History of drug injection.	□.....times/month	□
7. History of liver disease or jaundice.	□	□
8. History of having someone with liver disease or jaundice in the family.	□	□
9. History of a hemophilia and receiving blood or blood product.	□	□
10.Habitual alcohol drinking	□.....times/month	□
11.History of STDs within a year.	□.....times/month	□
12.History of sexual service.	□	□
13.History of extramarital sex relation.	□.....times/month	□
14.History of homosexual activity.	□.....times/month	□

The representative of blood donor

I voluntary donate some blood to the National Blood Center, Thai Red Cross Society for public benefit. In this donation, I hereby that all questions above have been answered accurately and I also confirm that my blood is safe for donating.

Donor Signature.....

Blood testing as the following :

1. ABO, Rh
2. VDRL
3. HBsAg
4. Anti-HCV
5. Anti-HIV

If the laboratory result is positive ;

☐ Please inform me by sending a letter to this address.....

.....

☐ Don't inform me.

Your can consult about your blood with authorise officers at any blood bank services.

Biography

Name Nantaporn Thammata

Date of Birth 10 May 1973

Place of Birth Tak , Thailand

Institution Attended

Boromarajonani Buddhacinaraj Nursing College , Phitsanulok , 1992-1995 : Diploma in Nursing and Midwifery (Equivalent of Bachelor of Science in Nursing)

Mahidol University , 1998-2000 : Master of Science (Public Health) , Major in Infectious Diseases

Position & Office

1995-Present , Department of Nursing , Umphang Hospital , Tak , Thailand.

Position : Register Nurse