# RISK FACTORS OF HIV AND HBV CO-INFECTION IN CHIANG RAI, THAILAND

**SAUWALUCK PONGWIRIYAKUL**

**MASTER OF SCIENCE**
**IN**
**PUBLIC HEALTH**

**SCHOOL OF HEALTH SCIENCE**
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THESIS COMMITTEE

.....CHAIRPERSON
(Assoc. Prof. Dr. Rachanee Sunsern)

.....ADVISOR
(Dr. Tawatchai Apidechkul)

.....CO-ADVISOR
(Assoc. Prof. Dr. Chomnard Pojanamart)

.....CO-ADVISOR
(Asst. Prof. Dr. Mitra Cassley)

.....EXTERNAL EXAMINER
(Assoc. Prof. Pipat Luksamijarulkul)

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Sauwaluck Pongwiriyakul

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| **Author** | Sauwaluck Pongwiriyakul |
| **Degree** | Master of Science (Public Health) |
| **Advisor** | Dr. Tawatchai Apidechkul |
| **Co-Advisor** | Assoc. Prof. Dr. Chomnard Pojanamart |
| | Asst. Prof. Dr. Mitra Cassely |

# ABSTRACT

This hospital based case-control study design aimed to investigate the risk factors of HIV/HBV co- infection in Chiang Rai province. Totally, 124 HIV-infected cases were recruited into the study from 9 ARV clinics; Mae Sai, Mae Chan, Chiang Saen, Khun Tan, Phaya Meng Rai, Theong, Mae Suai, Mae Lao, and Phan hospitals Completed structured questionnaires, 5 ml blood specimen and the in-depth interview were research instruments. Enzyme immunoassay and the immunochromatography methods were used for detecting HBV serological markers. Both of univariate and multivariate analyses were used for identifying the risk factors. The statistical significance level of 0.05 was used for identifying the association between independent and dependent variables. Results, 50.00% were male, 40.32% aged between 30 -39 years old, 62.90% married, 95.97% Buddhism, 18.55% illiterate, 10.48% unemployed. Twenty three point four percentages had a history of blood transfusion, 12.90% had history of jaundice, 24.19% had comorbidity, 29.03% had CD4 cell count $\leq$ 200 cells/$mm^3$, 33.87% had length of HIV infection > 3 years, 4.84% lived with HBV positive person in their family, 12.90% shared objects with

their family members, 0.81% IDU, 29.34% tattooed, 64.52% pierced. Twelve point one percentages had worked as commercial sex worker, 11.29% had the first sexual intercourse ≤ 15 years old, 6.45% homosexual, 37.90% had ≥ 10 sex partners, and 94.35% never or sometimes used condom before known the HIV status. After controlling the possible confounder factors by the multiple logistic regressions, it was found that 2 factors were associated with HIV/HBV co-infection: Years in school "No education" group had a greater risk than "≥ 13 years" group by 7.07 times (OR=7.07, 95% CI=1.77-28.24), and CD4 cell count "≤ 200 cells/$mm^3$"group was presented as a protective factor when compared to "≥ 200 cells/$mm^3$" group (OR=0.35, 95% CI=0.13-0.94).

An improvement in the socioeconomic status of the general population, in particular level of general education and sex education, should help toward reducing HIV/HBV co-infection. HIV-infected patients should receive HBV vaccination, continue to use condom, and practice safe sex in order to reduce the risk of co-infection with hepatitis.

**Keywords:** HIV/HBV/Co-Infection/Risk Factors

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# ABBREVIATIONS AND SYMBOLS

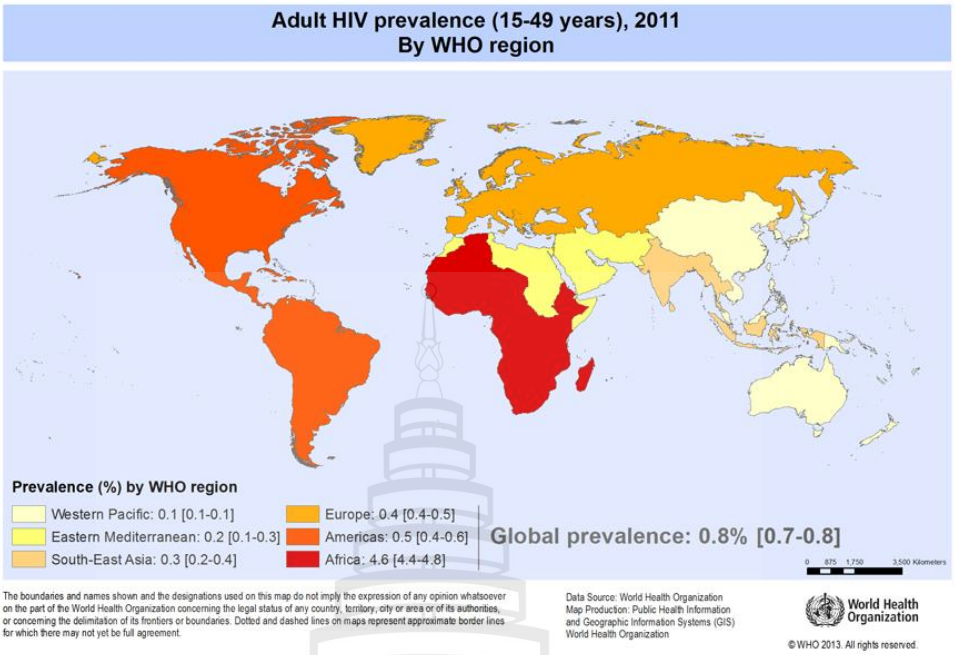
| | |
|---|---|
| AIDS | Acquired Immune Deficiency Syndrome |
| Anti-HBc | Antibody to hepatitis B core antigen |
| Anti-HBs | Antibody to hepatitis B surface antigen |
| ART | Antiretroviral Therapy |
| AZT | Azidothymidine |
| ARV | Antiretroviral |
| CD8 | Cluster of Differentiation Antigen Eight |
| CD4 | Cluster of Differentiation Antigen Four |
| EIA | Enzyme Immunoassay |
| HAART | Highly Active Antiretroviral Therapy |
| HBsAg | Hepatitis B Surface Antigen |
| HBV | Hepatitis B Virus |
| HCV | Hepatitis C Virus |
| HIV | Human Immunodeficiency Virus |
| HIV/HBV | HIV and HBV |
| IDU | Injecting Drug Use |
| IL-2 | Interleukin Two |
| IOC | Item Object Congruence |
| NNRTIs | Nonnucleoside Reverse Transcriptase Inhibitors |
| NRTIs | Nucleoside Reverse Transcriptase Inhibitors |
| PCR | Polymerase Chain Reaction |
| PIs | Protease Inhibitors |
| SES | Socioeconomic status |
| STDs | Sexual Transmitted Diseases |
| UNAIDS | United Nations and AIDS |
| WHO | World Health Organization |

# CHAPTER 1

# INTRODUCTION

## 1.1 Rationale and Background

Acquired immune deficiency syndrome, or acquired immunodeficiency syndrome (AIDS), is a disease which reduces the capacity of human immune system. Human immunodeficiency virus (HIV) is the main cause of the disease. HIV/AIDS acquisition affects every aspect of human life, such as physical, mental, social, and even economic aspects. It imposes heavy burdens on individuals, families, communities, and nations, causing international concern. Currently, there are more than 70 million people having been infected with HIV, and 35 million people have died of AIDS (World Health Organization [WHO], 2013c). In 2011, WHO estimated that there were 34 million people worldwide living with HIV and 1.7 million people having died of AIDS-related illnesses (WHO, 2013c). In 2008, 4.7 million (3.8-5.5 million) people in Asia were living with HIV, and approximately 330,000 (260,000-400,000) people were reported to have died of AIDS-related diseases at the same time. The number of HIV/AIDS cases peaked in Asia in the mid-1990s, and after that the annual HIV incidence subsequently declined by more than half. However, the epidemic has remained somewhat stable since 2000, while the annual number of AIDS-related deaths in South and South-East Asia in 2008 were approximately 12.00%, lower than the mortality peak in 2004 (Joint United Nations Programme on HIV/AIDS [UNAIDS], 2009).




**Source** WHO. (2013a). *Adult HIV prevalence (15-49 years).* Retrieved 29 July 2013, from http://www.who.int/gho/hiv/hiv_013.jpg

**Figure 1** A Global View of HIV Prevalence in 2011

The first case of AIDS in Thailand was reported in 1984 (Phanuphak, Locharernkul, Panmuong & Wilde, 1985). For the next few years, MSM (men who have sex with men), sex workers, injecting drug users, and tourists were more commonly affected than other groups. Between 1988 and 1989, the HIV prevalence among injecting drug users (IDUs) rose dramatically from almost zero through 40.00%. The prevalence among sex workers also increased, as supported by Weniger et al. (1991), who found that 44.00% of sex workers were infected with HIV. The rising level of infection among sex workers led to subsequent waves of the epidemic among the male clients of sex workers and through their wives, partners, and children (Viravaidya, Obremskey & Myers, 1996). HIV incidence in Thailand reached its peak in 1992, with approximately 115, 000 cases. A steep decline thereafter, which discontinued in 1997, was followed by another strike of 42, 000 cases in 1999. The

second surge, which coincided with the major economic crisis, brought on 60, 000 new infections (Punyacharoensin & Viwatwongkasem, 2009). From 1984 to September 2012, the cumulative number of AIDS cases was 276, 947. Sexual intercourse was the major route of HIV transmission. Overall, however, the trend of AIDS deaths in Thailand has dramatically decreased (Bureau of Epidemiology, 2012).

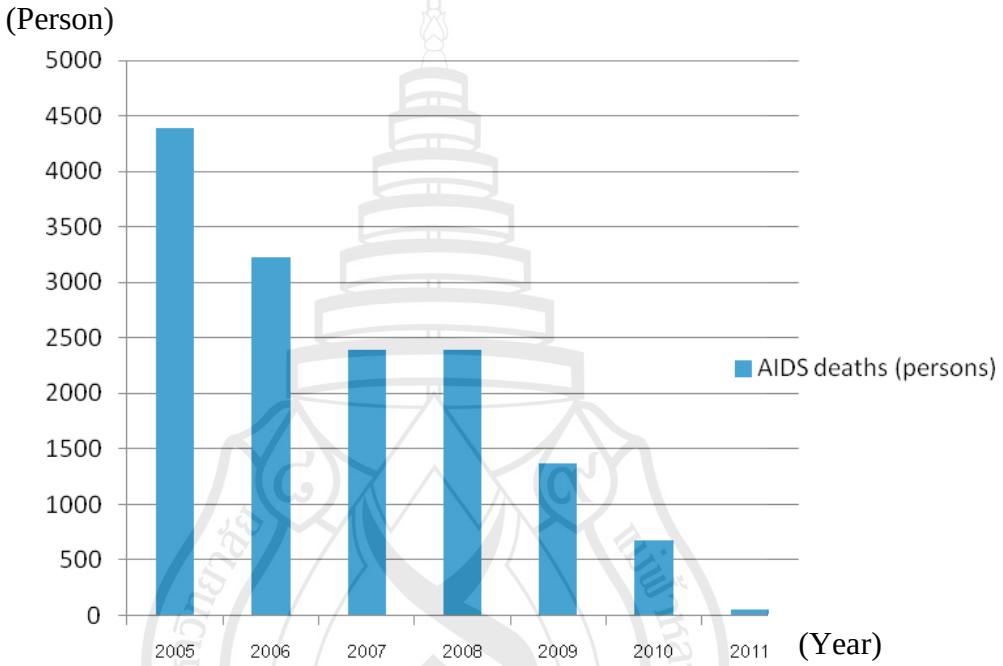



**Figure 2** AIDS Deaths in Thailand by Year (2005-2011)

In 2011, Chiang Rai Province, the northernmost province of Thailand, had a population of over 1, 200, 000 people (Wikipedia, 2013). The province has an area of about 12, 000 $km^2$ and is divided administratively into 18 districts. Located in what many refer to as the Golden Triangle area, where Myanmar, Laotian, and Thai borders converge, Chiang Rai is a gateway to southern China. The pattern of HIV distribution in Chiang Rai province is similar to the national trend. The number of HIV/AIDS patients in Chiang Rai province increased steadily after 1989, reached its peak in 1997-1998, dropped gradually until 2003 and started another cycle of increase in 2004

(Jongsthapongpanth & Bagchi-Sen, 2010). The cumulative number HIV/AIDS patients in Chiang Rai province during 1988 and October 2012 were 34, 352, and 15, 402 of them have died (Chiang Rai Provincial Public Health Office, 2012).

In 1987, the first antiretroviral therapy for AIDS, named azidothymidine (AZT), was approved for use in the United States. In 1996, the Highly Active Antiretroviral Therapy (HAART) was developed by UNAIDS. Later on, these therapeutic approaches brought promising changes to HIV infection, from an unavoidably fatal condition into a chronic manageable disease (Palmisano & Vella, 2011). The initial HAART regimens typically consisted of a combination of two NRTIs (Nucleoside Reverse Transcriptase Inhibitors) plus an NNRTIs (Nonnucleoside Reverse Transcriptase Inhibitors) or a PIs (Protease Inhibitors). In 2000, the combinations of antiretroviral drugs started to be used to treat people with HIV in Thailand (Kanshana & Simonds, 2002). The outcome was that patients having been treated with HAART could survive longer than before, because the treatment equipped the patients with greater capability of delaying the full-born state of AIDS. In subsequent years, the number of people accessing ARV increased dramatically, thereby significantly reducing the number of people dying from AIDS (Ormaasen, Sandvik, Dudman & Bruun, 2007).

There are two major types of death linked with HIV/AIDS: AIDS-related deaths and non-AIDS-related deaths. AIDS-related deaths include *Pneumocystis carinii* pneumonia (PCP), Cytomegalovirus (CMV) disease, non-Hodgkin lymphoma, Kaposi's sarcoma, encephalopathy, wasting syndrome, pulmonary/respiratory tract infection (RIT), meningitis, *Mycobacterium avium* complex (MAC) infection, and other AIDS-related malignancies.

Non-AIDS-related deaths, on the other hand, include drug overdose (in IDUs), suicide, violence, non-HIV related malignancies, hepatic diseases, septicemia, diabetes mellitus and cardiovascular conditions (Pacheco, Tuboi, Faulhaber, Harrison & Schechter, 2008). The pattern of AIDS mortality has also changed over time in developed countries since late 1996, as a result of the introduction of HAART and the utilization of preventive measures for opportunistic diseases, causing the number of HIV/AIDS deaths to dramatically decrease (Kumar, Kilaru & Roach, 2006; Borrell et al., 2006). Some deaths, however, were reported for non-AIDS-related causes

(Rodriquez et al., 2002). In addition, AIDS-related deaths are still being reported for diverse reasons, including failure of or late access to care, antiretroviral toxicities, and failure of HAART secondary to drug-resistant virus or advanced disease (Wood et al., 2003).

Several studies have shown that, prior to the introduction of HAART, AIDS mortality had been higher than during the era of HAART (Krentz, Kliewer & Gill, 2005; Ormaasen, Sandvik, Dudman & Bruun, 2007). In the pre-HAART period, 90.00% of all deaths were caused by AIDS-related factors, whereas only 67.00% of deaths are AIDS-related in the current HAART era. Moreover, deaths from non-AIDS-related conditions have increased and remained important causes of mortality among HIV/AIDS patients (Krentz et al., 2005).

The extension of life expectancy, thanks to HAART, has increased the number of HIV-infected patients in whom HCV or HBV disease progresses to liver fibrosis and cirrhosis (Cribier et al., 1995; Thio et al., 2002). Thio et al. (2002) reported that HIV itself accelerates the progression of HCV and HBV liver disease by increasing HCV and HBV load and hastening the onset of cirrhosis. Some studies reported 16.00-41.80% of deaths caused by liver diseases (Krentz et al., 2005; Salmon-Ceron et al., 2005; Teja, Sudha & Lakshmi, 2007; Martínez et al., 2007) and 2.00-8.00% by the HBV infection (Salmon-Ceron et al., 2005; Lewden et al., 2005). Chronic viral infections of the liver are one of the most important causes of hospitalization and mortality among HIV-infected patients during the HAART era in developed countries (Bica et al., 2001).

HBV was reported to have been transmitted to more than 2,000 people around the world, and today, about 350 million remain infected chronically and have become permanently carriers. In general, about 10.00% of HBV infected person will develop to a chronic HBV carriers stage within 6 months except people who have low immune response such as AIDS patients. The HIV-infected person has a greater opportunity to develop HBV carrier stage while compare to uninfected person. In 2012, three quarters of the world's population were on the stage of high levels of infection. Every year, over 4 million people are acute clinical cases of HBV, and 25.00% of those are carriers. One million people die of chronic active hepatitis, cirrhosis or primary liver cancer every year (WHO, 2013b). Normally, HBV is detectable in semen, saliva, and

nasopharyngeal fluids and can be transmitted through both sexual intercourse and exposure to infected blood. Thus HBV and HIV infections share common risk factors, and their co-infection is quite common. More than 80.00% of HIV-infected patients show some markers of past or current HBV infection (Solomon et al., 1990), and 8.00-11.00% of them are found to be hepatitis B carriers (Lee, 1997).

The stage of HIV/HBV co-infection is common due to shared routes of transmission. In the low endemic areas, such as North America, Australia, and Europe, HIV/HBV co-infection is usually acquired through sexual intercourse or percutaneous transmission in adults. The prevalence of chronicity or co-infection is 5.00-7.00% among HIV-infected individuals (Alter, 2006). In the countries with intermediate and high HBV epidemic areas, the main route of transmission of HBV is perinatal transmission, with 10.00-20.00% (Nyirenda et al., 2008; Diop-Ndiaye et al., 2008).

Liver diseases may progress more rapidly in those HIV/HBV co-infected patients and could lead to serious liver disease complications such as cirrhosis and liver cancer. There is an increase in the probability to develop hepatotoxicity after initiation of ARV drugs in HIV-infected patients co-infected with HBV. HIV/HBV co-infected patients can have a greater rate of chronicity; on the contrary, their decreased rates of antibody to hepatitis B e antigen (anti-HBe) and antibody to hepatitis B surface antigen (anti-HBs) seroconversion raise hepatitis B viral replication, probably due to the impairment of their bodies' immune response. Consequently, HIV/HBV co-infection may be associated with the liver fibrosis progression and may increase the rate of liver decompensation, cirrhosis, liver cancer or liver failure.

For the above reasons, it is important to focus on and identify the risk factors of HBV infections in order to prevent decompensated cirrhosis, Hepatocellular Carcinoma (HCC) and HAART hepatotoxicity, and to improve the survival rate among HIV-infected patients. A few studies of HIV/HBV co-infection in Thailand have been conducted, particularly in high epidemic areas like Northern Thailand. Some research, however, did not investigate the characteristics independently associated with HIV/HBV co-infection. This study was aimed to evaluate the risk factors which are associated with HIV/HBV co-infection in Thailand.

## 1.2 Objective

To determine the risk factors in HIV/HBV co-infection at an early HIV diagnosis.

## 1.3 Hypotheses

1.3.1 There was an association between sexual behavior and HIV/HBV co-infection.

1.3.2 There was an association between risk behavior and HIV/HBV co-infection.

1.3.3 There was an association between medical history and HIV/HBV co-infection.

1.3.4 There was an association between socio-demographic characteristics and HIV/HBV co-infection.

## 1.4 Conceptual Framework

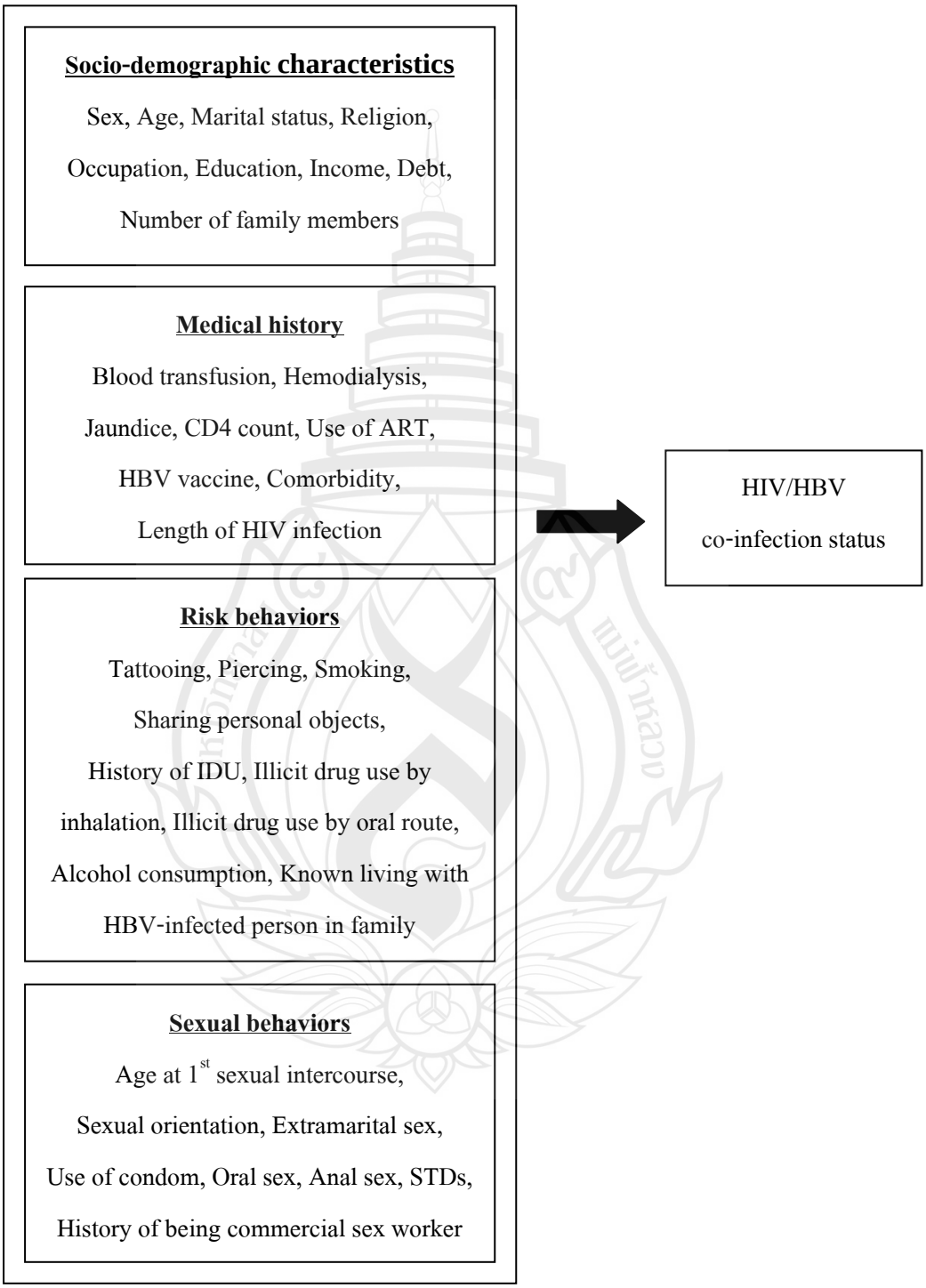



**Figure 3** Conceptual Framework

## 1.5 Operational Definitions

**1.5.1 HIV and HBV (HIV/HBV) Co-Infection** means HIV-infected patients who have been tested seropositive for hepatitis B surface antigen (HBsAg).

**1.5.2 Risk Factors** mean factors that have been risked for HIV and HBV co-infection in this study, which organized in four series including socio-demographic characteristics, medical history, risk behaviors, and sexual behaviors.

**1.5.3 Length of HIV Infection** means the number of years since the subjects became aware of their HIV status through the detection of anti-HIV in their blood to the time of collection of research data.

**1.5.4 CD4 Cell Count** means the recently counted CD4 level (cells/$mm^3$) in any subject at the time of the data collecting step.

**1.5.5 Comorbidity** means other personal diseases of the subjects, such as diabetes, hypertension, TB, migraine, and epilepsy.

**1.5.6 Condom Use** (before knowing HIV status) means the subjects' use of condoms before they became aware of their HIV infection.

**1.5.7 Condom Use** (after knowing HIV status) means the subjects' use of condoms after they became aware of their HIV infection.

**1.5.8 Homosexual** means men or women who have had sex with a person or persons of the same sex. Therefore, the term homosexual includes both of MSM (men who have sex with men) and WSW (women who have sex with women).

# CHAPTER 2

# LITERATURE REVIEWS

## 2.1 Human Immunodeficiency Virus (HIV)

HIV is a complex RNA virus of the genus *Lentivirus* within the *Retroviridae* family. The virus is an approximately 100 nm icosahedral structure with 72 external spikes that are formed by the two major envelope glycoproteins gp120 and gp 41. The lipid bilayer is also studded with a number of host-cell proteins during the budding process. HIV has a characteristic dense, cone-shaped nucleocapsid composed of the core protein p24. This nucleocapsid harbours two copies of the 9.8 kb single-stranded RNA genome which are associated with the viral enzymes reverse transcriptase (RT), RNase H, integrase and protease. In addition to structural genes HIV has genes whose products contribute to the complex regulation and replication of the virus. Of particular interest is the Nef (negative factor) protein. Deletions and mutations of this protein have been found in some HIV-infected individuals characterized as long-term non-progressors. Two major types of the AIDS virus, HIV-1 and HIV-2, have been identified. The major serological differences reside in the surface protein gp120. The HIV-1 and HIV-2 are further separated into subtypes (clades) due to the marked variability in the V3 (variable region) of the gp120 protein (Haaheim, Pattison & Whitley, 2002).

HIV is a blood-borne virus that can be spread through unprotected sex, sharing drug-injecting equipment and to a child during or shortly after birth from an infected mother. HIV cannot be cured, but can be managed by a combination of medications (Bor, Evans & Levitt, 2013).

### 2.1.1 HIV Epidemiology

AIDS was identified as a new disease entity in 1981 in the USA. The disease spread rapidly, first in the urban population on the east and west coast, later to all part of the country and to other continents. Although the incidence of AIDS in the homosexual population has received great public attention and still accounts for the majority of AIDS cases in the USA and in Western Europe, worldwide heterosexual transmission is the leading route for the HIV pandemic; in particular contacts with prostitutes, bisexual men and intravenous drug users. Sub-Saharan Africa, India, Thailand, the Russian Federation and China represent epidemic hot spots with rapidly increasing HIV prevalence. HIV is also transmitted from mother to child, *in utero*, intrapartum or perinatally via breast feeding. Transmission via whole blood or blood products has virtually ceased in industrialized countries after the introduction of blood screening. However, this is still a major concern in developing countries.

The total number of individuals living with HIV by the end of the year 2011 was reported by the WHO to be 34.00 million (31.40 million–35.90 million). An estimated 0.80% of adults aged 15-49 years worldwide were living with HIV, although the burden of the epidemic continues to vary considerably between countries and regions (UNAIDS, 2012). People 1.7 million died of AIDS in 2011, a 24.00% decreased since 2005. Deaths have declined due in part to ART scale up. HIV is a leading cause of death worldwide and the number one cause of death in Africa. New HIV infections overall have declined by more than 20.00% since 2001 and, in 25 low and middle income countries, new infections have declined by more than 50.00%. Still, there were about 2.5 million new infections in 2011 or more than 7,000 new HIV infections per day.

### 2.1.2 HIV Pathogenesis

Levy (1993) described that the virus initially enters an individual primarily by infecting either activated T cells, resident macrophages, or mucosal cells in the bowel or uterine cavity. Very few activated CD4 lymphocytes are circulating in the blood, and peripheral blood monocytes are not very susceptible to infection. In the initial days following acute infection, high levels of virus replication will take place in the lymph nodes and will be reflected by p25 antigenemia and viremia levels. CD8 cell

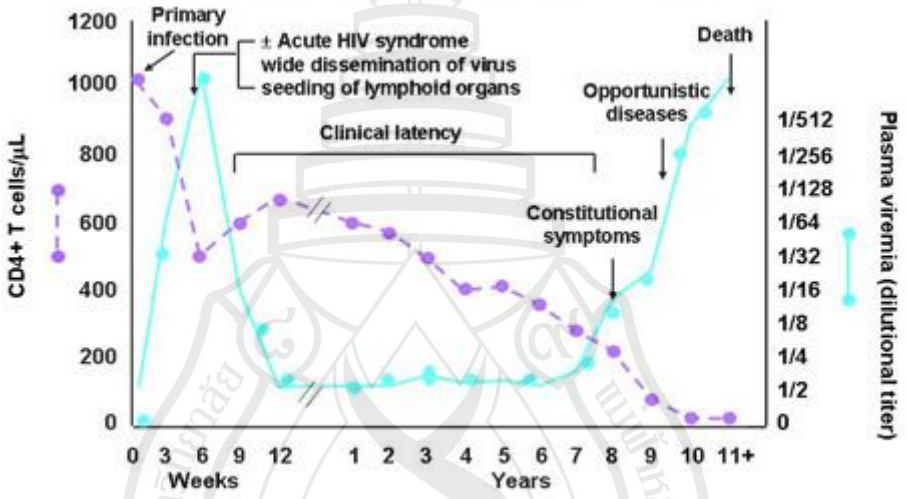
numbers rise, as is observed in other viral infections (Blumberg & Schooley, 1985). Generally, within one month, the viremia is reduced substantially, most probably as a result of immune reactions against the virus. Cellular immune responses could be the first effective antiviral activity, since in some cases CD8 cell HIV responses have been detected even prior to seroconversion (Clerici et al., 1992; Mackewicz & Levy, 1992). Moreover, virus levels in plasma appear to be reduced before neutralizing antibodies can be detected in recently infected individuals (Daar, Chernyavskiy & Moudgil, 1992).

Over the ensuing months to years, the CD8 cell number remains slightly elevated. Virus replication in the body persists, particularly in lymph nodes and PBMC, but levels mostly measured in the blood are low; in general, the virus is appreciatively suppressed. CD4 cell numbers usually rise to near-normal levels 3 to 4 months after the primary virus infection. They appear generally to decrease steadily during the persistent period at a rate estimated by some at 25 - 40 cell/$mm^3$ per year (Lang et al., 1989).

At a point when the individual develops symptoms, CD4 cell counts are usually below 300 cells/$mm^3$ and the levels of HIV in the blood are high compared with those during the asymptomatic state. At the same time, a reduction in antiviral CD8 cell responses can be demonstrated (Mackewicz & Levy, 1992). In some cases, the number of CD4 cells drops precipitously over just a few months, perhaps mirroring a return of high level virus production (Eyster, Gail, Ballard, Al-Mondhiry, & Goedert, 1987; Schellekens et al., 1992).

At the time of symptomatic infection, and certainly when the individual develops AIDS, the virus has characteristics distinct from the virus recovered soon after the infection. It takes on properties associated with virulence in the host, including an enhanced cellular host range, rapid kinetics of replication, and CD4 cell cytopathicity. It also appears to resist neutralization and becomes sensitive to enhancing antibodies. This virus, emerging later in the host, is related at the genomic level to the early virus (> 97.00%), but certain molecular changes in the regulatory (e.g., tat) and envelope regions are associated with these altered in vitro biologic properties.

With ongoing reduction in immunologic control of HIV infection, the more virulent variants replicate to higher levels and destroy large numbers of CD4 cells. They eventually eliminate the potential for any immune response to control opportunistic infections. In the terminal stages, CD8 cells, as well as CD4 cells, decrease in number, perhaps in part because of the loss of IL-2 production by of virus can be CD4 cells.




**Source** Fauci A. S., Pantaleo G., Stanley S. & Weissman D. (1996). Immunopathogenic mechanisms of HIV infection. *Annals of Internal Medicine, 124*(7). Retrieved July 25, 2013, from http://annals.org/article.aspx?articleid=709558

**Figure 4** The Natural History of HIV Infection

### 2.1.3 Laboratory Testing

Fearon (2005) described the laboratory diagnosis of HIV infections. It is identified either by the detection of HIV-specific antibodies in serum or plasma or by demonstrating the presence of the virus by nucleic acid detection using polymerase

chain reaction (PCR), p24 antigen testing or, rarely these days, by growing virus in cell culture. Antibody testing is the method most commonly used to diagnose HIV infection. With the highly sensitive HIV-1/HIV-2 enzyme immunoassay (EIA) tests, seroconversion can be detected within two to three weeks of infection in the majority of cases. In a small number of early seroconverters who are still in the 'window period', the p24 antigen may become positive before antibody is detectable. Therefore, to enable the laboratory to select appropriate testing, it is important to provide a clinical history that includes any recent high-risk behavior or symptoms consistent with seroconversion illness.

2.1.3.1 Enzyme Immunoassay (EIA)

EIA is commonly used as a screening assay for many infectious diseases, including HIV. These assays are used because they are highly sensitive and generally amenable to automation, facilitating high-volume testing. HIV EIAs have become increasingly more sensitive and specific since HIV testing began in the early 1980s. This has shortened the 'window period', or the time from exposure to seroconversion, from up to 12 weeks or more in the early days of diagnostic testing to the current “window period” of less than three weeks in most cases.

The small disadvantage of such a highly sensitive test is that the test produces false positives, the number and type of which vary with the assay used and the HIV prevalence in the tested population. All HIV diagnostic laboratories must confirm repeated EIA screen-positive results by a confirmatory assay, usually with Western blot. Laboratories may choose to first test with a second EIA assay, which uses a different part of the viral antigen for antibody capture, as part of their testing algorithm. Specimens that screen positive in the first assay but negative in the second assay should still be considered for confirmatory testing if the patient is symptomatic or high risk.

2.1.3.2 p24 antigen

p24 antigen tests are also EIA-based and use antibody to capture the disrupted p24 antigen from patient serum. Positive results that are repeatable must be confirmed with a neutralization procedure. In rare instances, the p24 antigen can be detected before HIV antibody in newly infected individuals. This test is useful for

specimens from patients that are high risk and symptomatic but HIV EIA-negative, or for specimens that are EIA-positive but Western blot-negative or -indeterminate. A follow-up HIV antibody test should be requested when a patient is p24 antigen-positive but antibody-negative. In a seroconverting patient, the follow-up specimen will be positive within a few weeks after the initial screen. It is important to remember that not all seroconverting patients will have detectable p24 antigen, and that this antigen may not be reliably found in individuals who are known to be HIV antibody-positive.

2.1.3.3 Western blot

The Western blot is an immunoblot that allows for the characterization of antibodies to each viral protein. Patient serum is reacted with a nitrocellulose strip containing all of the constitutive HIV virus proteins (core and envelope), arranged by molecular weight after polyacrylamide gel electrophoresis. Any specific antibodies present in the patient's serum will bind to the antigen, producing a coloured band when alkaline phosphatase-labelled, antihuman immunoglobulin G conjugate and colour development solution are added. These bands can be visualized, and positivity is assessed following the manufacturer's recommendations and based on the number and type of bands present. Generally, a specimen must show a positive reaction with a minimum of one core band and one envelope band to be judged positive by Western blot.

Specimens that have bands present but do not fulfill the criteria for positivity are called Western blot indeterminate, and a follow-up specimen should be requested, usually collected three to four weeks after the initial specimen. In follow up, patients will either show a definitive pattern indicating that they have seroconverted or will demonstrate the same banding pattern as previously observed. In the latter circumstance, the vast majorities of these patients are HIV-negative and have nonspecific antibody. In these cases, if the patient is considered to be at risk or is particularly anxious, a qualitative PCR may be recommended to confirm that the patient is truly HIV-negative. Because these indeterminate banding patterns may be seen in patients who are not infected, the Western blot does not make a good screening test for HIV. The test is also much more labour intensive and costly than

EIA tests and does not allow for the efficient processing of large numbers of specimens.

Specimens that have an unusual band pattern not reflective of EIA optical density values should be tested on an HIV-2. Generally, HIV-2 positive specimens exhibit antibody to many HIV-specific bands but lack antibody to the envelope components of HIV-1.

2.1.3.4 Qualitative PCR

PCR is a method that amplifies viral nucleic acid to allow for its detection in patient specimens. It is a particularly specific and sensitive test which can pick up very small numbers of viral particles. PCR is very useful in the diagnosis of HIV infection in babies born to infected mothers. Babies will carry maternal antibody up to approximately 15 months of age and, therefore, the antibody test is not a reliable indicator of infection in these children.

Because of the very specific nature of the primers used in the HIV-1 DNA PCR assay, caution should be used in assessing a negative result in an infant if the mother is likely to have HIV-2 or a non-B HIV infection. It may be prudent to ensure that the HIV DNA PCR assay is effective in detecting the mother's HIV infection if there is any likelihood that the case is not an HIV subtype B infection.

PCR may also be useful in resolving indeterminate Western blot results and testing immunocompromised individuals who may not mount an antibody response.

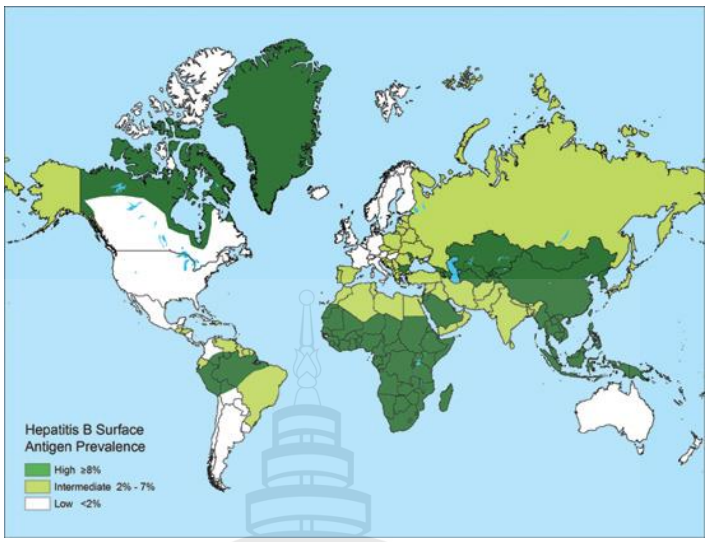
2.1.3.5 Quantitative RNA PCR and genotyping

Quantitative RNA PCR must only be used to monitor HIV-positive individuals before or during antiretroviral therapy. It is used in conjunction with CD4 counts and general clinical assessments to ascertain when therapy should be started. It is also used to help determine the patient's response to therapy. Genotyping is used to monitor the development or presence of drug resistance in patients before or during therapy. It is also used to assist physicians in their choice of antiretroviral drug combinations for the patient (Zhang & Versalovic, 2002; Hirsch et al., 2003). Quantitative PCR should not be used as a diagnostic test for HIV because false positives and false negatives can occur in these circumstances.

Thailand had been classified in the third group under the epidemic of HIV/ASIDS. There were at least 4 waves for explain the epidemic of HIV in Thailand. The first wave was reported in 1998 among IV drug users (IVDU). The second wave was female prostitutes, then the third wave happed in the clients who visited the prostitutes and finally came to their family and general population. Three years after being declared an epidemic among the III group countries, 300,000 Thai people had been estimated to be infected out of 55 million populations. Muaeng, Mae Chan, Phan, Mae Sai, and Wieng Pa Pao were the most top five district of cumulative number of AIDS patients in Chiang Rai province since 1988 to October 2012 (Chiang Rai Provincial Public Health Office, 2012).

## 2.2 Hepatitis B Virus (HBV)

Hepatitis B virus is a 42 nm particle belonging to the *Hepadnaviridae*. It has a 3.2 kilobase (kb), partially double-stranded, relaxed circular DNA genome and has been classified by molecular diagnostic techniques into six major genotypes (A-F) (Ngui, Hallet & Teo, 1999). HBV is the leading cause of chronic liver disease and liver-related death worldwide, with the majority of these cases occurring in areas of Africa and Asia where HBV prevalence is high (HBsAg prevalence ≥8%). Sexual transmission is followed by percutaneous transmission as the secondmost common mode of transmission (Edmunds et al., 1996).




**Source** Teshale, E. H. (2011). *Hepatitis B.* Centers for Disease Control and Prevention. Retrieved July 25, 2013, from http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/hepatitis-b

**Figure 5** Prevalence of Hepatitis B Surface Antigen

### 2.2.1 HBV Epidemiology

The distribution of HBV infection varies greatly throughout the world. In areas where the prevalence is high, such as Southeast Asia, China and Africa, more than half the population is infected at some time in their lives, and more than 8.00% are chronic carriers of the virus, the result of either neonatal transmission (vertical) or transmission from one child to another (horizontal). Areas with low levels of endemicity include North America, Western Europe, and Australia, where only a minority of people come into contact with the virus, as a result of horizontal transmission among young adults (Kane, 1995). The prevalence of chronic hepatitis B infection is about 5.00% worldwide, but differs between regions. Infection rates are low (0.10-2.00%) in the USA and Western Europe, intermediate (2.00-8.00%) in Mediterranean countries and Japan, and high (8.00-20.00%) in Southeast Asia and

sub-Saharan regions (Lavanchy, 2004). Additionally, HBV genotypes have a distinct geographical distribution: genotype A is prevalent in Northwestern Europe and the USA; genotype B and C in Asia; genotype D in the Mediterranean basin, Middle East, and India; genotype E in west Africa; genotype F in South and Central America; genotype G in the USA and France; and genotype H in Mexico and South America (Kao, 2007).

### 2.2.2 HBV Pathogenesis

HBV is not cytopathogenic. In acute infection, clinical hepatitis B becomes apparent after an incubation period of 45-180 days. The elimination of HBV by non-cytopathic mechanisms begins several weeks before the disease onset. HBV DNA clearance is mediated largely (up to 90.00%) by antiviral cytokines that are produced by cells of the innate and adaptive immune responses including tumor necrosis factor $\alpha$, interferon alfa, or interferon beta (Murray, Wieland, Purcell & Chisari, 2005; Guidotti et al., 1999; Bertoletti, Maini & Williams, 2003). After viral DNA declines, a cytolytic immune response with hepatocyte apoptosis and necrosis ensues, coincident with the onset of clinical hepatitis and a rise in serum alanine aminotransferase (ALT). The recognition of infected hepatocytes by virus-specific CD8 cytotoxic T cells, via class I human lymphocyte antigen (HLA-I) presenting HBV peptides, is presumed to be the main mechanism causing both liver damage and virus control. Cytotoxic T cells further recruit various antigen-non-specific inflammatory cells into the liver by secreting cytokines, initiating a cascade of immunological events leading to necroinflammation (Thimme et al., 2003; Visvanathan & Lewin, 2006; Rehermann, 2007). A vigorous, multispecific CD4 and CD8 response is associated with viral clearance (Maini et at., 2000). In individuals with chronic hepatitis B infection, the hepatitis B virus-specific CD4 and CD8 response is insufficient, and can cause a persistent inflammatory response that is ineffective for HBV clearance (Maini et at., 2000; McMahon et al., 1985).

### 2.2.3 Serological Markers of HBV

The first serologic marker to appear following exposure to HBV is HBsAg. On average, HBsAg can be detected 1 month after exposure to the virus. But the detection can range about 1 week to 9 weeks. When HBsAg is positive, HBV DNA can usually be detected in the patient's blood. The duration of HBsAg and HBV DNA is variable. About 50% of patients will be HBsAg and HBV DNA negative by 7 weeks after symptom appear and all patients who recover will be HBsAg and HBV DNA negative by 15 weeks after the appearance of symptom. HBeAg is generally detectable in patient with acute disease. The presence of HBeAg in serum correlates with higher titer of virus and greater infectivity while the present to the anti-HBe denotes the lower level of virus and the less infectivity. Symptoms when present occur on average 12 weeks after the exposure with a range of 9 weeks to 21 weeks. A diagnosis of acute hepatitis B can be made on the basis of the detection of IgM class anti-HBc. IgM anti-HBc is generally detectable at the time of onset symptom and decline to sub-detectable level within 6-8 months. Total anti-HBc persists as a marker of the past infection. Anti-HBs become detectable during convalescent and after the disappearance of HBsAg and generally indicate recovery and immunity from re-infection. Window period is the duration after the disappearance of HBsAg and before the appearance of anti-HBs. During this window period, IgM anti-HBc and total anti-HBc are the only serologic markers that presence.

2.2.3.1 Hepatitis B surface antigen (HBsAg): A protein on the surface of the HBV; it can be detected in high levels in serum during acute or chronic HBV infection. The presence of HBsAg indicates that the person is infectious. The body normally produces antibodies to HBsAg as part of the normal immune response to infection.

2.2.3.2 Hepatitis B surface antibody (anti-HBs): The presence of anti-HBs is generally interpreted as indicating recovery and immunity from HBV infection. Anti-HBs also develop in person who has been successfully vaccinated against hepatitis B.

2.2.3.3 Total hepatitis B core antibody (anti-HBc): Appears at the onset of symptoms in acute hepatitis B infection and persists for life. The presence of anti-HBc indicates previous or ongoing infection with HBV in an underfined time frame.

2.2.3.4 IgM antibody to hepatitis B core antigen (IgM anti-HBc): Positivity indicates recent infection with HBV (less than or equal to 6 months). Its presence usually indicates acute infection.

2.2.3.5 Hepatitis B e antigen (HBeAg): A secreted product of the nucleocapsid gene of the hepatitis B virus is found in serum during acute and chronic hepatitis B infection. Its presence indicates that the virus is replicating and the infected person has high levels of HBV.

2.2.3.6 Hepatitis B e antibody (HBeAb or anti-HBe): Produced by the immune system temporarily during acute HBV infection or consistently during or after a burst in viral replication. Spontaneous conversion from e antigen to e antibody (a change known as seroconversion) is a predictor of long-term clearance of HBV in patients undergoing antiviral therapy and indicates lower levels of HBV.

In conclusion, HBV is transmitted by percutaneous or mucosal exposure to the blood or body fluids of an infected person, most often through injection-drug use, from sexual contact with an infected person, or from an infected mother to her newborn during childbirth. Transmissions of HBV also occur among persons who have prolonged but nonsexual interpersonal contact with someone who is HBV-infected (e.g., household contacts).

The risk for chronic HBV infection decreases with increasing age at infection. Among infants who acquire HBV infection from their mothers at birth, as many as 90.00% become chronically infected, whereas 30.00-50.00% of children infected at age 1-5 years become chronically infected. This percentage is smaller among adults, in whom approximately 5.00% of all acute HBV infections progress to chronic infection.

## 2.3 HIV/HBV Co-Infection

Vallet-Pichard and Pol (2004) said that co-infections by hepatotropic viruses and the HIV were frequent given the shared (sexual, mother-to-child and parenteral) routes of transmission. In studies conducted in the “AIDS era” (pre-HAART), the late consequences of the hepatitis virus-related chronic liver disease were indeed

overshadowed by extra-hepatic causes of deaths, related to severe immune deficiency, namely opportunistic infections, lymphomas or wasting syndrome (Lefkowitch, 1994) and the impact of hepatitis virus infection on mortality of HIV-infected patients was low (Cacoub et al., 2001). The development of HAART [regimens composed of mucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs) and/or non-nucleoside reverse transcriptase inhibitors (NNRTIs)] have resulted in a significant decrease in morbidity and mortality among HIV-infected patients (Pallela et al., 1998). This clear benefit allowed the expression of liver-related complications associated with HCV or HBV chronic infection. Liver disease is nowadays one of the leading causes of morbidity and mortality in co-infected patients (Bica et al., 2001).

### 2.3.1 Definition of HIV/HBV Co-Infection

A person who is infected with both HBV and HIV in the same time is said to have an HIV/HBV co-infection. The detected HBV marker in several studies of HIV/HBV co-infection was HBsAg (Kouassi-M'Bengue et al., 2011; Sungkanuparph et al., 2004; Pal et al., 2011; Hussain, Kulshreshtha, Sinha, Yadav & Katoch, 2006; Gupta & Singh, 2006; Filho et al., 2009; Nyirenda et al., 2008; Guriev & Spinu, 2010; Adewole et al., 2009) and some studies included anti-HBc (Asl, Avijgan & Mohamadnejad, 2004; Solomon et al., 1990).

People co-infected with HIV/HBV have higher level of hepatitis B viremia, progress to chronic hepatitis B approximately 5 times among people infected with HBV only and have a higher risk of cirrhosis and hepatocellular carcinoma (Hoffmann & Thio, 2007). HIV/HBV co-infection increases the morbidity and mortality compared to patients caused by either infection alone.

### 2.3.2 Impact of Co-Infection on the Natural History of HBV and HIV

Singh & Wong (2009) described the impact of co-infection on the natural history of HBV and HIV. The rate of progression and complications from viral hepatitis are accelerated in patients with HIV co-infection (Puoti, Torti, Bruno, Filice & Carosi, 2006; Thio, 2009). After acquiring HBV infection, HIV-infected individuals are 6 times more likely to develop chronic hepatitis B than HIV negative

individuals (Bodsworth, Cooper & Donovan, 1991; Hadler et al., 1991; Gatanaga, Yasuoka, Kikuchi, Tachikawa & Oka, 2000). This was more likely to occur in HIV-infected men with lower CD4 cells (Bodsworth et al., 1991). Decreased rates of clearance of Hepatitis Be Antigen (HBeAg) and increase HBV replication are also seen, with higher HBV DNA viral load (Colin et al., 1999; Gilson et al., 1997; Krogsgaard et al., 1987). In addition, HIV-infected individuals are more likely to lose previously developed protective anti-HBs antibody and develop acute hepatitis B infection this risk is also associated with lower CD4 counts (Laukamm-Josten et al., 1988).

HIV also hastens the progression of HBV related liver disease. Cirrhosis is more common despite lower ALT levels than in HBV mono-infection and is also more common with lower CD4 counts (Colin et al., 1999; DiMartino et al., 2002). HIV-HBV co-infected men are greater than 17 times more likely to die of liver related causes compared to those mono-infected with HBV (Thio et al., 2002). The impact of co infection is especially important in regions with widespread use of ART (Hoffman & Thio, 2007). As the use of ART becomes more prevalent in parts of the world with high HBV endemicity and long term survival increases, it is likely that liver disease from chronic hepatitis B in HIV-infected population may emerge as a greater public health problem than before (Hoffman & Thio, 2007). It is unclear at present if the risk of hepatocellular carcinoma (HCC) is increased, but there is some evidence that HIV infected individuals with lower CD4 counts are at greater risk of developing HCC (Clifford et al., 2008). For individuals on ART, co infection with chronic hepatitis B increases the risk of hepatotoxicity from ART three-fold to five-fold (Puoti et al., 2003; Sulkowski, Thomas, Chaisson & Moore, 2000; Livry et al., 2003).

### 2.3.3 Impact of HBV Co-Infection on HIV Disease Progression

HBV is mainly a hepatotropic virus, but it has also been shown to be a lymphotropic virus so that HIV-1 and HBV meet at the cellular level in co-infected patients (Laure et al., 1985). Some authors have suggested that HBV could alter the course of HIV infection, inducing faster progression to AIDS. Recent studies have been conducted to investigate the molecular relationship between the two viruses.

A recent study has demonstrated that HBV-X protein (HBx) super-induces ongoing HIV replication and HIV-1 long-terminal repeat (LTR) transcription (Gómez-Gonzalo et al., 2001). These results obtained *in vitro* support the hypothesis that HBx could contribute to a faster progression to AIDS in HIV/HBV co-infected individuals.

Several cross-sectional and longitudinal studies designed to assess the impact of HBV co-infection on HIV disease progression have been published in the last 15 years. Two longitudinal studies did not show any impact of HBV co-infection on CD4 depletion, progression to full-blown AIDS, or AIDS induced mortality (Scharschmidt et al., 1992). These results have been confirmed in an analysis of data from a cohort of British homosexuals, but this study has not excluded an effect on the development of certain HIV-related complications, such as thrombocytopenia (Gilson et al., 1997).

On the other hand, two studies identified an association between HBV infection and a more severe evolution of HIV disease. In a Scandinavian cohort of homosexual men, the influence of previous or present hepatitis B infection was associated with a more rapid HIV disease progression (Eskild et al., 1992). Another study revealed that HBV co-infection was associated with reduced survival inpatients with full-blown AIDS (Ockenga et al., 1997).

### 2.3.4 Impact of HIV Co-Infection on HBV Disease Progression

There are many evidences that co-infection with HIV significantly modifies the natural history of HBV infection. In patients with HBV infection, HIV co-infection is associated with higher chronicization rate of acute HBV, (Eskild et al., 1992; Weller et al., 1986; Krogsgaard et al., 1987; Bodsworth, Donovan & Nigthingale, 1989; Koblin, Taylor, Rubinstein & Stevens, 1992) higer levels of HBV replication even in the presence of hepatitis Delta virus super-infection, (Eskild et al., 1992; Weller et al., 1986; Krogsgaard et al., 1987; Bodsworth et al., 1989; Koblin et al., 1992; Goldin et al., 1990; Perrillo, Regenstein & Roodman, 1986; Colin et al., 1999; McDonal, Harris, Waters & Thomas, 1987), lower rate of spontaneous loss of HBeAg and/or HBsAg and seroconversion to anti-HBe and anti-HBs (Eskild et al., 1992; Weller et al., 1986).

There are contradictory data on the activity of inflammatory liver disease in co-infected patients. Many studies from northern Europe and USA cohorts of

homosexuals showed a significantly less severe necroinflammatory activity in HIV co-infected patients (Bodsworth et al., 1989; Perrillo et al., 1986). This observation seems to be related to the immunological pathogenesis of liver damage in chronic HBV infection. However, immunesuppression related to HIV is associated with reactivation of HBV infection in patients who have lost detectable HBsAg (Lazizi et al., 1988; Waite et al., 1988; Colin et al., 1999). On the contrary, some studies performed in Californian and French cohorts, who included many infection drugs users, showed increased necroinflammatory activity in HIV seropositive subjects (Bonacini, Govindarajan & Redeker, 1991; Housset et al., 1992). Contradictory data have also been published about the impact of HIV on hepatitis B progression towards cirrhosis and hepatocellular carcinoma: some northern European and USA studies did not reveal an unfavorable impact of HIV co-infection on hepatitis B evolution, (Gilson et al., 1997; Krogsgaard et al., 1987; Bodsworth et al., 1989; Perrillo et al., 1986) while two French studies (Colin et al., 1999; Housset et al., 1992) clearly identified a more rapid progression towards cirrhosis in HIV/HBV co-infected subjects.

These discrepancies could be related to differences in the prevalence of infecting HBV genotypes and of mutant HBeAg defective HBV strains, to differences in the degree of immunesuppression, and to the different prevalence of co-factors associated with liver injury (alcohol, HCV, HDV, etc.) in the various cohorts. In the presence of high prevalence of HCV and HDV co-infections, HBV co-infection has been related with a significantly higher incidence of end stage liver disease related death in HIV-seropositives, independently from other factors, (Puoti et al., 2002) with an adjusted OR of 9.00 (95% confidence interval 3.80-21.70). Long-term follow-up prospective studies of HIV/HBV co-infected patients, considering immune status, influence of other potential causes of liver injury, and the genotype of infecting HBV strains, is required to determine if HBV-related liver disease may influence the prognosis of co-infected patients.

In conclusion, co-infection with HIV and HBV is common due to shared routes of transmission. In areas of low endemicity, the prevalence of chronic

co-infection is around 5.00-7.00% among HIV-infected individuals (Alter, 2006). In countries with intermediate and high HBV endemicity, the main routes of transmission of HBV are perinatal or in early childhood; in these countries HBV co-infection rates are 10.00-20.00% (Nyirenda et al., 2008; Diop-Ndiaye et al., 2008).

## 2.4 Researches Related to the Study

Sellier et al. (2010) reported a consistent relation between HIV/HBV co-infection and liver diseases, and that the co-infection is also related to all-cause mortality. These results have been confirmed by another cohort study design which showed a high liver-related mortality in 72 HIV-infected individuals with chronic hepatitis B (Bonacini, Louie, Bzowei & Wohl, 2004). Moreover, a cross-sectional study by Sungkanuparph et al. (2004) found that the prevalence of HBV co-infection in Thai HIV-infected patients was 8.70%, relatively high when compared with that in other countries.

### 2.4.1 HIV/HBV Co-Infection and General Characteristics

Hussain et al. (2006) assessed the risk factors of co-infections with HIV, HBV, HCV, and syphilis among patients attending sexually transmitted disease (STD) clinics. They found the incidence of HIV and BBV co-infection was greater among males than females. In addition, the study of Gupta and Singh (2006) found that more male HIV-infected patients were co-infected with HBV than female were, with a statistical significance. However, Adewole et al. (2009) revealed opposite results, showing that females formed a larger proportion of HIV/HBV co-infection victims than males did. The study, conducted in Nigeria, showed that females were more prone to HIV/HBV co-infection than males were.

A cross-sectional study of Sungkanuparph et al. (2004) in Thailand showed that the elderly age group and history of IDU were significantly associated with HBV infection among HIV-infected patients.

With marital status taken into account, Adewole et al. (2009) found the largest proportion of HIV/HBV co-infection was HIV-infected patients who were married.

Nonetheless, the study by Garner (2000), Gregson, Zhuwau, Anderson and Chandiwana (1999), and Hill, Cleland and Ali (2004), which took place in South Africa, Zimbabwe, and Brazil, respectively, showed that members of Pentecostal and AIC (African Initiated Church) churches exhibited a reduced risk of HIV infection, due to their reduced likelihood of having extramarital partners when compared with members of other religious groups. Trinitapoli and Regnerus (2004) showed that independence of denomination and attendance at religious services were also associated with the decreasing odds of both risk behavior and perceived risk, an effect that was particularly strong for members of Pentecostal churches. However, Gray (2004) suggested that the restrictions on sexual behavior and the consumption of alcohol, as well as the practice of circumcision, may have contributed to Muslims in Africa experiencing reduced levels of HIV-contracting risk.

A cross-sectional study by Arvanitidou, Constantinidis, Doutsos, Mandraveli and Katsouyannopoulos (1998), which attempted to detect HBV markers to determine the prevalence of HBV infection and assess the risk of exposed sewage workers becoming infected, found that exposure to sewage was independently associated with positivity for HBV infection. Sali, Bashtar and Alavian (2005), evaluating some possible risk factors for the spread of HBV infection in Tehran, Iran, found that occupations could be considered a risk factor for HBV infection, particularly those involving any kind of contact with infected blood. In addition, the study noted that certain jobs displayed greater potential of putting people at risk of adopting some risky lifestyles, among them were health care workers (HCWs), barbers, large-vehicle drivers, sewage workers, detainees, and prisoners (Arvanitidou et al., 1998; Meheus, 2000; Jahani, Motevalian & Mahmoodi, 2003; Candan, Alagözlü, Poyraz & Sümer, 2002; Rischitelli, Harris, McCauley, Gershon & Guidotti, 2001). Finally, Kouassi-M'Bengue et al. (2011) reported in their study the prevalence of co-infection which varied across occupations and social classes.

A systematic review by Kirby, Obasi, and Laris (2006) concluded that a large majority of school-based sex education and HIV education interventions reduced the risky sexual behavior in developing countries.

Poverty and lack of economic opportunities were commonly cited as important contributors to the AIDS epidemic. The study by Lurie et al. (1995) found female sex

workers who had a lower socio-economic status were more likely than those with a higher socio-economic status to be infected with HIV-1, syphilis, and hepatitis B. The study by Shelton, Cassell, and Adetunji (2005) identified a strong positive relationship between household wealth and HIV infection prevalence in the United Republic of Tanzania. However, Parkhurst (2010) argued that socio-economic status might be associated with sets of behavior that were either preventive of or contributing to HIV infection.

Beyond the individual realm, Fiore et al. (2001) stated that HIV was an important individual illness that affected victims' families. Moreover, French et al. (2003) concluded that intra-familial transmission needed to be considered in the high epidemic areas of HIV/AIDS HIV-1 in particular. A study of hepatitis B in the Croatia family identified kinship degrees as the factor that influenced intra-familial transmission. Moreover, the use of a common shaver was identified as one of the most frequent routes of infection transmission among family members (Milas et al., 2000). In Thailand, the studies by Punyagupta, Olson, Harinasuta, Akarawong and Varawidhya (1973) reported that children positive for HBsAg were commonly found in families in which an older member was HBsAg positive. They concluded, therefore, that close contact in the family was an important source of infection.

### 2.4.2 HIV/HBV Co-Infection and Medical History

A close observation of patients' medical history showed significant relation to HIV/HBV co-infection. Hemodialysis patients were identified as a high-risk population for HBV infection (Dienstag, 1998). At the same time, HIV/AIDS were also found at a high proportion in this group of population. Khameneh and Sepehrvand (2008) evaluated the prevalence of HBV in chronic hemodialysis patients in Iran, and found that 6.50% of HBsAg positive patients were among this group of population.

Before 1970, approximately 6.00% of multi-transfused recipients had acquired transfusion-transmitted HBV. Over the last four decades, the risk of transfusion-transmitted hepatitis B virus has been steadily reduced, yet HBV transmission has remained the most frequent transfusion-transmitted viral infection. The reduced risk of HBV transfusion transmission was mainly due to the HBsAg negativity of donated

blood collected either during the pre-seroconversion “window period” (WP), defined as the time between infection and detection of a viral antigen or antibody marker, or during the late stages of infection (Candotti, Chaar & Allain, 2011). Saraswat et al. (1996) studied a prospective design to determine the incidence and type of post-transfusion hepatitis in 41 open-heart surgery patients who had received 3 or more units of HBsAg-negative blood, and found 14.60% developed post-transfusion hepatitis. They argued that screening of donated blood units for HBsAg using ELISA did not eliminate all blood units infected with hepatitis B virus.

Landrum et al. (2010), studying the hepatitis B vaccination and risk of hepatitis B infection in HIV-infected individuals, found that, overall, HBV vaccination after diagnosis of HIV was not associated with reduction in the risk of HBV infection. The rate of HBV and poor immunogenicity in HIV patients might have been the main cause of the unpreventability of HBV infection, despite HBV vaccination. However, with regard to history of sexually transmitted infection (STI), males were associated with a greater risk of HBV infection after vaccination than females.

A retrospective study by Wiwanitkit and Suwansaksri (2005), which focused on the cause of jaundice among Thai HIV-infected patients, revealed that alcohol-related liver diseases appeared to be the most common cause of jaundice, followed by opportunistic infections, neoplasm, drug-induced hepatitis, and viral hepatitis B; moreover, 46.15% of the HIV-infected patients with jaundice showed a CD4 count of <200 cells/μL.

Another retrospective study, by Smiatacz and Zielińska (1996), found that HIV-infected patients who were anti-HBc seropositive had a slower CD4 count decline rates compared with the anti-HBc seronegative group. Moreover, Mesner et al. (2012) studied the CD4 reconstitution in HIV/HBV co-infected patients, and found a greater increase in the CD4 count in HIV-infected patients with chronic HBV than in those who were HBV negative. However, Hoffmann et al. (2009) found that in HIV-infected patients receiving long-term HAART, the HBV status did not influence HIV suppression or CD4 increase.

### 2.4.3 HIV/HBV Co-Infection and Risk Behavior

Rahimi-Movaghar, Razaghi, Sahimi-Izadian and Amin-Esmaeili (2010), who studied the HIV, HCV, and HBV co-infections among injecting drug users in Tehran, Iran, found that 7.80% of IDUs had HIV/HBV co-infection. In Thailand, there were 529 HIV-infected patients, with an HBV prevalence rate of 8.70%. History of intravenous drug use was also associated with HBV co-infection (Sungkanuparph et al., 2004). Another two studies conducted in Italy and Canada have shown that intravenous drug use was independently associated with hepatitis B (Stroffolini et al., 2000; Roy et al., 1999; Sagliocca et al., 1997). Therefore, the increased risk caused by sharing contaminated needles and other injection equipment among IDUs seems rather obvious and has been well documented (Centers for Disease Control and Prevention [CDC], 1982).

Samuel, Doherty, Bulterys and Jenison (2001) assessed the seroprevalence and risk factors for HBV, HCV, and HIV-1 infection among IDUs in New Mexico. They found the receipt of tattoos in prison or jail was associated with HBV infection, and identified the prevalence of HBV at 61.10%, whereas that of HIV was found at 0.50%. Another case-control study, conducted in Italy and Africa, found that percutaneous exposure, including body piercing and ear piercing, was associated with viral hepatitis (Mele et al., 1995; Abdool Karim et al., 1988).

The meta-analyses by Fisher, Bang, and Kapiga (2007) in Africa found that alcohol drinkers were more likely to be HIV seropositive than non-drinkers. The study by Mbulaiteye et al. (2000) in Uganda found that participants who lived in a household where alcohol was sold were associated with a history of alcohol-drinking experience. The rate of HIV prevalence among adults living in households selling alcohol was 15.00%, compared with 8.00% among those living in non-alcohol-selling households. Moreover, individuals who had drunk alcohol were twice as likely to experience HIV prevalence than those who had not (Mbulaitey et al., 2000).

Cigarette smoking was another habit more commonly found among HIV-infected patients than among uninfected persons. Estimates indicated that as many as 50.00-70.00% of HIV-infected persons were current smokers (Shuter & Bernstein, 2008; Tesoriero, Gieryic, Carrascal & Lavigne, 2010). Smoking was also associated with substantial morbidity among HIV-infected patients. In fact, smoking and HIV

infection were independent risk factors in many of the comorbid illnesses, such as bacterial pneumonia, chronic obstructive pulmonary disease, and lung cancer (Kohli et al., 2006; Crothers et al., 2006; Kirk et al., 2007). This was supported by Crothers et al. (2009), who found that current cigarette smoking was associated with significantly increased mortality in HIV-infected patients. Moreover, the impact of smoking on mortality was accentuated in the HIV-infected patients when contrasted with the HIV-negative patients.

### 2.4.4 HIV/HBV Co-Infection and Sexual Behavior

Initiation of sexual activity usually introduces an individual to the risk of acquiring sexually transmitted diseases (STDs). There were two cross-sectional studies conducted by Hallett et al. (2007) in Zimbabwe showed the median first-sex age of the Zimbabweans was 19 years for men and 18 years for women. They also found that women who began to have sex earlier than others of their age were more likely to be infected with HIV. Pettifor, van der Straten, Dunbar, Shiboski, and Padian (2004) found that a significantly higher risk profile among Zimbabwean women was related to their early sexual intercourse, multiple lifetime partners, and failure to complete high school. Another study in Tanzania showed that women who had their first sexual intercourse at age 18-19 or 20 and over were less likely to have STDs, HIV-1, and HSV-2 than women who had their first sexual intercourse before their 18$^{th}$ birthday (Ghebremichael, Larsen & Paintsil, 2009).

Gupta and Singh (2006) studied the prevalence of HBV and HCV infection among HIV-infected patients. They found the predominant mode of acquiring HIV infection was heterosexual contact (80.00%), followed by transfusion of blood products (6.00%), IDU (2.30%) and the rest were unknown. The rate of HBsAg co-infection was 5.32 in HIV-positive patients.

The study by Margolis et al. (2006) found that men who drank heavily or who had risky sex partners were more likely to report unprotected sex with multiple partners. Moreover, a study of people with HIV/AIDS in Botswana found that 20.00% of them reported two or more sex partners in the previous 3 months before participating in the study (Kalichman et al., 2007). They also found that steady sex

partners of participants with multiple partners were significantly less likely to be protected by condoms than steady partners of individuals with only one sex partner.

Significantly higher rates of HIV infection have been documented among sex workers and their clients, as compared with most other population groups within a given country. HIV infection often spreads among sex workers before spreading into the general population (UNAIDS technical update, 2002). A cross-sectional study by Bacon et al. (2006) showed that HIV prevalence among MSM-IDUs was 12.00%, and 68.00% of them reported being paid by another man for sex. Moreover, a retrospective cohort study conducted by Gray et al. (1997) found that the HIV-1 seroprevalence among Thai female commercial sex workers was 52.00%, rising from 29.00% at the initial test in 1989 to 53.00-63.00% during 1900-1993.

The risk of HIV transmission from an infected sex partner through oral sex is much less than the risk of HIV transmission from anal or vaginal sex. Measuring the exact risk of HIV transmission as a result of oral sex was very difficult. Additionally, because most sexually active individuals practice oral sex in addition to other forms of sex, such as vaginal and/or anal sex, when transmission occurs, it was difficult to determine whether infection was caused by oral sex or by other more risky sexual activities. Several co-factors might also increase the risk of HIV transmission through oral sex, including oral ulcers, bleeding gums, genital sores, and the presence of other STDs (CDC, 2009). Even so, Hawkins (2001) concluded that unprotected oral sex carried a risk of HIV transmission, owing to the frequency with which it was practiced and given the fact that those with the highest risk of acquiring HIV often had protected anal or vaginal sex.

Men who have sex with men (MSM) have much higher risk of HIV acquisition than those in the heterosexual population, even in countries with generalized epidemics (Baral, Sifakis, Cleghorn & Beyrer, 2007). A study among MSM found a much higher liver-related mortality rate among HIV/HBV co-infected patients than among those with HIV mono-infection and those with HBV mono-infection (Thio, 2003). A cross-sectional study by Berry et al. (2012) found that HIV infection was associated with unprotected receptive anal sex among MSM in Kazakhstan.

The best evidence for condoms acting as an effective means of hepatitis B prevention is in a series of studies of female sex workers in high prevalence countries (Sánchez et al., 1998; Bhave et al., 1995; Tanaka et al., 1996). In one report from Peru, there was 40.00% lower prevalence and 66.00% reduction in incidence of serological evidence of hepatitis B in women reporting consistent condom use for vaginal sex, compared with women who did not use condoms regularly (Sánchez et al., 1998).

# CHAPTER 3

# MATERIALS AND METHODS

The hospital-based case-control study design was conducted, aimed to identify the risk factors in HIV/HBV co-infection among HIV patients who were diagnosed between 2006 and 2012 in Chiang Rai province, Thailand. This study was divided into 2 phases: quantitative and qualitative phases. The quantitative phase was conducted to test the hypotheses of the study. The qualitative phase was done to explain the phenomenon of HIV/HBV co-infection.

## 3.1 Study Design

Using the hospital-based case-control approach, this study was conducted on a population of HIV-infected patients who had been recently diagnosed. The target population consisted of HIV-infected patients aged 18-65 years who had been living in Chiang Rai province for at least 2 years prior to the commence of the study. Both the cases and controls were selected from the HIV-infected patients who had been diagnosed between 2006 and 2012 and who had visited ARV clinics at any one of the study sites.

### 3.1.1 Study Sites

There were 18 hospitals' ARV clinics in Chiang Rai (in 18 districts). The researcher used a simple random sampling technique to select the study sites. After the sampling, 9 hospitals' ARV clinics were selected as the study sites, and all of the subjects were recruited from these clinics, which were under Mae Chan, Mae Sai, Chiang Saen, Khun Tan, Phaya Meng Rai, Theong, Mae Suai, Mae Lao and Phan Hospitals, as shown in figure 6.


**Figure 6** Map of Chiang Rai Province, Thailand

### 3.1.2 Sample Size Estimation

The sample size estimation was calculated separately in each study phase.

3.1.2.1 Sample size estimation for the quantitative phase

The quantitative phase needed a sample size of at least 240 cases (120 cases and 120 controls) in order for association to be observed. The alpha had been set at 0.05 and 80.00% power. The sample size was calculated by Epi Info, with an added 10.00% for any error in the study. Therefore, in total, 120 subjects were used for cases and the other 120 subjects for controls. The sample size estimation was calculated based on Schlesseman's formula, as shown below (Schlesselman, 1982).

$$n = \frac{\left[ Z_{\alpha}\sqrt{(1+m)\,\bar{p}'(1-\bar{p}')} + Z_{\beta}\sqrt{p_1(1-p_1)+mp_0(1-p_0)} \right]^2}{(p_1-p_0)^2}$$

$$\bar{p}' = \frac{p_1 + p_0/m}{1+1/m}$$

$$p_1 = \frac{p_0\psi}{1+p_0(\psi-1)}$$

$$n_c = \frac{n}{4}\left(1+\sqrt{1\frac{2(m+1)}{nm|p_0-p_1|}}\right)^2$$

Where:

| | | |
|---|---|---|
| n | = | Sample size |
| $n_c$ | = | Continuity corrected sample size |
| $\alpha$ | = | Level of type I error set at 0.05 (5%) |
| $Z_{\alpha}$ | = | Standard score for $\alpha$ ($Z_{0.95}$ =1.96) |
| $\beta$ | = | Level of type II error set at 0.20 (20.00%) |
| $Z_{\beta}$ | = | Standard score for power of test ($Z_{0.20}$ = 0.84) |
| 1-$\beta$ | = | The power of statistics set at 0.80 (80.00%) |
| $P_0$ | = | The probability of exposure in controls ($P_0$=2.60%) (Chimparlee, Oota, Phikulsod, Tangkijvanich & Poovorawan, 2011) |
| P1 | = | The probability of exposure in cases ($P_1$=8.70%) (Sungkanuparph et al., 2004) |
| m | = | Number of controls per case subjects |
| Ψ(psi) | = | The odds ratio (OR) of exposure in cases relative to controls. |

3.1.2.2 Sample size estimation for the qualitative phase

In the qualitative phase, despite the absence of one single ideal or most appropriate sample size estimation method, data saturation, defined by many as the point at which the data collection process no longer offered any new or relevant data, was used as a means of estimating a sample size. The purposive sampling technique was used for selecting of subjects into the study.

### 3.1.3 Study Population

The population for this study was HIV-infected patients who had been diagnosed between 2006 and 2012, aged 18-65 years, and had visited any one of the 9 hospitals' ARV clinics in Chiang Rai province.

3.1.3.1 Inclusion criteria

1. Inclusion criteria for cases:

1) Voluntarily participated into the study.

2) Having tested positive for an HBsAg marker in a serum test by immunochromatographic technique;

3) Having lived in Chiang Rai province at least 2 years before the interview date;

4) Being able to communicate in Thai; and

5) Holding a Thai identity card.

2. Inclusion criteria for controls:

1) Voluntarily participated into the study.

2) Having tested negative for HBsAg, Anti-Hbs, and Anti-HBc in a serum test by the immunochromatographic technique and enzyme immunoassay, respectively;

3) Having lived in Chiang Rai province at least 2 years before the interview date;

4) Being able to communicate in Thai; and

5) Holding a Thai identity card.

3.1.3.2 Exclusion criteria

1. Exclusion criteria for cases:

1) Being HIV-infected subjects who could certify, or were proved by medical records, that their HIV infection was from their mothers;

2. Exclusion criteria for controls:

1) Being HIV-infected subjects who had tested positive for either anti-HBs or anti-HBc in serum.

2) Being HIV-infected subjects who could certify, or were proved by medical records, that their HIV infection was from their mothers.

## 3.2 Research Instruments

The research instruments were developed according to the quantitative and qualitative phases. The research instruments for the quantitative phase consisted of a structured questionnaire, 5 mL of blood specimen, and laboratory testing. The research instruments for the qualitative phase, on the other hand, consisted of an interview guideline and a sound recorder.

### 3.2.1 Research Instruments for the Quantitative Phase

3.2.1.1 The structured questionnaire

The structured questionnaire was created by the researcher according to the conceptual framework developed by the researcher. It was composed of 4 parts: socio-demographic characteristics, medical history, risk behavior, and sexual behavior. See appendix B.

1. The questions for socio-demographic characteristics addressed 9 issues, namely, age, gender, marital status, religion, habitat, number of family members, education, occupation, monthly income, and debt.

2. The questions for medical history addressed 8 issues, namely, history of blood transfusion, hemodialysis, jaundice, HBV vaccination, use of ARV drug, recent CD4 cell count, length of HIV infection, and comorbidity.

3. The questions for risk behavior addressed 9 issues, namely, known living with HBV-infected member in the same family, sharing personal objects with family members, history of IDU, history of drug abuse by inhalation and oral route, tattooing, piercing, alcohol drinking, and smoking.

4. The questions for sexual behavior addressed 8 issues, namely, age at first sexual intercourse, sexual orientation, history of STDs, history of commercial sex work, number of partners, history of oral and anal sex, and use of condoms.

3.2.1.2 Blood specimen

A five-mL specimen of blood was obtained from each of the subjects. The blood was drawn from the median cubital vein, stored in a serum test tube, and transferred to the laboratory. Each blood test tube was centrifuged at 3,500 rpm for 5 minutes to separate the serum. Each subject's serum was coded and tested for HBsAg and anti-HBs markers. The remaining serum was stored at -20 $^{o}$C and tested for anti-HBc when the number of subjects reached 91 samples according to the laboratory testing reason.

3.2.1.3 Laboratory method

There were 3 methods for identifying the HBV status of all subjects: HBsAg, anti-HBs, and total anti-HBc. Following is the principle for each method.

1. The detection for the HBsAg in this study was done using the immunochromatographic test method named Determine® or Alera™ with 99.95% specificity and 95.16% sensitivity. These assays were tests for the qualitative detection and are also routinely used to diagnose the stage of HBV infection. By this assay, the subject's serum is added to the sample pad. As the serum migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antibody conjugate. This mixture continues to migrate through the solid phase to the immobilized antibodies at the patient window site. If HBsAg is present, the antigen will bind to the antibody-selenium colloid and to the antibody at the patient window, forming a red line at the patient window site. In the absence of HBsAg, the antibody-selenium colloid will flow past the patient window, and no red line is formed at the patient window site. To ensure assay validity, a procedural control is incorporated in the device and labeled "Control". If the control bar does not turn red by assay completion, the test result is invalid and the sample should be retested. For the test procedure and interpretation of the test, see appendix D.

2. The detection for the anti-HBs in this study was done using the immunochromatographic test method named NanoSign Anti-HBs with greater than 99.00% specificity and sensitivity. This assay is a test for the qualitative detection of anti-HBs in human serum or plasma. Basically, a nitrocellulose membrane is immobilized with HBsAg. Another HBsAg is conjugated to colloidal gold particles. This conjugate is then placed on a polyester or fiberglass as a conjugate pad. When

the specimen is dropped into the device, the solubilized conjugate migrates with the sample by passive diffusion, and both the conjugate and sample come into contact with the HBsAg that they immobilized on the nitrocellulose membrane. For the test procedure and interpretation of the test, see appendix D.

3. The detection for the anti-HBc in this study was done using the EIA named Monolisa$^{TM}$ Anti-HBc Plus with greater than 99.83% specificity and 100.00% sensitivity. The detection of antibodies to hepatitis B nucleocapsid or core antigen is a major marker for the presence of past (anti-HBc Total) or recent (anti-HBc IgM) infection by the HBV. The detection limit of this test was $\geq$ 0.60 anti-HBc IgG PEI Unit, and $\geq$ 4.00 anti-HBc IgM PEI unit. By this approach, enzyme immunoassay (indirect ELISA type) is used for the simultaneous detection of total antibodies to HBV core in human serum or plasma. This test is based upon the use of a solid phase prepared with a recombinant HBc antigen. The serum to be tested and the control serum are added to the wells. If antibodies to HBc are present, they will bind to the antigens fixed on the solid phase. The peroxidase-labeled antibodies to human IgG and IgM are added after a washing step. They in turn bind to the specific antibodies captured on the solid phase. After removal of the unbound enzymatic conjugate, the antigen-antibody complex is revealed by addition of substrate. After the reaction has been stopped, the absorbance values are read using a spectrophotometer at 450/620-700 nm. The absorbance measured for a sample allows the presence or absence of antibodies to HBc to be determined. The color intensity is proportional to the quantity of anti-HBc antibodies bound on the solid phase. The test procedure, calculation, interpretation, specificity and sensibility of the test see appendix D. For the test procedure, calculation and interpretation of the test, see appendix D.

### 3.2.2 Research Instruments for the Qualitative Phase

3.2.2.1 The in-depth interview

The interview included unstructured questions used to explore the risk of STDs infection among the subjects. The qualitative data obtained from this in-depth interview was used to confirm the results of the quantitative analysis. The interview was composed of questions concerning socio-economic status, risk behavior, and sexual behavior. See appendix C.

1. The socio-economic status interview focused on subject's income and educational level.

2. The risk behavior interview focused on the knowledge of separating personal objects in the family, use of illicit drugs, and alcohol drinking.

3. The sexual behavior interview focused on homosexuality, history of being or using the service of a commercial sex worker, and having multiple sex partners.

3.2.2.2 Audio recording

A mobile phone was used for the recording of the conversation between the researcher and each of the subjects during an in-depth interview.

### 3.2.3 Methods of Validity and Reliability Testing

3.2.3.1 Method of validity testing for the structured questionnaire

The validity was tested by the 3 experts and adjusted using the Item Objective Congruence Index (IOC) technique (Rovinelli & Hambleton, 1977). The IOC technique was used for appropriate question identification. Questions were adjusted and corrected if the IOC value was less than 0.50. Finally, there was no question that had the IOC value less than 0.50. IOC was scaled by the following formula.

$$\text{IOC} = \frac{\sum R}{n}$$

Where

R = the score from all experts in each item

*n* = the number of experts

3.2.3.2 Method of reliability testing for the interview guideline

The interview was developed by the researcher and the researcher's advisor. Before being used in the field, all of the questions were tested for reliability by means of pilot-testing with 5 persons at Mae Chan Hospital. The correlation coefficient was 0.75 which means the interview guideline has high reliability.

## 3.3 Data Gathering Process

The process of data gathering involved the following steps.

### 3.3.1 Contacting the ARV Clinics' Staff Members

All ARV clinics' staff members were informed about the objectives and the selection of subjects to be recruited into the study. Then a date for data collection was appointed.

### 3.3.2 Collection of Quantitative Data

All persons matching the inclusion and exclusion criteria were invited to participate in the project on a voluntary basis, after informed consent had been obtained. To guarantee privacy during data collection and confidentiality of the information obtained from the questionnaire and interview, a code was used to anonymously refer to each respondent. The structured questionnaire was used in conjunction with a face-to-face interview to collect data. Each data collecting session, which lasted about 30 minutes, took placed in a private room to ensure confidentiality. After the interview, a 5-mL blood sample was collected for the purpose of HBV marker detection.

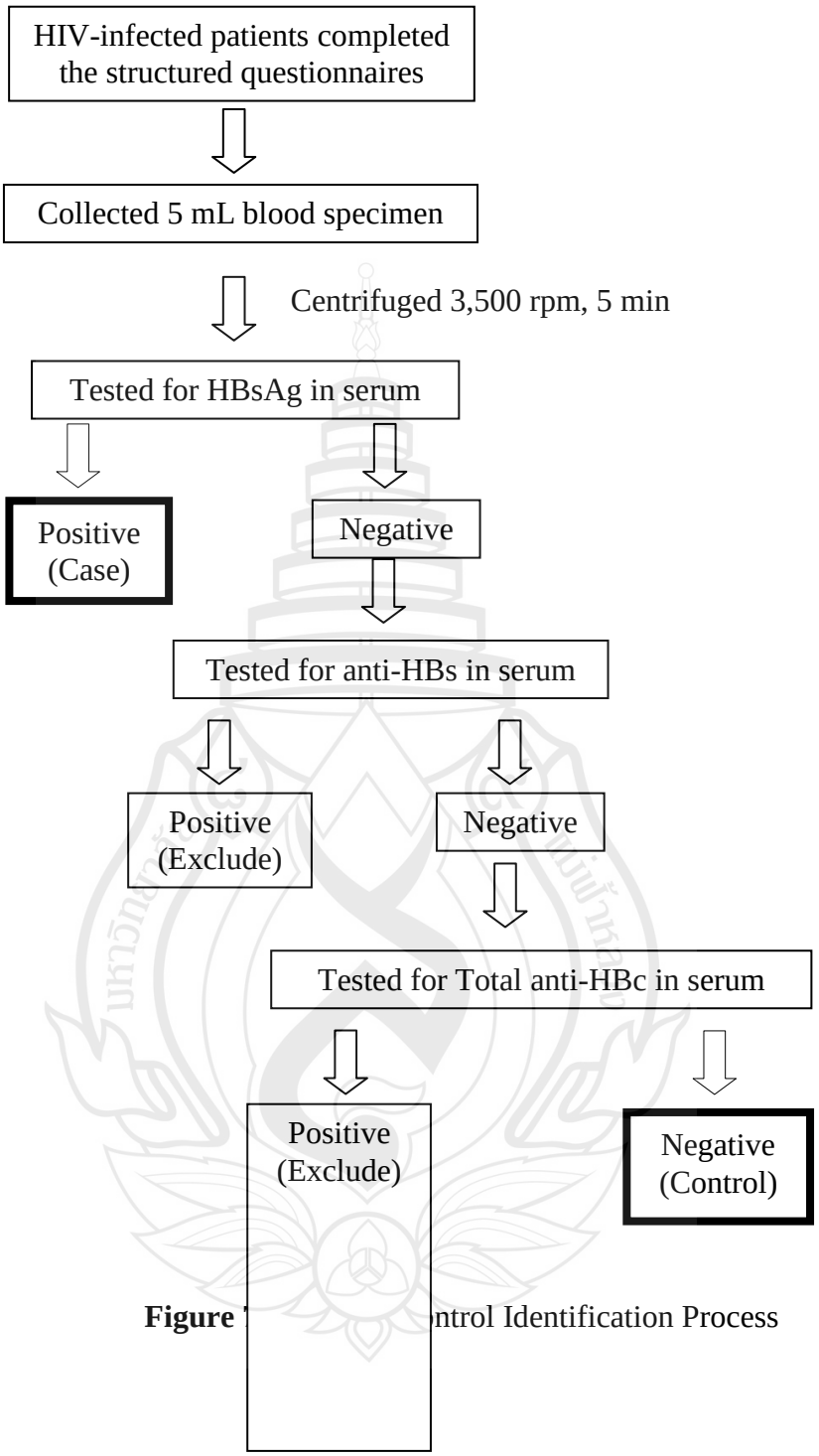



**Figure** ntrol Identification Process

### 3.3.3 Collection of Qualitative Data

After the selection of cases and controls, the qualitative phase started by contacting the staff at the ARV clinics to make appointments with the subjects. In this phase, the selection of subjects was by means of the purposive selection method. Subjects form case and control groups were recruited for the in-depth interview which conducted in a private and confidential room. The in-depth interview began with recording general information, followed by asking interview questions. Before the in-depth interview, the researcher explained the objectives of the study and each of the subjects gave the permission for the conversation between the researcher and subject to be recorded. This step of data collection took about 25-30 minutes.

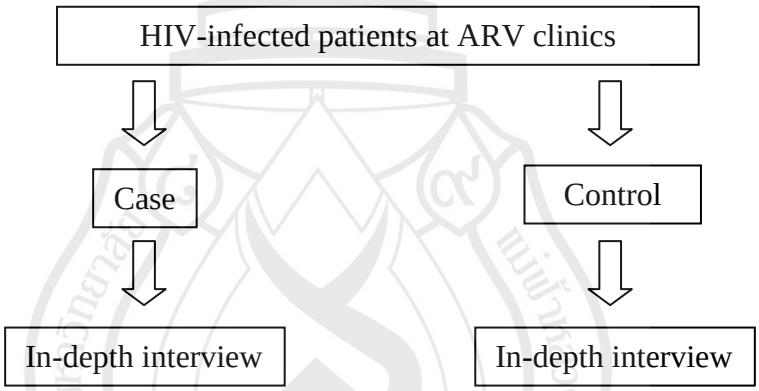



**Figure 8** Collection of Qualitative data

## 3.4 Statistical Analysis

The data were subject to double entry and screened for all missing value and uncorrected cells using Microsoft Excel. The analysis in the quantitative phase was conducted by using statistics program.

This step of analysis involved 4 main groups of characteristics, each put into the model separately according to the conceptual framework.

Descriptive statistics (means, standard deviation, frequency, and percentage) was used to describe the general characteristics of the subjects.

Univariate analysis was then used to identify risk factors associated with the HIV/HBV co-infection at $\alpha = 0.10$. All significant variables were considered and maintained in the model for multivariate analysis.

Multivariate analysis was conducted to identify the risk factors associated with the HIV/HBV co-infection by controlling all possible confounder factors. Variables that remained associated with HIV/HBV co-infection at $\alpha=0.05$ in the statistical model were determined as the risk factors.

## 3.5 Ethical Consideration

Before this study was conducted, it had been approved as conforming to the research protocol by the Ethics in Human Research Committee of Mae Fah Luang University. No aspects of the research were found in contradiction to The Declaration of Helsinki and Guidelines of National Research Ethics 2011 (Project No. REH-54022, document No. 22/2554), and permission to collect the data in 9 hospitals in Chiang Rai province was granted by the Provincial Chief of Public Health.

# CHAPTER 4

# RESULTS

After both the questionnaire data and 5-mL blood specimen had been obtained from the selected subjects, a total of 355 HIV-infected patients were recruited into the study, which took place during May 2011 to December 2012. All of them voluntarily signed a written informed consent before the data were collected. However, after having been screened using the laboratory method, forty one subjects (11.55%) tested positive for the HBsAg marker. The other 314 subjects, who tested negative for the HBsAg marker, were tested for anti-HBs. There were 77 subjects (21.69%) who were found positive for the anti-HBs marker. The remaining 237 subjects, who had tested negative for anti-HBs, were tested for total anti-HBc. Finally, there were 83 subjects (23.38%) who showed negative results for the total anti-HBc marker (see Figure 9).

Therefore, only 124 subjects (41 cases and 83 controls) were qualified for the analysis, according to the objectives of the study.

All obtained data were presented under the following topics.

Section 4.1 Comparison of general characteristics by group (inclusion and exclusion)

Section 4.2 General characteristics of subjects

Section 4.3 Comparison of characteristics by group (case-control)

Section 4.4 Univariate analysis of risk factors in HIV/HBV co-infection

Section 4.5 Multivariate analysis of risk factors in HIV/HBV co-infection

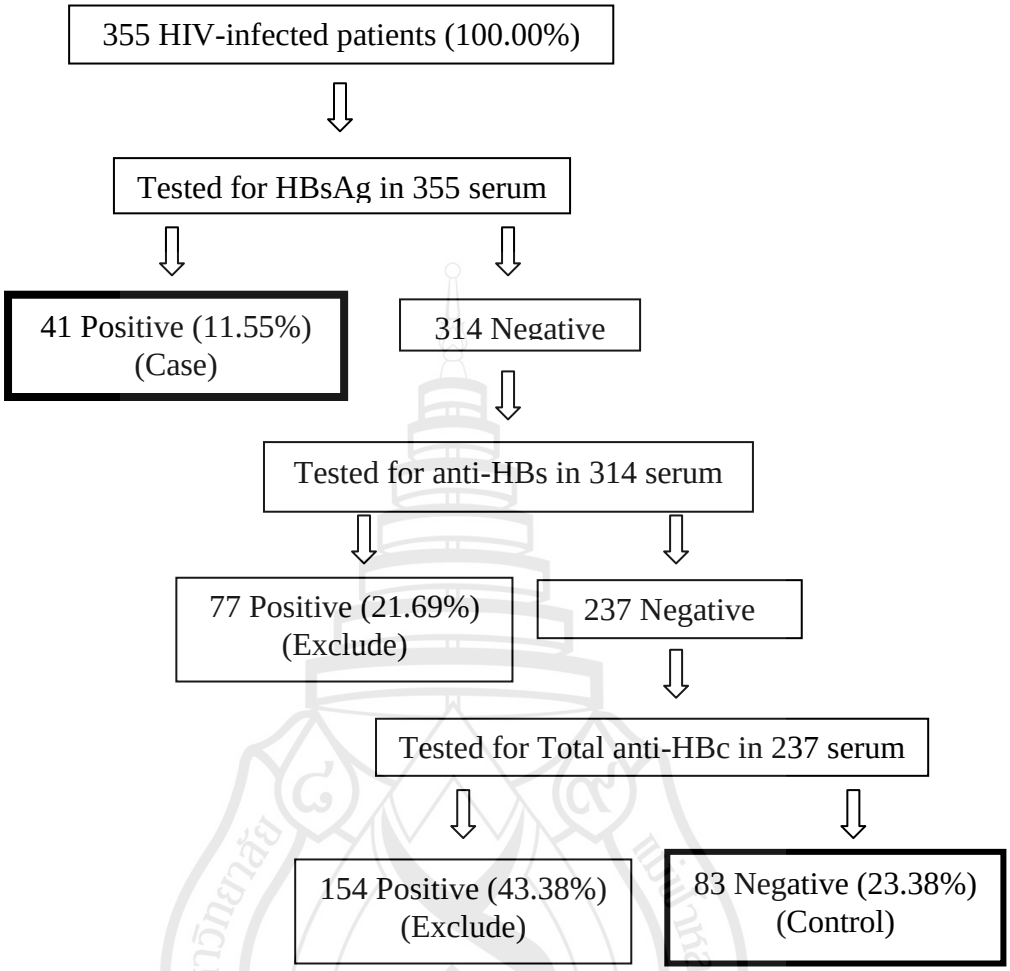



**Figure 9** Process of Identifying Case and Control

## 4.1 Comparison of General Characteristics by Inclusion and Exclusion

This section described the comparison of general characteristics by group (inclusion and exclusion). There were 231 (65.07%) subjects identified for the exclusion group (positive for anti-HBs and total anti-HBc) and 124 subjects identified for the inclusion group (positive for HBsAg, negative for anti-HBs and total anti-HBc).

Nine general characteristics, as shown in Table 4.1, were used to compare between the inclusion and exclusion group. Based on the chi-square test at $\alpha$=0.05, two significant difference between the two groups were marital status and occupation.

**Table 4.1** General Characteristics Comparison Between Inclusion and Exclusion Group

| General characteristics | Inclusion | | Exclusion | | $\chi^2$-test | p-value |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| **Total** | **124** | **100.00** | **231** | **100.00** | | |
| Sex | | | | | 0.01 | 0.907 |
| Male | 62 | 50.00 | 117 | 50.65 | | |
| Female | 62 | 50.00 | 114 | 49.35 | | |
| Age (years old) | | | | | 3.46 | 0.326 |
| ≤29 | 11 | 8.87 | 13 | 5.65 | | |
| 30-39 | 50 | 40.32 | 79 | 34.35 | | |
| 40-49 | 43 | 34.68 | 99 | 43.04 | | |
| ≥50 | 20 | 16.13 | 39 | 16.96 | | |
| Marital status | | | | | 5.99 | 0.050* |
| Single | 13 | 10.48 | 27 | 11.69 | | |
| Married | 78 | 62.90 | 115 | 49.78 | | |
| Divorced/widowed | 33 | 26.62 | 89 | 38.53 | | |
| Religion | | | | | 0.10 | 0.752 |
| Buddhism | 119 | 95.96 | 220 | 95.24 | | |
| Others | 5 | 4.04 | 11 | 4.76 | | |
| Number of family member (persons) | | | | | 0.03 | 0.857 |
| ≤4 | 95 | 76.61 | 175 | 75.76 | | |
| ≥5 | 29 | 23.39 | 56 | 24.24 | | |

**Table 4.1** (Continued)

| General characteristics | Inclusion n | Inclusion % | Exclusion n | Exclusion % | $\chi^2$-test | p-value |
|---|---|---|---|---|---|---|
| Years in school | | | | | 5.18 | 0.075 |
| No education | 23 | 18.55 | 59 | 25.54 | | |
| 1-6 | 68 | 54.83 | 132 | 57.14 | | |
| ≥7 | 33 | 26.62 | 40 | 17.32 | | |
| Occupation | | | | | 4.02 | 0.045* |
| Unemployed | 13 | 10.48 | 43 | 18.61 | | |
| Employed | 111 | 89.52 | 188 | 81.39 | | |
| Income | | | | | 0.21 | 0.647 |
| Yes | 113 | 91.13 | 207 | 89.61 | | |
| No | 11 | 8.87 | 24 | 10.39 | | |
| Debt | | | | | 2.58 | 0.108 |
| Yes | 62 | 50.00 | 95 | 41.13 | | |
| No | 62 | 50.00 | 136 | 58.87 | | |

**Note.** *; Significant Level at $\alpha = 0.05$

## 4.2 General Characteristics of Subjects

This section describes the general characteristics of the 355 subjects initially recruited into the study. The subjects were nearly evenly divided into males (179, or 50.42%) and females (176, or 49.58%). The two largest age groups were the 40-49-year-old group (40.20%) and the 30-39-year-old group (36.54%). Almost all of the subjects (95.49%) were Buddhists. More than half (54.37%) were married, and most (76.06%) lived in families of ≤ 4 persons. In terms of education, while more than half (56.35%) had spent 1 to 6 years in school, 23.05% had not received any education. Concerning occupations, 44.51% worked as daily employees, 18.31% had their own businesses, and 15.49% were unemployed. Full information is provided in Table 4.2.

The mean number of family members was 3.58 persons. The mean family income and family debt were 5,779.96 Baht/month and 60,482.68 Baht, respectively.

**Table 4.2** General Characteristics of Subjects

| General characteristics | n | % |
|---|---|---|
| **Total** | **355** | **100.00** |
| Sex | | |
| Female | 176 | 49.58 |
| Male | 179 | 50.42 |
| Age (years old) | | |
| ≤29 | 24 | 6.88 |
| 30-39 | 129 | 36.54 |
| 40-49 | 142 | 40.20 |
| ≥50 | 58 | 16.38 |
| *Min 18, Max 65, Mean 40.92, S.D. 8.11* | | |

**Table 4.2** (Continued)

| General characteristics | n | % |
|---|---|---|
| Marital status | | |
| Single | 40 | 11.27 |
| Married | 193 | 54.37 |
| Divorced/widowed | 122 | 34.36 |
| Religion | | |
| Buddhism | 339 | 95.49 |
| Others | 16 | 4.51 |
| Number of family members (persons) | | |
| ≤4 | 270 | 76.06 |
| ≥5 | 85 | 23.94 |
| Years in school | | |
| No education | 82 | 23.05 |
| 1-6 | 200 | 56.35 |
| 7-12 | 68 | 19.16 |
| ≥ 13 | 5 | 1.44 |
| Occupation | | |
| Unemployed | 55 | 15.49 |
| Agriculture | 38 | 10.71 |
| Monthly employee | 35 | 9.86 |
| Daily employee | 158 | 44.51 |
| Business | 65 | 18.31 |
| Others | 4 | 1.12 |

**Table 4.2** (Continued)

| General characteristics | n | % |
|---|---|---|
| Income (Baht/month), Mean 5,779.96, S.D. 7,322.25 | | |
| None | 35 | 9.86 |
| ≤ 4,999 | 152 | 42.82 |
| ≥ 5,000 | 168 | 47.32 |
| Debt | | |
| Yes | 157 | 44.23 |
| No | 198 | 55.77 |

Regarding medical history, 24.51% had a history of blood transfusion, and the average number of units of blood received was 2.40 units. Forty-two (11.83%) had a history of jaundice, but none had had hemodialysis. As many as 93.52% had received ARV drug, but nearly all (97.46%) had not received HBV vaccination. In addition, 30.42% had comorbidity, with the three most diagnosed illnesses being hypertension (26.67%), diabetes mellitus (6.67%), and stomachache (6.67%). These are shown in Table 4.3.

**Table 4.3** Medical History of Subjects

| Medical history | n | % |
|---|---|---|
| **Total** | **355** | **100.00** |
| History of blood transfusion | | |
| Yes | 87 | 24.51 |
| No | 198 | 75.49 |
| History of jaundice | | |
| Yes | 42 | 11.83 |
| No | 313 | 88.17 |
| History of hemodialysis | | |
| Yes | 0 | 0.00 |
| No | 355 | 100.00 |
| ARV drug | | |
| Yes | 332 | 93.52 |
| No | 23 | 6.48 |
| History of HBV vaccination | | |
| Yes | 9 | 2.54 |
| No | 346 | 97.46 |
| Comorbidity | | |
| Yes | 108 | 30.42 |
| No | 247 | 69.58 |
| CD4 cell count (cells/$mm^3$), Mean 254.50 | | |
| ≤ 200 | 121 | 34.08 |
| ≥ 201 | 189 | 53.24 |
| Missing | 45 | 12.68 |
| Length of HIV infection (years), Mean 3.03 | | |
| ≤ 3 | 263 | 74.08 |
| > 3 | 88 | 24.79 |
| Missing | 4 | 1.13 |

The next factor concerned the subjects' risk behavior. Although only 2.54% had lived together with HBV-infected persons in their family, most of whom were their spouses, forty-five subjects (12.68%) had shared personal objects with other members of their families. Towels were identified as the most shared personal object, followed by toothbrushes, water glasses, soap and others (razors and nail clippers). Seven subjects (1.97%) had been IDUs, and 3 of these 7 (42.86%) had shared needles or other injection equipment with other IDUs. The most used illicit drug ingested via inhalation was amphetamine, followed by glue or thinner and marijuana. The most used illicit drug ingested orally was also amphetamine, followed by ecstasy and tobacco. In addition, 32.11% of the subjects had been tattooed, while almost twice as many (60.00%) had had body parts pierced. Before the subjects came to know their HIV status, 85.92% had drunk alcohol, with 30.70% drinking alcohol every day and 44.79% smoking. However, after the subjects had been informed of their HIV status, only 38.44% continued drinking alcohol and 23.01% continued smoking. Details are given in Table 4.4.

**Table 4.4** Risk Behaviors of Subjects

| Risk behaviors | n | % |
|---|---|---|
| **Total** | **355** | **100.00** |
| Known living with HBV –infected person in family | | |
| Yes | 9 | 2.54 |
| No | 346 | 97.46 |
| Sharing personal objects in family | | |
| Yes | 45 | 12.68 |
| No | 310 | 87.32 |
| History of IDU | | |
| Yes | 7 | 1.97 |
| No | 348 | 98.03 |
| History of illicit drug use, by means of inhalation | | |
| Yes | 74 | 20.85 |
| No | 281 | 79.15 |
| History of illicit drug use, by oral route | | |
| Yes | 37 | 10.42 |
| No | 318 | 89.58 |
| History of tattooing | | |
| Yes | 114 | 32.11 |
| No | 241 | 67.89 |
| History of piercing | | |
| Yes | 213 | 60.00 |
| No | 142 | 40.00 |
| Alcohol drinking (before knowing HIV status) | | |
| Yes | 305 | 85.92 |
| No | 50 | 14.08 |

**Table 4.4** (Continued)

| Risk behaviors | n | % |
|---|---|---|
| Frequency of alcohol drinking | | |
| None | 50 | 14.08 |
| Less than 1 time/month | 78 | 21.97 |
| 1-2 times/month | 60 | 16.91 |
| 1-2 times/week | 58 | 16.34 |
| Everyday | 109 | 30.70 |
| Smoking | | |
| Yes | 159 | 44.79 |
| No | 196 | 55.21 |

The factor of sexual behavior showed the following. About one-sixth of the subjects (15.77%) had a history of being commercial sex workers, with the average working period of 4.83 years, and the longest working period was 26 years. Forty-nine (13.88%) had their first sexual intercourse at ≤15 years of age, the mean and minimum age at the first sexual intercourse being 18.76 and 12 years old, respectively. Two hundred and ten (59.15%) had a history of STDs and the most found symptoms were vesicular lesion/rash in the sexual organ/anus (33.24%), followed by pus secretion from the sexual organ (15.77%). Twenty-six (7.32%) were homosexual, with 18.31% having had oral sex, 6.76% having had anal sex, and 89.30% having 2 or more sex partners. Before the subjects became aware of their HIV status, 158 (44.51%) had never used condoms, while 182 (51.26%) had used them occasionally. However, after all of the subjects had become aware of their HIV status, only 8 (2.25%) still did not use condoms and the same number (8, 2.25%) used them sometimes, whereas as many as 189 (54.24%) always used them. More detailed information is shown in Table 4.5 below.

**Table 4.5** Sexual Behaviors of Subjects

| Sexual behaviors | n | % |
|---|---|---|
| **Total** | 355 | 100.00 |
| History of being commercial sex worker | | |
| Yes | 56 | 15.77 |
| No | 299 | 54.23 |
| Having first sexual intercourse at age ≤ 15 years old | | |
| Yes | 49 | 13.88 |
| No | 304 | 86.12 |
| History of STDs | | |
| Yes | 210 | 59.15 |
| No | 145 | 40.85 |
| Being homosexual | | |
| Yes | 26 | 7.32 |
| No | 329 | 92.68 |
| Oral sex | | |
| Yes | 65 | 18.31 |
| No | 290 | 81.69 |
| Anal sex | | |
| Yes | 24 | 6.76 |
| No | 331 | 93.24 |
| Number of partners | | |
| One | 38 | 10.70 |
| 2-9 | 166 | 46.76 |
| ≥10 | 151 | 42.54 |

**Table 4.5** (Continued)

| Sexual behaviors | n | % |
|---|---|---|
| Condom use (before knowing HIV status) | | |
| Never | 158 | 44.51 |
| Sometimes | 182 | 51.26 |
| Always | 4 | 1.12 |
| Missing | 11 | 3.01 |
| Condom use (after knowing HIV status) | | |
| Quit sexual intercourse | 101 | 28.45 |
| Never | 8 | 2.25 |
| Sometimes | 8 | 2.25 |
| Always | 189 | 53.24 |
| Missing | 49 | 13.81 |

## 4.3 Comparison of Subject's Characteristics by Group (case-control)

There were 83 subjects identified for the control group (negative for HBsAg, anti-HBs and total anti-HBc) and 41 subjects identified for the case group (positive for HBsAg).

Seventy-seven (21.69%) subjects who had had tested positive for anti-HBs and 154 subjects (43.38%) who had tested positive for total anti-HBc were excluded.

Nine general characteristics, as shown in Table 4.6, were used to compare between the cases and controls. Based on the chi-square test at $\alpha$=0.05, the only significant difference between the two groups was the number of years spent in school (p-value = 0.011).

**Table 4.6** General Characteristics Comparison Between Cases and Controls

| **General characteristics** | **Case** | | **Control** | | **$\chi^2$-test** | **p-value** |
|---|---|---|---|---|---|---|
| | **n** | **%** | **n** | **%** | | |
| **Total** | **41** | **100.00** | **83** | **100.00** | | |
| Sex | | | | | 0.04 | 0.849 |
| Male | 21 | 51.21 | 41 | 49.39 | | |
| Female | 20 | 48.79 | 42 | 50.61 | | |
| Age (years old) | | | | | 3.30 | 0.348 |
| ≤ 29 | 3 | 7.32 | 8 | 9.64 | | |
| 30-39 | 16 | 39.02 | 34 | 41.96 | | |
| 40-49 | 12 | 29.27 | 31 | 37.35 | | |
| ≥ 50 | 10 | 24.39 | 10 | 12.05 | | |
| Marital status | | | | | 1.68 | 0.431 |
| Single | 3 | 7.32 | 10 | 12.05 | | |
| Married | 29 | 70.73 | 49 | 59.04 | | |
| Divorced/widowed | 9 | 21.95 | 24 | 28.91 | | |

**Table 4.6** (Continued)

| General characteristics | Case | | Control | | $\chi^2$-test | p-value |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| Religion | | | | | 0.11 | 0.736 |
| Buddhism | 39 | 95.12 | 80 | 96.39 | | |
| Christian | 2 | 4.88 | 3 | 3.61 | | |
| Number of family member (persons) | | | | | 0.03 | 0.853 |
| ≤ 4 | 31 | 75.61 | 64 | 77.10 | | |
| ≥ 5 | 10 | 24.39 | 19 | 22.90 | | |
| Years in school | | | | | 9.04 | 0.011* |
| No education | 13 | 31.71 | 10 | 12.05 | | |
| 1-6 | 22 | 53.66 | 46 | 55.43 | | |
| ≥ 7 | 6 | 14.63 | 27 | 32.52 | | |
| Occupation | | | | | 0.19 | 0.662 |
| Unemployed | 5 | 12.20 | 8 | 9.64 | | |
| Employed | 36 | 87.80 | 75 | 90.36 | | |
| Income | | | | | 1.21 | 0.272 |
| Yes | 39 | 95.12 | 74 | 89.16 | | |
| No | 2 | 4.88 | 9 | 10.84 | | |
| Debt | | | | | 0.04 | 0.849 |
| Yes | 21 | 51.21 | 41 | 49.39 | | |
| No | 20 | 48.79 | 42 | 50.61 | | |

**Note.** *; Significant Level at $\alpha = 0.05$

The next criterion used to compare between the cases and controls was six aspects of medical history, as shown in Table 4.7. Based on the chi-square test at $\alpha$=0.05, the CD4 cell count and length of HIV infection were identified as significant statistical differences (p-values = 0.025 and 0.039, respectively).

Twenty-nine subjects had a history of blood transfusion and the average amount of blood transfused was 2.76 units. None had received HBV vaccine. There were 30 (24.19%) who showed comorbidities. The majority of comorbidity illnesses were hypertension (20.07%), diabetes mellitus (16.72%), and dyslipidemia (10.02%).

**Table 4.7** Medical History Comparison Between Cases and Controls

| **Medical history** | **Case** | | **Control** | | **$\chi^2$-test** | **p-value** |
|---|---|---|---|---|---|---|
| | **n** | **%** | **n** | **%** | | |
| **Total** | **41** | **100.00** | **83** | **100.00** | | |
| History of blood transfusion | | | | | 0.41 | 0.524 |
| Yes | 11 | 26.83 | 18 | 21.68 | | |
| No | 30 | 73.17 | 65 | 78.32 | | |
| History of jaundice | | | | | 0.16 | 0.686 |
| Yes | 6 | 14.63 | 10 | 12.05 | | |
| No | 35 | 85.37 | 73 | 87.95 | | |
| ARV drug | | | | | 1.11 | 0.292 |
| Yes | 37 | 90.24 | 79 | 95.18 | | |
| No | 4 | 9.76 | 4 | 4.82 | | |
| Comorbidity | | | | | 0.23 | 0.630 |
| Yes | 11 | 26.83 | 19 | 22.90 | | |
| No | 30 | 73.17 | 64 | 77.10 | | |
| CD4 cell count (cells/$mm^3$), Mean 273.87 | | | | | 5.04 | 0.025* |
| $\leq 200$ | 7 | 18.92 | 29 | 40.28 | | |
| $\geq 201$ | 30 | 81.08 | 43 | 59.72 | | |
| Length of HIV infection (years) | | | | | 4.26 | 0.039* |
| $\leq 3$ | 32 | 78.05 | 48 | 59.26 | | |
| $> 3$ | 9 | 21.95 | 33 | 40.74 | | |

**Note.** *; Significant Level at $\alpha = 0.05$

The third criterion for comparison between cases and controls was nine aspects of risk behavior, as presented in Table 4.8. However, this risk-behavior-based comparison between cases and controls showed no factors with statistical differences.

There were 6 (4.84%) subjects who had lived together with HBV-positive persons in their family, and all of them were their husbands. Sixteen (12.90%) had shared personal objects with their family members, with towels being the personal object most frequently shared, followed by toothbrushes, razors, water glasses, and others (soap and nail clippers), respectively. The illicit drug most frequently ingested by means of inhalation was amphetamine, followed by glue or thinner, marijuana, and ketamine, while the illicit drug most frequently ingested by oral route was amphetamine and ecstasy. After all of the subjects had become aware of their HIV status, 38.94% and 26.05% of the subjects continued drinking alcohol and smoking.

**Table 4.8** Risk Behaviors Comparison Between Cases and Controls

| **Risk behaviors** | **Case** | | **Control** | | **$\chi^2$-test** | **p-value** |
|---|---|---|---|---|---|---|
| | **n** | **%** | **n** | **%** | | |
| **Total** | **41** | **100.00** | **83** | **100.00** | | |
| Known living with HBV –infected person in family | | | | | 0.82 | 0.366 |
| Yes | 3 | 7.32 | 3 | 3.61 | | |
| No | 38 | 92.68 | 80 | 96.39 | | |
| Sharing objects in family | | | | | 0.03 | 0.869 |
| Yes | 5 | 12.20 | 11 | 13.25 | | |
| No | 36 | 87.80 | 72 | 86.75 | | |
| History of IDU | | | | | 0.50 | 0.480 |
| Yes | 0 | 0.00 | 1 | 1.20 | | |
| No | 41 | 100.00 | 82 | 98.80 | | |

**Table 4.8** (Continued)

| Risk behaviors | Case | | Control | | $\chi^2$-test | p-value |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| History of illicit drug use, by means of inhalation | | | | | 0.02 | 0.899 |
| Yes | 8 | 19.51 | 17 | 20.48 | | |
| No | 33 | 80.49 | 66 | 79.52 | | |
| History of illicit drug use, by oral route | | | | | 0.03 | 0.869 |
| Yes | 5 | 12.20 | 11 | 13.25 | | |
| No | 36 | 87.80 | 72 | 86.75 | | |
| History of tattooing | | | | | 0.27 | 0.607 |
| Yes | 11 | 26.83 | 26 | 31.32 | | |
| No | 30 | 73.17 | 57 | 68.68 | | |
| History of piercing | | | | | 0.38 | 0.537 |
| Yes | 28 | 68.30 | 52 | 62.65 | | |
| No | 13 | 31.70 | 31 | 37.35 | | |
| Alcohol drinking (before knowing HIV status) | | | | | 0.23 | 0.631 |
| Yes | 35 | 85.37 | 68 | 81.93 | | |
| No | 6 | 14.63 | 15 | 18.07 | | |
| Smoking (before knowing HIV status) | | | | | 0.68 | 0.409 |
| Yes | 20 | 48.79 | 34 | 41.96 | | |
| No | 21 | 51.21 | 49 | 59.04 | | |

The final criterion used to compare between the cases and controls was nine aspects of sexual behavior, as presented in Table 4.9. However, no variable was found with significant statistical differences.

There were 15 subjects who had been CSWs, with the mean of their working period being 4.13 years and the longest working period being 9 years. The average age at their first sexual intercourse was 18.93 years, and the minimum age at their first sexual intercourse was 12 years. The disorder most commonly found among the

subjects who had a history of STDs was vesicular lesion/rash in the anus/sexual organ (38.71%), followed by pus secretion from the sexual organ (17.74%) and pain in the sexual organ, testis and abdomen (12.93%).

**Table 4.9** Sexual Behaviors Comparison Between Cases and Controls

| **Sexual behaviors** | **Case** | | **Control** | | **$\chi^2$-test** | **p-value** |
|---|---|---|---|---|---|---|
| | **n** | **%** | **n** | **%** | | |
| **Total** | **41** | **100.00** | **83** | **100.00** | | |
| History of being commercial sex worker | | | | | 0.37 | 0.543 |
| Yes | 6 | 14.63 | 9 | 10.84 | | |
| No | 35 | 85.37 | 74 | 89.16 | | |
| Age at first sexual intercourse (years old) | | | | | 2.20 | 0.138 |
| ≤15 | 7 | 17.50 | 7 | 8.43 | | |
| >15 | 33 | 82.50 | 76 | 91.60 | | |
| History of STDs | | | | | 0.21 | 0.650 |
| Yes | 27 | 65.85 | 58 | 69.87 | | |
| No | 14 | 34.15 | 25 | 30.13 | | |
| History of being homosexual | | | | | 1.11 | 0.292 |
| Yes | 4 | 9.76 | 4 | 4.82 | | |
| No | 37 | 90.24 | 79 | 95.18 | | |
| Oral sex | | | | | 0.36 | 0.547 |
| Yes | 7 | 17.07 | 18 | 21.68 | | |
| No | 34 | 82.93 | 65 | 78.33 | | |
| Anal sex | | | | | 0.82 | 0.366 |
| Yes | 3 | 7.32 | 3 | 3.61 | | |
| No | 38 | 92.68 | 80 | 96.39 | | |

**Table 4.9** (Continued)

| Sexual behaviors | Case | | Control | | $\chi^2$-test | p-value |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| Number of partners | | | | | 0.37 | 0.831 |
| 1 | 5 | 12.20 | 10 | 12.05 | | |
| 2-9 | 19 | 46.34 | 43 | 51.81 | | |
| ≥10 | 17 | 41.46 | 30 | 36.14 | | |
| History of extra marital sexual intercourse | | | | | 0.00 | 0.981 |
| Yes | 36 | 87.80 | 73 | 87.95 | | |
| No | 5 | 12.20 | 10 | 12.05 | | |
| Condom use (before knowing HIV status) | | | | | 0.15 | 0.703 |
| Always | 1 | 2.44 | 3 | 3.75 | | |
| Never/sometimes | 40 | 97.56 | 77 | 96.25 | | |

## 4.4 Results of the Qualitative Phase and Univariate Analysis of Risk Factors and HIV/HBV Co-Infection

Results of the qualitative phase were presented under this section. The objective of the in-depth interview in this study was to confirm and acquire an understanding of risk factors in HIV/HBV co-infection among informants. There were 16 HIV-infected patients participating in the in-depth interview. Seven were purposive selected from case group and the other 9 were purposive selected from the control group. There were 4 and 6 males in the case and control groups, respectively. There were several factors that contributed to the risk of STDs. In this study, all informants were asked to supply information regarding their behavior of alcohol drinking; use of sexual service; history of tattooing; use of illicit drugs by inhalation, oral, and injection routes; and sexual behavior. Most of the informants agreed that these types of risk behavior were the factors that contributed to the STDs infection.

The next step of the study was to analyze all of the four main aspects of the subjects, namely, general characteristics, medical history, risk behavior, and sexual behavior, to a univariate analysis at $\alpha$=0.10.

When general characteristics were co-analyzed with HIV/HBV co-infection, the most statistically significant association was found in the “years in school” category, among the subjects who had not received any education, at OR=5.85, 90% CI 2.12-16.14. This can be seen in Table 4.10.

**Table 4.10** Univariate Analysis of Risk Factors in HIV/HBV Co-Infection and General Characteristics

| General characteristics | Case | | Control | | OR | 90% CI |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| **Total** | **41** | **100.00** | **83** | **100.00** | | |
| Sex | | | | | | |
| Male | 21 | 51.21 | 41 | 49.39 | 1.08 | 0.57-2.02 |
| Female | 20 | 48.79 | 42 | 50.61 | 1.00 | |
| Age (years old) | | | | | | |
| ≤ 29 | 3 | 7.32 | 8 | 9.64 | 1.00 | |
| 30-39 | 16 | 39.02 | 34 | 41.96 | 1.26 | 0.37-4.25 |
| 40-49 | 12 | 29.27 | 31 | 37.35 | 1.03 | 0.30-3.59 |
| ≥ 50 | 10 | 24.39 | 10 | 12.05 | 2.67 | 0.70-10.13 |
| Marital status | | | | | | |
| Married | 29 | 70.73 | 49 | 59.01 | 1.97 | 0.63-6.23 |
| Divorced/widowed | 9 | 21.95 | 24 | 28.91 | 1.25 | 0.36-4.40 |
| Single | 3 | 7.32 | 10 | 12.05 | 1.00 | |
| Religion | | | | | | |
| Christian | 2 | 4.88 | 3 | 3.64 | 1.37 | 0.29-6.35 |
| Buddhism | 39 | 95.12 | 80 | 96.39 | 1.00 | |
| Number of family member (persons) | | | | | | |
| ≤ 4 | 31 | 75.61 | 64 | 77.10 | 1.00 | |
| ≥ 5 | 10 | 24.39 | 19 | 22.90 | 1.09 | 0.52-2.27 |
| Years in school | | | | | | |
| No education | 13 | 31.71 | 10 | 12.05 | 5.85 | 2.12-16.14* |
| ≤ 6 | 22 | 53.66 | 46 | 55.43 | 2.15 | 0.91-5.07 |
| ≥ 7 | 6 | 14.63 | 27 | 32.52 | 1.00 | |
| Occupation | | | | | | |
| Unemployed | 5 | 12.20 | 8 | 9.64 | 1.30 | 0.48-3.52 |
| Employed | 36 | 87.80 | 75 | 90.36 | 1.00 | |

**Table 4.10** (Continued)

| General characteristics | Case | | Control | | OR | 90% CI |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| Income | | | | | | |
| Yes | 39 | 95.12 | 74 | 89.16 | 1.00 | |
| No | 2 | 4.88 | 9 | 10.84 | 0.42 | 0.11-1.59 |
| Debt | | | | | | |
| Yes | 21 | 51.21 | 41 | 49.39 | 1.08 | 0.57-2.02 |
| No | 20 | 48.79 | 42 | 50.61 | 1.00 | |

**Note.** *; Significant Level at $\alpha$=0.10

All of key informants were asked about the association between income and the risk behavior for STDs infection. Some of them stated that the chance of STDs infection depended on each person's behavior. However, the differences in income led the disparate expressions of behavior. A poor person may have a greater risk of STDs because of their prostitution career. At the same time, a rich person may have a greater chance of access to sexual service. These were supported by the following comments.

"I think poor people have greater risk of STDs infection."

"Some poor people became commercial sex workers because they needed money."

"Rich people may have a greater risk of STD infection because they have a lot of money to spend on having nights out without having to fear anything."

Some key informants indicated that STDs infection was a result of an individual's behavior. However, behavioral differences also resulted from differences in education. They reasoned that some illiterate people may not have knowledge of STDs, while some educated people may have more chances to have be with other people and have sexual intercourse with them, as in the following comments.

"Although they have high education, they could be infected also if they have bad sexual behavior."

"Educated people know many people, so they may have more chances to have sexual intercourse with many sex partners."

"Illiterate persons do not have knowledge, so they tend to trust others too easily, without fear of STD infection."

Two areas of statistically significant association were identified between the risk factors in HIV/HBV co-infection and the subjects' medical history, namely, CD 4 cell count (CD4 $\leq$ 200 cells/$mm^3$ group OR=0.35, 90% CI 0.16-0.77) and the length of HIV infection ($\leq$ 3 years group OR=2.44, 90% CI 1.19-5.04), as shown in Table 4.11.

**Table 4.11** Univariate Analysis of Risk Factors in HIV/HBV Co-Infection and Medical History

| Medical history | Case | | Control | | OR | 90% CI |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| **Total** | 41 | 100.00 | 83 | 100.00 | | |
| History of blood transfusion | | | | | | |
| Yes | 11 | 26.83 | 18 | 21.68 | 1.32 | 0.64-2.74 |
| No | 30 | 73.17 | 65 | 78.32 | 1.00 | |
| History of jaundice | | | | | | |
| Yes | 6 | 14.63 | 10 | 12.05 | 1.25 | 0.50-3.12 |
| No | 35 | 85.37 | 73 | 87.95 | 1.00 | |
| ARV drug | | | | | | |
| Yes | 37 | 90.24 | 79 | 95.18 | 1.00 | |
| No | 4 | 9.76 | 4 | 4.82 | 2.14 | 0.64-7.15 |
| Comorbidity | | | | | | |
| Yes | 11 | 26.82 | 19 | 22.90 | 1.24 | 0.60-2.54 |
| No | 30 | 73.18 | 64 | 77.10 | 1.00 | |
| CD4 cell count (cells/$mm^3$) | | | | | | |
| ≤ 200 | 7 | 18.92 | 29 | 40.28 | 0.35 | 0.16-0.77* |
| ≥ 201 | 30 | 81.08 | 43 | 59.72 | 1.00 | |
| Length of HIV infection (years) | | | | | | |
| ≤ 3 | 32 | 78.05 | 48 | 59.26 | 2.44 | 1.19-5.04* |
| > 3 | 9 | 21.95 | 33 | 40.74 | 1.00 | |

**Note.** *; Significant Level at $\alpha$=0.10

When the risk factors in HIV/HBV co-infection were analyzed in conjunction with the subjects' risk behavior, no variable was identified as showing statistically significant association, as Table 4.12 shows.

**Table 4.12** Univariate Analysis of Risk Factors in HIV/HBV Co-Infection and Risk Behaviors

| Risk behaviors | Case | | Control | | OR | 90% CI |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| **Total** | **41** | **100.00** | **83** | **100.00** | | |
| Living with HBV-infected patient in family | | | | | | |
| Yes | 3 | 7.32 | 3 | 3.61 | 2.11 | 0.53-8.38 |
| No | 38 | 92.68 | 80 | 96.39 | 1.00 | |
| Sharing personal objects in family | | | | | | |
| Yes | 5 | 12.20 | 11 | 13.25 | 0.91 | 0.35-2.35 |
| No | 36 | 87.80 | 72 | 86.75 | 1.00 | |
| History of illicit drug use, by means of inhalation | | | | | | |
| Yes | 8 | 19.51 | 17 | 20.48 | 0.94 | 0.43-2.07 |
| No | 33 | 80.49 | 66 | 79.52 | 1.00 | |
| History of illicit drug use, by oral route | | | | | | |
| Yes | 5 | 12.20 | 11 | 13.25 | 0.91 | 0.35-2.35 |
| No | 36 | 87.80 | 72 | 86.75 | 1.00 | |
| History of tattooing | | | | | | |
| Yes | 11 | 26.83 | 26 | 31.32 | 0.80 | 0.40-1.61 |
| No | 30 | 73.17 | 57 | 68.68 | 1.00 | |
| History of piercing | | | | | | |
| Yes | 28 | 68.30 | 52 | 62.65 | 1.28 | 0.66-2.50 |
| No | 13 | 31.70 | 31 | 37.35 | 1.00 | |
| Alcohol drinking (before knowing HIV status) | | | | | | |
| Yes | 35 | 85.37 | 68 | 81.93 | 1.29 | 0.54-3.06 |
| No | 6 | 14.63 | 15 | 18.07 | 1.00 | |
| Smoking (before knowing HIV status) | | | | | | |
| Yes | 20 | 48.79 | 34 | 41.96 | 1.37 | 0.73-2.58 |
| No | 21 | 51.21 | 49 | 59.04 | 1.00 | |

In some of the key informants' opinion, drinking alcohol, tattooing and illicit drug use were major types of behavior leading to the risk of STDs infection. This was supported by the following comments.

"If someone is drunk, s/he may have sexual intercourse without protection."

"Then they could be infected with HIV."

"People who have been tattooed are at risk of STD infection because the same needle could have been used on many clients, and the needle may have been contaminated with someone's blood."

"Using illicit drugs by injection could certainly infect a person with STDs."

"Injecting drugs could infect a person with STDs if the users share needles and syringes with others."

"People could be infected with STDs if they ingest drugs by inhalation. Infection may be through the nasal fluid."

However, some informants said that people would not be infected with STDs if they ingested the illicit drugs by oral route. They also mentioned some advantages of alcohol drinking, such as relaxation, comfort, socializing, and fun. Moreover, one of the informants said that alcohol could kill some parasites in his body. The comments are presented as follows.

"I have to drink alcohol because I love to eat raw meat."

"I think alcohol can kill some parasites."

When the risk factors in HIV/HBV co-infection were analyzed in conjunction with the subjects' sexual behavior, no variable was identified as showing statistically significant association, as Table 4.13 shows.

**Table 4.13** Univariate Analysis of Risk Factors in HIV/HBV Co-Infection and Sexual Behaviors

| Sexual behaviors | Case | | Control | | OR | 90% CI |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| **Total** | **41** | **100.00** | **83** | **100.00** | | |
| History of being commercial sex worker | | | | | | |
| Yes | 6 | 14.63 | 9 | 10.84 | 1.41 | 0.56-3.57 |
| No | 35 | 85.37 | 74 | 89.16 | 1.00 | |
| Age at first sexual intercourse (years old) | | | | | | |
| ≤ 15 | 7 | 17.50 | 7 | 8.43 | 1.00 | |
| > 15 | 33 | 82.50 | 76 | 91.60 | 0.43 | 0.17-1.12 |
| History of STDs | | | | | | |
| Yes | 27 | 65.85 | 58 | 69.87 | 0.83 | 0.43-1.62 |
| No | 14 | 34.15 | 25 | 30.13 | 1.00 | |
| History of being homosexual | | | | | | |
| Yes | 4 | 9.76 | 4 | 4.82 | 2.14 | 0.64-7.15 |
| No | 37 | 90.24 | 79 | 95.18 | 1.00 | |
| Oral sex | | | | | | |
| Yes | 7 | 17.07 | 18 | 21.68 | 0.74 | 0.33-1.67 |
| No | 34 | 82.93 | 65 | 78.33 | 1.00 | |
| Anal sex | | | | | | |
| Yes | 3 | 7.32 | 3 | 3.61 | 2.11 | 0.53-8.38 |
| No | 38 | 92.68 | 80 | 96.39 | 1.00 | |
| Number of partners | | | | | | |
| 1 | 5 | 12.20 | 10 | 12.05 | 1.00 | |
| 2-9 | 19 | 46.34 | 43 | 51.81 | 0.88 | 0.32-2.42 |
| ≥10 | 17 | 41.46 | 30 | 36.14 | 1.13 | 0.41-3.18 |

**Table 4.13** (Continued)

| Sexual behaviors | Case | | Control | | OR | 90% CI |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| History of extra marital sexual intercourse | | | | | | |
| Yes | 36 | 87.80 | 73 | 87.95 | 0.99 | 0.38-2.58 |
| No | 5 | 12.20 | 10 | 12.05 | 1.00 | |
| Condom use (before knowing HIV status) | | | | | | |
| Always | 1 | 2.44 | 3 | 3.75 | 1.00 | |
| Never/sometimes | 40 | 97.56 | 77 | 96.25 | 1.56 | 0.23-10.70 |

Concerning CSWs, the key informants were divided into two groups. The first group said that CSWs had a greater risk of STDs infection than ordinary women because they had more sexual partners and some clients refused to use condoms, as the following comments indicated.

"Women working as commercial sex workers have many sex partners."

"Someone who is HIV-infected may visit her."

"Although there are condoms at the brothel, some clients do not use them."

On the other hand, the second group contradicted that ordinary women had a greater risk of STDs infection than women who worked as CSWs. They gave the following supporting reasons.

"CSWs would force their clients to use condoms."

"If the client refuses, a CSW will not have sex with him."

"Ordinary women do not use condoms because they think that their partners are healthy [not infected with HIV]."

"CSWs usually check their own health, so they should be more concerned about HIV infection."

The key informants agreed that having sexual intercourse with the same sex was associated with STD infection. Although some informants did not know whether or not the people with whom they had had sexual intercourse were infected with STD,

most of the informants admitted that men who had sex with men (MSMs) were more vulnerable to STD infection, as supported by the following statements.

“Men do not have enough lubrication fluid, so tearing of the rectal tissue and bleeding usually occurs.”

“That’s how they could be infected with STDs.”

“If their mouths are exposed to the fluid, they could be infected because there were some viruses in that fluid.”

However, most of the informants had no knowledge of the process of sexual intercourse between women and women. But there was one informant who gave commented that women who had sex with women were at risk of STD infection, as stated below.

“They could be infected with STDs if they share the same sex toy, such as a fake penis.”

It was commonly agreed by the informants that having many sex partners at one time was associated with STD infection. All of the informants admitted that having many sex partners at any one time could cause them to be infected with STDs more easily than having only one sex partner, as shown in the statements below.

“I did not know who among my sex partners had STDs; some of them might have been infected with STDs.”

“My sex partners may have had sexual intercourse with other people also.”

“Today she has sexual intercourse with me, but tomorrow I do not know whether she will have sexual intercourse with some other guys.”

## 4.5 Multivariate Analysis of Risk Factors and HIV/HBV Co-Infection

Multivariate analysis of risk factors of HIV/HBV co-infection among subjects was conducted using the unconditional logistic regression, at a significant $\alpha$ level of = 0.05 (Table 4.14). After all of the possible confounder factors (namely, sex, age, marital status, religion, occupation, income, debt, number of family members, blood transfusion, hemodialysis, jaundice, use of ARV, HBV vaccination, comorbidity, length of HIV infection, tattooing, piercing, smoking, drinking alcohol, sharing personal objects, illicit drug use, age at first sexual intercourse, sexual orientation, extramarital sex, use of condom, oral sex, anal sex, STDs, and CSW) were taken into analysis, two variables were found to have statistically significant association with HIV/HBV co-infection. First, under the "years in school" category, the uneducated subjects showed 7.07 times (95%CI=1.77-28.24) greater risk of HIV/HBV co-infection than those having spent seven or more years in school. Second, the subjects with the CD4 cell count of $\leq$ 200 cells/$mm^3$ had a lower risk of HIV/HBV co-infection compared to those with $\geq$ 201 cells/$mm^3$ (OR=0.35, 95%CI=0.13-0.94).

**Table 4.14** Multivariate Analysis of Risk Factors in HIV/HBV Co-Infection

| Risk factors | $OR_{adj}$ | 95%CI |
|---|---|---|
| Years in school | | |
| No education | 7.07 | 1.77-28.24* |
| 1-6 | 2.21 | 0.72-6.76 |
| $\geq$ 7 | 1.00 | |
| CD4 cell count (cells/$cm^3$) | | |
| $\leq$ 200 | 0.35 | 0.13-0.94* |
| $\geq$ 201 | 1.00 | |

**Note.** *: Significant Level at $\alpha$=0.05

# CHAPTER 5

# DISCUSSION AND CONCLUSION

This study aimed to determine the risk factors in HIV/HBV co-infection in Chiang Rai Province, Thailand. Conducted during May 2011 to December 2012, this study involved a total of 124 subjects who received treatment at 9 ARV clinics and who met the study criteria. Logistic regression analysis with 95% confident interval was used to determine the association of significant factors in HIV/HBV co-infection.

## 5.1 Discussion

The discussion is composed of two parts. The first part discusses the research methodology, while the second part discusses the results of both the quantitative and qualitative phases.

### 5.1.1 Discussion of Research Methodology

This research study, designed as a case-control study, was conducted to determine the risk factors in HIV/HBV co-infection in Chiang Rai Province, Thailand. The study sample consisted of HIV-infected patients, and the data were collected using a face-to-face interview technique that inquired about the subjects' general characteristics, medical history, risk behavior, and sexual behavior. However, since the questions were asked some time after the events had occurred, the answers obtained from the interviewees might not have been complete due to the recall-bias or memory lapses. The researcher, therefore, attempted to reduce this kind of bias by checking individual's medical records for some information of interest. Before implementation, all instruments used in this study had been tested for validity and reliability.

In total, 356 HIV-infected patients were invited to participate in this study, but 1 patient (0.28%) refused to do so. All of the other 355 subjects answered the questionnaire willingly, and none was too shy to provide personal data.

All of the participating subjects, who had been recruited from 9 ARV clinics by means of random sampling, well represented the population. Based on the standard healthcare procedure during the time of study, once a person was found to be HIV positive, s/he was advised to seek treatment at an ARV clinic. For this reason, the recruitment of the subjects into the study was done in the ARV clinics, where it was most practical for people with HIV characteristics to be identified. Another advantage was that the staff of all the 9 ARV clinics very cooperatively facilitated in the step of data gathering.

After compared the difference of socio-demographic characteristics between inclusion and exclusion group, marital status and occupation were found the statistical significant degree.

However, there were two major limitations to this study. The first concerned the number of subjects, which fell short of the initial estimate (120 cases and 120 controls). The researcher was unable to obtain the estimated numbers due to the limitation of time and budget. This was the major setback for the researcher's attempt to identify association between the independent and dependent variables.

The second limitation was the necessity to employ two sets of socio-demographic criteria for exclusion and inclusion purposes. Since the exclusion criteria for this study were the subjects' positivity for anti-HBs and for total anti-HBc, it was not possible to identify whether the subjects became infected with HBV before or after the HIV infection.

Finally, 41 cases and 83 controls were obtained, yielding a 1:2 case-control ratio. This might have affected the results of the study because the number of subjects was smaller than the expected number, and data on some variables, such as the history of hemodialysis, HBV vaccination, and IDU, were not sufficient to analyze. In addition, some pieces of information were missing during data collection, such as those regarding condom use (before knowing HIV status), length of HIV infection, and CD4 cell count. As some of the subjects were new HIV-infected patients, they had not had a CD4 cell count, while some others could not remember when they had

become HIV-infected and, therefore, were unable to tell whether they had used condoms or not.

### 5.1.2 Discussion of Results

Several factors were not found significant in the univariate analysis: gender; age group; religion; family size; income; debt; history of blood transfusion; history of jaundice; HBV vaccination; ARV drug; comorbidity; living with HBV-infected patients in family; sharing personal objects in family; IDU; use of other illicit drugs; tattooing; piercing; alcohol drinking; smoking; age at first sexual intercourse; sexual orientation; oral sex; anal sex; history of being sex workers; history of STDs; number of partners; extramarital sex; and condom use.

Although male subjects were more likely to be co-infected with HBV, the difference was not statistically significant. The results of this study corresponded to the study of Soběslavský (1980), which found that Thai healthy males had a higher rate of HBV infection than females. In addition, several other studies stated that males frequently formed the majority of people with the risk of HIV/HBV co-infection or HBV infection (Hussain et al., 2006; Gupta & Singh, 2006; Filho et al., 2009; Khameneh & Sepehrvand, 2008; Sali et al., 2005; Stover et al., 2003; Dursun et al., 2005; Akcam, Uskun, Avsar & Songur, 2009).

This study, however, identified high-risk factors more commonly found among males than females, namely, IDU, blood transfusion, tattooing and multiple sexual partners. This finding was contrary to Kouassi-M' Bengue et al. (2011), Guriev and Spinu (2010), and Adewole et al. (2009), who reported that females were the main group with the risk of HIV/HBV co-infection.

Regarding to age-groups, although Kouassi-M' Bengue et al. (2011), Sungkanuparph et al. (2004), Gupta and Singh (2006), and Adewole et al. (2009) identified the highest risk age group as being between 20-40 years, this study found that the majority of HIV/HBV co-infection cases were in the age group of 30-49 years. Moreover, HIV-infected patients aged between 50-60, 40-49, and 30-39 years were more likely to be co-infected with HBV than those aged between 18-29 years, but the difference was not statistically significant. This finding was in line with some studies

that identified increased age as being associated with more opportunity of exposure to HBV in their lives (Dursun et al., 2005; Stover et al., 2003).

With marital status taken into analysis, this study found that the married and divorced/widowed groups were more likely to have HBV co-infection than the single group, but without a statistically significant margin. This study's findings corresponded to that conducted by Sali et al. (2005) and Ashraf et al. (2010), who reported that married people were more associated with HBV infection and faced greater risk factors in HBV infection, compared with the general population. Moreover, the study by Khameneh and Sepehrvand (2008) found the possibility of HBsAg positivity in hemodialysis patients were more likely in the married group than in the single or widowed group. Adewole et al. (2009) also said that married people were the largest proportion affected with HIV or co-infection with HBV. Jones, Marmor and Nyambi (2005), who studied the risk factors in HIV seroprevalent infection in women, found that married women had a greater risk than single ones (OR=0.44 95%CI=0.30-0.66).

This study also revealed that the subjects who were unemployed were more likely to be HBV/HBV co-infected, but with a statistically insignificant margin. On the other hand, Khameneh and Sepehrvand (2008), who studied hemodialysis patients, found the percentage of HBsAg was higher among the employed than the unemployed. Some studies showed that police officers, barbers, drivers, sewage workers, and healthcare workers faced higher risk of HBV or HIV infection (Sali et al., 2005; Arvanitidou et al., 1998; Bell, 1997; Jeffries, 1995).

Regarding the factor of debt, this study found that HIV-infected patients who were in debt were more likely to have HBV co-infection, but the margin was not statistically significant. Moreover, no income group was identified as likely related to the decrease in the risk of HIV/HBV co-infection in this study, but the difference was not statistically significant. This finding differed slightly from that of an earlier study by Asl et al. (2004) and Lurie et al. (1995), which showed that HIV and HBV infection was more commonly found among gypsies and sex workers, who had low socioeconomic status, than among other groups.

Concerning the context of the family, Punyagupta et al. (1973) suggested that family contact was an important source of hepatitis B virus infection. Another study,

in Yemen, found that a large family size seemed to be associated with higher exposure to infection with HBV among general population (Bawazir et al., 2011). This study found that subjects who lived with five or more family members or lived in a family with HBV-infected people were more likely to have HBV co-infection, but the difference showed no statistical significance.

A study by Dursun et al. (2005) suggested that rural crowded families would tend to increase HBV familial transmission. However, Milas et al. (2000) showed that there was no significant difference in the mean rate of HBV infection between small families (up to four members) and large families (more than four members) among general population in Croatia. They concluded that the size of the family had no impact on the familial dissemination of HBV infection. Our study also found that subjects living with HBV-infected people in their families were more likely to have HBV co-infection, but the margin showed no statistical significance. Furthermore, although Talaat et al. (2007) supported that fact that exposure to household items used by hepatitis patients raised the risk of being infected with HBV, this study showed that history of sharing personal objects in the family was likely to decrease the risk of HIV/HBV co-infection, but such was of no statistical significance.

This study also found that subjects who had blood transfusion were more likely to have HBV co-infection, although the margin was not statistically significant. In Thailand, Nucleic Acid Amplification Technologies (NAT) is used to detect HBV during the window period and to identify donors with occult hepatitis. This method is a main contributor to the decrease in HBV (Chimparlee, Oota, Phikulsod, Tangkijvanich & Poovorawan, 2011). Nyirenda et al. (2008) studied the prevalence of infection with HBV, HCV and co-infection with HIV in Malawi, and found no correlation between a positive HBV, HCV or HIV test and a history of receiving blood transfusion.

Moreover, a study by Bawazir et al. (2011) showed an inverse association between a history of receiving blood transfusion and HBV infection. A study conducted among hemodialysis patients found the mean percentage of HBsAg positive was higher in patients who had a history of blood transfusion than those who did not (Khameneh & Sepehrvand, 2008). In this study, however, there was no case with a history of hemodialysis.

WHO recommends that all infants and people in high-risk groups should be vaccinated. According to WHO, high-risk people include those with high-risk sexual behavior; partners of or people having household contact with infected people; IDUs; people who frequently require blood and blood components; recipients of solid organ transplantation; healthcare workers; and travelers to countries with a high rate of hepatitis B (WHO, 2013b). Vaccination against HBV may be effective but response rates are reduced in HIV-infected patients (Brook, 2006). Some studies found that HBV vaccination after the HIV diagnosis showed no association with reduction of the risk of HBV infection. One contributing factor in the lack of protection for HBV might be the high rate of exposure and poor initial immunogenicity of the vaccine in HIV-infected patients (Landrum et al., 2010).

HIV-infected patients not having received ARV drugs were more likely to be infected with HBV, but without statistical significance. Most of the subjects in our study had regularly received ARV drugs, which could have increased their immunity.

Jaundice was a condition caused by hepatic pathology due to viruses, drugs, and alcohol abuse. This study found that subjects who had jaundice were more likely to have HBV co-infection, but not with a statistically significant margin.

The association between IDU and HIV/HBV co-infection could not be identified in this study due to the small number of subjects. The type of intravenous drug identified as related to the risk of HIV/HBV co-infection was not favorite among Northern Thais. According to Kilmarx, Limpakarnjanarat, Saisorn, Mock, and Mastro (2000), IDU was rare in Northern Thailand, even though IDU was found as a risk factor in HBV infection among chronic hepatitis B patients, female HIV-infected patients, and high-risk HIV-uninfected women groups (Sali et al., 2005; Stover et al., 2003). Sali et al. (2005) also suggested that IDU was an important means of HBV spread. Stover et al. (2003) found reverse association with HIV-infected and high-risk HIV-uninfected women who had smoked crack cocaine. My study showed, on the other hand, that the use of illicit drugs by inhalation and oral routes had reverse association with HIV/HBV co-infection, but to a statistically insignificance degree.

Similar association was also identified in patients with a history of tattooing and STDs in this study. However, Nyirenda et al. (2008) found no correlation between a positive HBV, HCV or HIV test and the presence of scarification marks, consistent

with the study in Egypt by Talaat et al. (2007), who found no an association between HBV infection and tattooed and pierced ears. However, my study found that HBV co-infection was found more among subjects who had had their body parts pierced than those who had not, although the margin was not statistically significant.

Chu et al. (2011) found the main comorbidity disorders among HIV-infected adults were hypertension, dyslipidemia, and diabetes, with the prevalence levels of 26.00, 48.00, and 13.00%, respectively. Moreover, Ryan (2010) also found that the metabolic effects of combined therapies for HIV infection increased the risk of insulin resistance, type 2 diabetes, and poor cardiovascular disease outcomes. My study found that the subjects who had comorbidity were more likely to be HBV co-infection; however, the increased likelihood showed no statistical significance.

Concerning smoking and alcohol drinking, this study found the subjects who had been smokers and/or alcohol drinkers before they became aware of their HIV status were more likely to have HBV co-infection, but without a statistically significant margin. This is supported by Beckett, Burnam, Collins, Kanouse and Beckman (2003), who found that 35.00% to 53.00% of the heterosexuals and MSMs identified as HIV positive had used alcohol with sex. Bryant (2006) also found that drinking alcohol was associated with the increased sexual risk of HIV infection.

People who have first intercourse at an early age tend to have many sex partners. In this study, the subjects who had had first sexual intercourse at the age of 15 or older were more likely to face reduced risk of HBV co-infection; however, there margin was not statistically significant. Similarly, the subjects who had same-sex intercourse were found more likely to have HIV/HBV co-infection, but without a statistically significant margin. A study among HIV-infected MSMs in Taiwan showed that their prevalence of HBsAg was higher than MSMs who tested seronegative for HIV (Tseng et al., 2012). However, sexual orientation was not found to be associated with HBV co-infection in my study, as only a small number of subjects identified themselves as MSMs.

Another factor frequently shown to be related to HIV is a history of being CSWs. Kilmarx et al. (2000), who studied the rate of mortality among female sex workers in Chiang Rai, found that 32.00% of them were HIV-1 infected. Being CSWs was also identified as a risk factor in HBV infection among HIV-infected and

high-risk HIV-uninfected women groups (Stover et al., 2003). Moreover, Lurie et al. (1995) found that female sex workers who had lower socioeconomic status were more likely to be infected with HIV-1, syphilis, and hepatitis B than those having higher socioeconomic status. As my study shows, subjects who had a history of being commercial sex workers were more likely to have HBV co-infection, although the difference was not of statistical significance.

Subjects with experience of oral sex displayed reverse association with HIV/HBV co-infection, even though some other studies, such as that by Van der Eijk et al. (2004), suggested to the contrary because the saliva of hepatitis B patients contained considerable amounts of HBV DNA. This corresponds to the study by Kidd-Ljunggren, Holmberg, Bläckberg and Lindqvist (2006), who reported that highly viremic HBV carriers that had high titers of HBV DNA could be found in other body fluids, such as urine, saliva, nasopharyngeal fluids, and tears. Moreover, a case report by Hui et al. (2005) identified a human bite as a possible route of HBV transmission. They found the HBV in a man who had been bitten by another HBV-infected man had identical genotype and sequence.

This study showed that extramarital sex behavior was also found in reverse association with HIV/HBV co-infection, but not to a statistically significant degree. Subjects who had two or more sex partners (from both the '2-9 partners' and '≥ 10 partners' groups) were likely to face decreased risk of HBV co-infection compared with subjects who had only one partner. However, this variable did not produce a statistically significant difference.

Use of condoms is also frequently studied with reference to HIV situations. In this study, subjects who had never or sometimes used condoms before they knew their HIV status were more likely to have HBV co-infection, but not to a statistically significant degree.

Next, subjects who had known their HIV status for three years of less at the time of interview had a greater risk of HBV co-infection than those who had known their HIV status for more than three years. It was possible that the subjects who had been aware of their HIV status for 3 years or longer had more skills in preventing themselves from contracting STDs. However, this variable was not statistically significant in the process of multivariate analysis.

Finally, after controlling all the possible confounder factors in the multiple logistic regression analysis, only 2 factors were found significant to HIV/HBV co-infection.

This study found HIV-infected patients with a CD4 cell count of ≥ 201 cells/$mm^3$ had a greater risk of HIV/HBV co-infection with a statistical significant degree. The one importance issue in this study was the researcher did not know the exactly date of HIV infection. Therefore the identification for CD4 level could difficult in the step of analysis. However, this finding was against the theory since the longer HIV infection, the lower CD4 cell count. Normally, around 80.00-90.00% of HIV-infected individual who had infected longer than 5 years had the CD4 cell count ≤ 200 cells/ $mm^3$ which means they would be AIDS. The mean of CD4 cell count in cases was higher than in controls (308.70 vs 255.97 cells/$mm^3$), but not to a statistically significant degree. This finding did not correspond to the study by Sungkanuparph et al. (2004) in Thailand, where they found the median serum CD4 cell count of HIV/HBV co-infection groups was lower than the non-co-infected group (HIV-infected only), but the difference was not statistically significant. However, Hoffmann et al. (2009) found that HBV did not influence the increase in CD4 in HIV-infected patients. A study of HIV/HBV co-infected patients in India found that HBV genotypes A, C, and D were related to CD4 cell count of less than 200 cells/$mm^3$ (Pal et al., 2011). According to previous studies of HBV genotype in Thailand, HBV genotypes C (73.00-91.00%) and B (8.00-21.00%) were predominant (Tangkijvanich et al., 2005; Jutavijittum, Yousukh, Jiviriyawat, Kunachiwa & Toriyama, 2008). My study found that HIV-infected patients with a CD4 cell count of less than 200 cells/$mm^3$ were presented as the preventive factor in HIV/HBV co-infection. However, it may be because of the natural history of HIV/HBV co-infection that the previous research found a greater number of CD4 and a slower rate of CD4 count decline among co-infected patients (Mesner et al., 2012; Smiatacz & Zielińska, 1996). Moreover, Salmon-Ceron et al. (2005), studying deaths caused by liver diseases, found that HIV/HBV co-infected patients had better immunovirological status than HIV-infected patients who were free of chronic viral hepatitis, as reflected by higher CD4 cell counts (112 vs 60 cells /$mm^3$).

In this study, subjects who had no education were at a greater risk for HIV/HBV co-infection with a statistical significant degree. Dursun et al. (2005) found that education level had a statistically significant effect on HBV seropositivity in urban areas. They suggested that the use of health facilities increased with the education level. In addition, Stover et al. (2003) found that a lower level of education (less than high school) was associated with HBV infection in HIV-infected and high-risk HIV-uninfected women.

Although some key informants from the in-depth interview remarked that education was not associated with STD infection, our quantitative study revealed that the higher the education level, the greater the chance that the risk of HIV/HBV co-infection could be reduced. However, some key informants agreed that education played a significant part in the prevention of STD infection, noting that people with higher education tended to know how to protect themselves from STD infection and were more likely to have self-restraint than those without education.

## 5.2 Conclusion

This study, which adopted a hospital-based case-control design, aimed to investigate the risk factors in HIV/HBV co-infection among patients who were diagnosed between 2006 and 2012 in 9 hospitals of Chiang Rai province. The research instruments consisted of a structured questionnaire, in-depth interview, and 5 mL of blood specimen used to identify HBV markers. In total, 124 subjects were included in the study.

Results show that patients with low or no education tended to face a greater risk of HIV/HBV co-infection than those who had spent seven or more years in school (OR=7.07, 95%CI=1.77-28.24). Next, the patients who had a CD4 cell count of 201 cells/$cm^3$ or more had a greater risk of HIV/HBV co-infection than those having a CD4 cell count of 200 cells/$cm^3$ or lower (OR=0.35, 95%CI=0.13-0.94).

Nonetheless, people with less education might face a greater risk of HIV/HBV co-infection. The first reason could be because these two diseases often share the same route of transmission. Another reason is lowly educated people tend to be less

aware of the risk of co-occurrence of these diseases than people with higher education. To prevent HIV/HBV co-infection or HIV infection, therefore, proper education and encouragement, as well as more regular implementation of preventive programs, are needed for people who are at risk.

To be on the safe side, HBV immunization is necessary for young people living in such an area as Northern Thailand. The immunity may reduce the complication of liver diseases resulting from the ARV drug.

## 5.3 Recommendations

### 5.3.1 Recommendations for Subjects

The outcomes of this study confirm that HIV-infected people should be identified for HBV status and, if testing HBsAg negative, should be provided with HBV vaccination.

All subjects should be concerned about having their CD4 levels detected as part of their health monitoring, even though a CD4 cell count had reverse association with HIV/HBV co-infection. Since HIV, HBV infection and ARV are causes of hepatitis, it is much more important for the subjects to monitor their health, particularly those who have received the ARV drug.

### 5.3.2 Recommendations for Policy Makers and Healthcare Personnel

A healthcare setting should be conducive to detection of HBV infection among HIV-positive patients, particularly before starting an ARV drug program.

Based on an outcome of the research, uneducated people tend to become HIV/HBV co-infected. Healthcare providers, therefore, should pay attention to patients' socioeconomic status during the screening of risk population for HIV/HBV co-infection. Moreover, health education should be provided on a more frequent basis for those attending in ARV clinics.

Next, use of condoms and avoidance of multiple sex partner behavior should be emphasized among those who are HIV-positive.

Lastly, for long-term reduction of HIV/HBV co-infection, it is recommended that the Thai government introduce an HBV vaccination program for all HIV-positive and HBsAg-negative people and implement more campaigns promoting use of condoms among HIV-positive people and other risk groups.

### 5.3.3 Recommendations for Future Studies

More studies need to be done to more thoroughly examine the risk factors of HIV/HBV co-infection and also on the consequence of HIV/HBV co-infection among subjects who have received the ARV drug.

Future study should also be focused on the prospective cohort study as a means of identifying the association between people who have received HBV vaccine and HIV/HBV co-infection. Because HBV vaccination was first administered to Thai new born babies in 1992, it may be assumed that a large number of people who are HIV-positive at the present time have not received the HBV vaccine.

The researcher should concern the other HBV markers for identifying HBV status in the future study. The case group should be included subjects who had positive results of HBsAg, anti-HBs, and HBV DNA which means cases of carriers and chronic hepatitis B.

Finally, further investigation is recommended for the CD4 and HBV status among HIV-infected patients, as the findings in this study suggested reverse association, compared with other stronger study designs (like an experimental study design).

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# APPENDICES

# APPENDIX A

## Informed Consent Form

**Title: Risk factors of HIV and HBV co-infection in Chiang Rai, Thailand**

Date ……/……./……

I have been recruited to participate in a study of "Risk factors of HIV and HBV co-infection in Chiang Rai, Thailand". If I consent to participate in this study, I will complete a series of questionnaires and/or in-depth interview. These include questions of sociodemographic characteristics, medical history, risk behaviors, and sexual behaviors. The time to complete these questionnaires and/or in-depth interview should be about 15-30 minuets.

Beyond my participation, it is likely that I will indirectly benefit from my participation in this study such as the knowledge gained from this study may contribute to understanding of HIV and HBV co-infection and confirmation of my HBV status. I have an exhibit of the objective of the study and allowed the researcher to check my medical record and take blood sample.

Moreover, I understand that all individual research results will be kept confidential and only the results of the study will be published. Answering some questions about self-concept might create transitory discomfort. I understand that I am completely free to refuse to answer any question and I am free to cease participating at any time after the study has started without penalty.

I have read and understand this consent form, and I agree to participate in this study.

In case of the volunteer is illiterate; I cannot read the documents, but the researcher have read the informed consent to me with the local language. Therefore, I understand all things and sign or to fingerprint hereby volunteer to participate in this study.

Signature……..(Participant)

Signature…….……..(Witness)

# APPENDIX B

## Questionnaire

**Title: Risk factors of HIV and HBV co-infection in Chiang Rai Thailand**

**Check in the box or fill in the space according to your information.**

**Code…….. (Fill in by the researcher)**

**Section 1** Socio-demographic characteristic

1. Gender
   - □ Male
   - □ Female
2. Age ……. Years
3. Marital status
   - □ Single
   - □ Married
   - □ Widowed or divorced
4. Religion
   - □ Buddhism
   - □ Christian
   - □ Muslim
   - □ Others
5. Present habitat in …….. district
6. Number of family member ……... persons

7. Education

□ No education

□ Primary school in …….. grade

□ Secondary school in …….. grade

□ Vocational or diploma certificate

□ Bachelor's degree

□ Others

8. Occupation

□ Unoccupied

□ Agriculture

□ Monthly employee

□ Daily employee

□ Business

□ Government officer

□ Retired

□ Student

□ Monk

9. Income per month …….. Baht

10. Debt ……... Baht

**Section 2** Medical history

11. Have you ever had blood transfusion?

□ Yes

□ No

12. Have you ever had hemodialysis?

□ Yes

□ No

13. Have you ever had jaundice?

□ Yes

□ No

14. Have you ever received hepatitis B virus vaccination?

□ Yes

□ No

15. Have you ever received antiretroviral drug?

□ Yes

□ No

16. Have you ever had other comorbidities?

□ Yes, there are ……..

□ No

**Section 3** Risk behavior

17. Have you ever lived with HBV-infected person in your family?

□ Yes

□ No, please skip the question no. 18

18. If yes, what is the relationship with you? ……

19. Have you ever shared the personal objects with other family members?

□ Yes, I shared ……

□ No

20. Have you ever used illicit drug by injection?

□ Yes

□ No, please skip the question no. 21

21. Have you ever shared the injecting equipment with other drug users?

□ Yes

□ No

22. Have you ever used illicit drug by inhalation?

□ Yes, I used ……..

□ No

23. Have you ever used illicit drug by oral route?

□ Yes, I used ……..

□ No

24. Have you ever had tattooed?

□ Yes

□ No

25. Have you ever had pierced?

□ Yes

□ No

26. Before knowing your HIV status, have you ever drunk alcohol?

□ Yes

□ No, please skip the question no. 27

27. How often?

□ Less than a time per time

□ 1-2 times per month

□ 1-2 times per week

□ Mostly everyday

28. After known your HIV status, have you ever drunk alcohol?

□ Yes

□ No, please skip the question no. 29

29. How often?

□ Less than a time per time

□ 1-2 times per month

□ 1-2 times per week

□ Mostly everyday

30. Before knowing your HIV status, have you ever smoked?

□ Yes

□ No

31. After known your HIV status, have you ever smoked?

□ Yes

□ No

**Section 4** Sexual behavior

32. You had the first sexual intercourse at aged ……. years

33. Have you ever been a commercial sex worker?

□ Yes

□ No

34. Have you ever had these symptoms? Please write "/" in the box.

□ Wound or rash at the genital organ or anus

□ Pus from the genital organ

□ Pain and swell at the genital organ

□ Lymphadenopathy at the groin

□ Scabies or crab lice at the genital organ

□ Leucorrhea

□ Pain at lower abdominal or testis

□ Never had these symptoms

35. Have you ever had sexual intercourse with the same own gender?

□ Yes

□ No, please skip the question no. 36

36. What is your sexual preference?

□ Top

□ Bottom

□ Both

37. Have you ever had oral sex?

□ Yes

□ No

38. Have you ever had anal sex?

□ Yes

□ No

39. How many of your sex partners? ……. Persons

40. Before knowing your HIV status, have you ever used condom?

□ Never

□ Yes, sometimes

□ Yes, always

41. After known your HIV status, have you ever used condom?

□ Never

□ Yes, sometimes

□ Yes, always

**END OF THE QUESTION**

**THANK YOU**

---

This section for the researcher only

CD4 count ……. cells/$mm^3$

HIV diagnosis in year ……

# APPENDIX C

## In-Depth Interview Guideline

Title: Risk factors of HIV and HBV co-infection in Chiang Rai Thailand

Code…….. (Fill in by the researcher)

Date ……/……/……..…

**Section 1** General information and risk behavior interview guideline

1. Do you think that illiterate or had low education people will have more risk of HIV or other sexual transmitted diseases compared to high education people? Why?

2. Do you think that poor people will have more risk of HIV or other sexual transmitted diseases compared to rich people? Why?

3. Do you think that alcohol drinking will have an association with HIV or other sexual transmitted diseases? Why?

4. Do you think that tattooing or piercing will have an association with HIV or other sexual transmitted diseases? Why?

5. Do you think being injecting drug users or use other illicit drugs will have an association with HIV or other sexual transmitted diseases? Why?

**Section 2** Sexual behavior interview guideline

1. Do you think being commercial sex worker will have an association with HIV or other sexual transmitted diseases? Why?

2. Do you think being homosexual will have an association with HIV or other sexual transmitted diseases? Why?

3. Do you think that people who have many sex partners will have an association with HIV or other sexual transmitted diseases? Why?

# APPENDIX D

## Laboratory Procedures

**1. Test for HBsAg (Immunochromatographic technique)**

1.1 Test procedure

1) Removed the protective foil cover from each test.

2) Applied 50 μL of sample (precision pipette) to the sample pad (marked by the arrow symbol).

3) Waited a minimum of 15 minutes (up to 24 hours).

4) Read result.

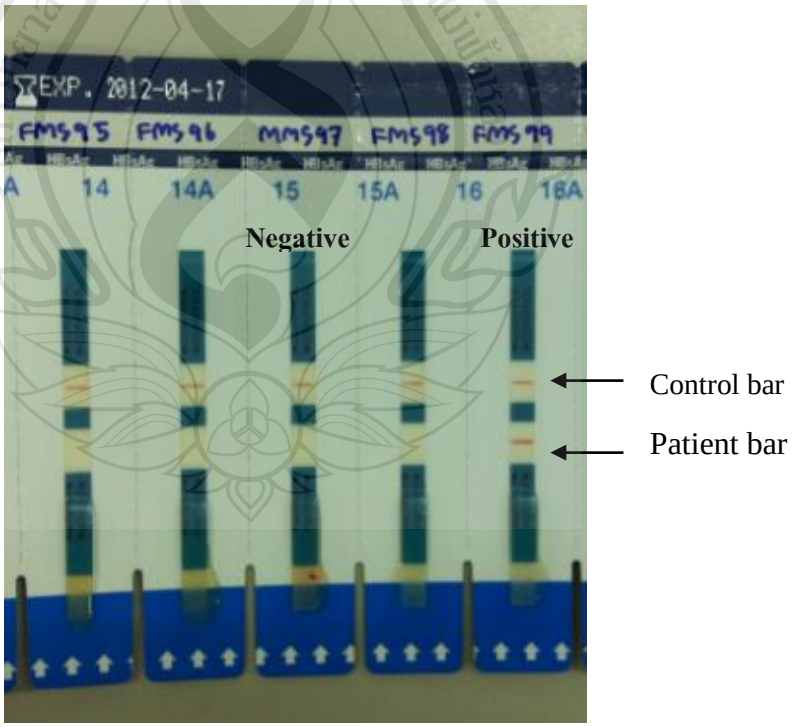


**Figure 10** Detection of HBsAg Marker in Serum

1.2 Interpretation of result

1) Positive: Red bars appear in both the control window and the patient window of the strip.

2) Negative: One red bar appears in the control window of the strip and disappear in the patient window of the strip.

3) Invalid: No red bar in the control window of the strip.

1.3 Specificity and sensitivity of Determine® or Alera™

1) 99.95% specificity
2) 95.16% sensitivity

**2. Test for anti-HBs (Immunochromatographic technique)**

2.1 Test procedure

1) Placed all specimens and test devices and allow them to room temperature prior to use (15-30 minutes).

2) Prepared the test device and mark the patient's code on the top of the device. Perform the test immediately after removing the device from foil pouch.

3) Applied 100 μL of serum or plasma into the sample well (S) in the test device.

4) After 10-15 minutes, read result.

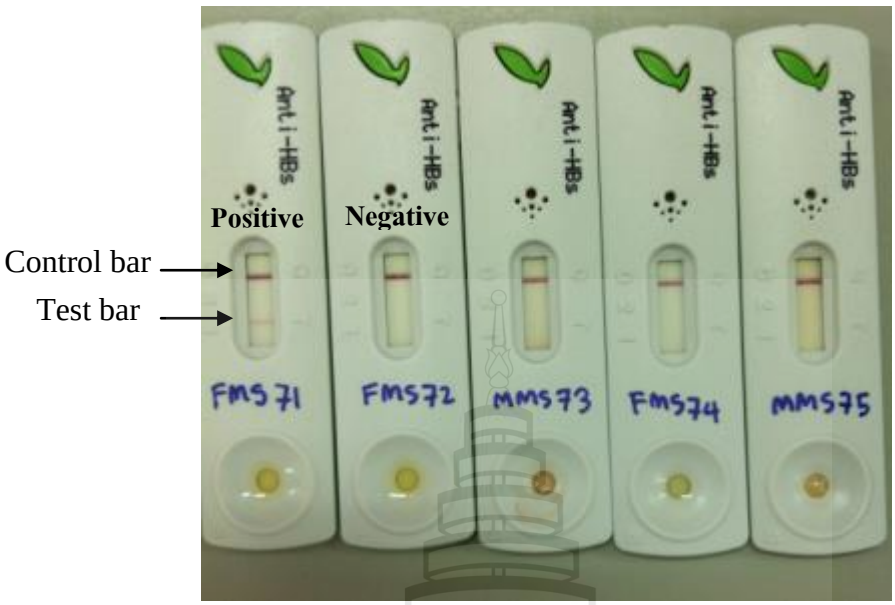


**Figure 11** Detection of Anti-HBs Marker in Serum

2.2 Interpretation of results

1) Positive: Red bars appear in both the control window and the patient window of the strip.

2) Negative: One red bar appears in the control window of the strip and disappear in the patient window of the strip.

3) Invalid: No red bar in the control window of the strip

2.3 Specificity and sensitivity of the test

1) Greater than 99.00% specificity.

2) More than 99.00% sensitivity.

**3. Test for anti-HBc (Enzyme immunoassay)**


**Figure 12** Monolisa™ Anti-HBc Plus Kit

3.1 Test procedure

1) Before using the reagents of the Monolisa™ Anti-HBc Plus kit, allowed them to stabilize at room temperature (18-30°C) for 30 minutes.

2) Diluted washing solution (20X concentrate) 1:20 in distilled water to obtain the ready for use washing solution. Prepared 800 mL for one plate of 12 strips.

3) Removed the microplate frame and ready to use strips from their protective bag.

4) Added quickly, directly and in succession: 200 µL of diluent into each well, 20 µL of negative control serum in A1 and B1 well, 20 µL of positive control serum in C1, D1 and E1 well, 20 µL of the first sample in F1 well and 20 µL of the second sample in G1, etc.

5) Covered the wells with adhesive film by pressing over the whole surface to ensure tightness.

6) Incubated the microplate in a thermostat-controlled water-bath or in a dry microplate incubator 30 min ± 5 minutes at 37°C ± 1 °C.

7) Removed the adhesive film. Aspirated the contents of all wells into a liquid waste container and add a minimum of 0.37 mL of washing solution to each well. Aspirated again. Repeated the washing step three times (4 washes). The residual volume must be lower than 10 μL (if necessary, blot the microplate by turning it upside down on absorbent paper). If an automatic washing device is used, follow the same operating cycle.

8) Distributed quickly 200 μL of the conjugate solution into all wells. The conjugate must be shaken gently before use.

9) Covered with new adhesive film and incubate for 60 ± 5 minutes at 37 ± 1 $^{o}$C.

10) Removed the adhesive film, empty all wells by aspiration and wash 4 times as previously described. The residual volume must be lower than 10 μL (if necessary, blot the microplate by turning upside down on absorbent paper).

11) Prepared the substrate solution by diluting reagent (R9) 1:11 using reagent R8. Prepared 10 mL for 1-12 strips.

12) Quickly dispensed into each well 100 μL of prepared development solution, freshly prepared before use. Allow the reaction to develop in the dark for 30 ± 5 minutes at room temperature (18-30 $^{o}$C). Do not use adhesive film during this incubation.

13) Add 100 μL stopping solution by using the same sequence and rate of distribution as for the substrate solution. Homogenize the reaction mixture.

14) Carefully wiped the plate bottom. At least 4 minutes after stopping solution addition and within 30 minutes of stopping the reaction, read the optical density at 450/620-700 nm using a plate reader.

15) Before recording the results, check the correlation between the reading and the microplate and sample distribution and identification plan.

3.2 Calculation and interpretation of the results

The presence or absence of anti-HBc antibodies is determined by comparing for each sample the recorded with that of the calculated cut-off value. Calculated the mean of the absorbance values for the positive control serum (OD R4).

$$\text{Mean of OD R4} = \frac{\text{Total optical density (C1, D1 and E1 well)}}{3}$$

1) Calculation of the cut-off value (Vs)

$$\text{Vs} = \frac{\text{mean of OD R4}}{5}$$

2) The validation criteria are as follows:

A) For the negative control: each individual measured the absorbance value must be less than 0.100.

B) For the positive control: each absorbance value must be greater than, or equal to, 1.000 and less than, or equal to 2.900. If one of the positive control value is out of these norms or differs by more than 30% from the mean value, carry out the calculation again with the two remaining positive control values. The test should be repeated if more than one positive control value is outside the limits set above.

3.3 Interpretation of the results

Sample with an optical density less than the cut-off value is considered to be negative with the Monolisa™ Anti-HBc PLUS test. Sample with an optical density higher than, or equal to, the cut-off value are considered to be initially positive with the Monolisa™ Anti-HBc PLUS test.

3.4 Specificity and sensitivity of the test

1) Greater than 99.83% specificity.

2) 100.00% sensitivity.

# CURRICULUM VITAE

# CURRICULUM VITAE

| | |
|---|---|
| **NAME** | Ms. Sauwaluck Pongwiriyakul |
| **DATE OF BIRTH** | 16 December 1983 |
| **ADDRESS** | 333, Thasud<br>Muang District,<br>Chiang Rai, Thailand<br>57100 |

**EDUCATIONAL BACKGROUND**

| | |
|---|---|
| 2006 | Bachelor of Sciences<br>Medical Technology<br>Chiang Mai University |

**WORK EXPERIENCE**

| | |
|---|---|
| July 2007-Present | Scientist<br>Scientific and Technological Instrument<br>Center, Mae Fah Luang University |
| January-June 2007 | Medical Technologist<br>Nan Hospital |
| March-December 2006 | Research Assistant<br>Program for HIV Prevention and Treatment<br>Chiang Mai University |