EPIDEMIOLOGY AND DETERMINANTS OF SEROLOGICALLY DIAGNOSED HIV-1 AND HIV-1&2 IN TERTIARY HOSPITALS OF EASTERN PENINSULAR MALAYSIA

DR. SITI AISHAH BINTI MUHADI

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PATHOLOGY (MEDICAL MICROBIOLOGY)

UNIVERSITI SAINS MALAYSIA

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Symbols / Abbreviations	Meaning
e.g	exempli gratia
n	Frequency
%	Percentage
<	Less than
>	More than
ml	Millilitre
g	Gram
dl	Densilitre
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral Therapy
Ag/ab	Antigen/ antibody
CMV	Cytomegalovirus
CD4	Cluster of differentiation 4
CCR5	Chemokine receptor type 5
CI	Confidence interval
CXCR4	CX Chemokine receptor 4
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent
ECLIA	Electrochemiluminescence immunoassay
HIV-1	*Human Immunodeficiency Virus type 1*
HIV-2	*Human Immunodeficiency Virus type 2*
HIV-1&2	*Human Immunodeficiency Virus type 1 & 2*
HBV	Hepatitis B virus
HCV	Hepatitis B virus
HIS	Hospital Information System
INNOLIA	Immunoblot Line immunoassay
IVDU	Intravenous drug user
LIS	Laboratory Information System
MSM	Men who have sex with men
NAT	Nucleic Acid Test
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NNRTI	Non Nucleoside Reverse Transcriptase Inhibitor
OD	Odd ratio
PA	Particle agglutination
PI	Protease inhibitor
PCR	Polymerase chain reaction
PLHIV	People were living with HIV
PrEP	Pre prophylaxis exposure
RNA	Ribonucleic acid
SD	Standard deviation
STD	Sexually transmitted disease
TB	Tuberculosis
WB	Western Blot

ABSTRAK

Pengenalan: Jangkitan *Human immunodeficiency virus* (HIV) adalah merupakan penyebab utama morbiditi and mortaliti di seluruh dunia. Terdapat dua jenis jangkitan HIV iaitu HIV-1 dan HIV-2; yang berbeza dari aspek epidemiologi dan faktor risiko mengikut kawasan.

Tujuan: Kajian ini bertujuan mengenal pasti epidemiologi dan faktor risiko kepada HIV-1 and HIV-2 dalam kalangan pesakit HIV di hospital berpakar di Pantai Timur Semenanjung Malaysia.

Kaedah: Ini adalah kajian keratan rentas dalam kalangan pesakit HIV-1 and HIV-1&2 yang berumur 12 tahun ke atas daripada bulan Januari 2016 sehingga Disember 2018 yang dijalankan di hospital berpakar di Pantai Timur Semenanjung Malaysia. Data di-perolehi daripada borang permohonan makmal, fail rekod perubatan, sistem informasi makmal, dan sistem informasi hospital. SPSS versi 24 telah digunakan untuk menganalisis penelitian deskriptif data sosio demografi. Hubung kait di antara jenis HIV dengan penyakit HBV, HCV, TB dan syphilis di analisis secara Pearson chi square dan hubung kait di antara jenis HIV dengan parameter haematologi secara ujian T sample tidak bersandar. Dalam mengenal pasti faktor penyebab kepada HIV-1&2, beberapa pemboleh ubah telah dianalisis dengan analisis regresi linear sederhana, pemboleh ubah yang memperolehi nilai $p < 0.25$ adalah signifikan secara statistik dan akan diteruskan dengan analisis regresi berganda. Pemboleh ubah yang memperolehi nilai $p < 0.05$ adalah dikira sebagai signifikans secara statistik.

Keputusan: Daripada 519, pesakit HIV, 344(66.3%) adalah HIV-1 dan 175 (33.7%) adalah HIV-1&2. Pesakit HIV adalah tinggi dalam kalangan lelaki Melayu, tidak berkahwin dan bekerja di sektor pekerjaan tidak professional bagi kedua-dua kumpulan.

Kebanyakan pesakit HIV-1 telah di diagnosis sebagai pesakit tuberkulosis juga. Manakala, kebanyakan pesakit HIV-1&2 adalah tiada simptom semasa diagnosis dibuat. Purata umur diagnosis adalah sedikit tinggi dalam kalangan pesakit HIV-1&2 (39, SD =9) berbanding purata umur diagnosis pesakit HIV-1 (38, SD=11). Cara transmisi yang paling biasa dalam kalangan HIV-1 adalah melalui aktiviti seksual 131(45.7%) manakala dalam kalangan HIV-1&2 adalah penggunaan dadah secara jarum intravena 56(45.2%). Jangkitan bersama dengan tuberkulosis (p=0.005) dan hepatitis C (p<0.001) adalah signifikan secara statistik dalam kalangan HIV-1 berbanding HIV-1&2. Penggunaan dadah secara jarum intravena adalah faktor risiko yang signifikan dalam kalangan pesakit HIV-1&2 (Adjusted OR: 3.5, 95% CI=1.875-5.227, p<0.001).

Kesimpulan: Kadar HIV-1&2 adalah tinggi dalam kajian ini. Kebanyakan HIV-1&2 tidak mempunyai simptom klinikal semasa diagnosis HIV dan pengguna dadah secara jarum intravena adalah faktor risiko yang signifikan secara statistik dalam kalangan HIV-1&2. Simptom klinikal pesakit HIV-1&2 adalah kurang teruk berbanding pesakit HIV-1. Walau bagaimanapun, ujian molekular HIV perlu dijalankan untuk mengesahkan jenis HIV.

Kata Kunci: HIV-1, HIV-2, Serologi

ABSTRACT

Introduction: *Human immunodeficiency virus* (HIV) infection is known as the leading cause of mortality and morbidity worldwide. There are two types of HIV infection, HIV-1 and HIV-2, which are geographically different in epidemiology and determinants.

Objective: To determine the epidemiology and determinants of HIV-1 and HIV-1&2 among HIV patients in tertiary hospitals of Eastern Peninsular Malaysia.

Method: A cross sectional study of serologically diagnosed HIV -1 and HIV-1&2 from January 2016 until December 2018 of three tertiary hospitals in Eastern Peninsular Malaysia, aged >12 years were included. Data were obtained from laboratory request forms, medical record folders, Laboratory Information System (LIS) and Hospital Information System (HIS).All collected data were analysed by SPSS version 24 which include descriptive statistic for sociodemographic data, Pearson chi square for association between type of HIV with HCV, HBV, syphilis and tuberculosis and Independent T test for association between type of HIV with haematological parameters. The risk factor associated with HIV-1&2, were determined by Simple Linear Regression Model, variables with p value <0.25 were subjected to Multiple Logistic Regression Model. A p value of <0.05 was considered as statistically significant.

Results: Out of 519 serologically diagnosed HIV, 344 (66.3%) were HIV-1 and 175 (33.7%) were HIV-1&2. HIV positive were highly distributed in Malay male, single and worked in nonprofessional sector in both groups. Most HIV-1 patients were presented with tuberculosis. However, HIV-1&2 patients were mostly asymptomatic at diagnosis. The mean age was slightly higher, in HIV-1&2 (39, SD =9) compared to HIV-1 (38, SD=11). The commonest mode of transmission for HIV-1 was by sexual contact 131(45.7%) whereas IVDU 56(45.2%) in HIV-1&2. Co-infection with tuberculosis

(p=0.005) and HCV (p<0.001) were significantly higher in HIV-1 as compared to HIV-1&2. IVDU was a significant determinant to develop HIV-1&2 (Adjusted OR: 3.5, 95% CI=1.875-5.227, p<0.001).

Conclusion: Proportion of HIV-1&2 was high in this study. Most HIV-1&2 was asymptomatic at diagnosis and IVDU was the significant determinant of serologically diagnosed of HIV-1&2. Patient with HIV-1&2 present less severe compare to patient with HIV-1. However, further molecular diagnostic study should be tested to confirm the type of HIV in future.

Keywords: HIV-1, HIV-2, Serology

SECTION A
INTRODUCTION

1.1 Background

Human immunodeficiency virus (HIV) is a *Lentivirus*, family Retroviridae known as the leading cause of mortality and morbidity worldwide. Based on global HIV and AIDS statistic, a total of 77.3 million people have become infected with HIV. In 2017, estimated about 1.8 million newly diagnosed HIV individuals worldwide, on the average of a 5,000 new infections per day (1).

Globally, a total of 35.4 million people have died from AIDS-related illnesses with estimated about 940 000 people died from AIDS-related illnesses in year 2017(1).

In Malaysia, a total of 3,347 newly diagnosed case of HIV in 2017 with national ratio was 10.3 people for every 100,000 population. Based on that, there were 23.3 people diagnosed to have HIV for every 100,000 in Terengganu followed by Pahang and Kelantan, 19.5 and 18.7 respectively (2). In comparison to year 2010, national ratio was reduced from 12.9 people for every 100,000 population with tremendously reduced in Kelantan state from 28.8 people for every 100,000 population (3).

1.2 Type of HIV

There are two types of HIV infection, HIV-1 and HIV-2. HIV-1 accounts for around 95% of all infections worldwide. The strains of HIV-1 can classify into four groups. M is the ‘major’ group and is responsible for the majority of the global HIV epidemic. The other three groups - N, O and P - are quite uncommon (4). HIV-2 is estimated to be more than 55% genetically distinct from HIV-1. HIV-2 initially restricted to West Africa, but has been seen in other countries with links to West Africa which has spread to all continents (5).

The proportion of HIV-1 mono-infection was from 79.5% to 95%, while HIV-2 mono-infection was from 1% to 2% and HIV-1&2 dual infection was from 3% to 19 % based on study in West Africa (6-7).

1.3 Pathogenesis

Pathogenesis of HIV infection is the result of a complex interplay between the virus and the immune system, particularly the mechanisms responsible for T-cell homeostasis and regeneration. There are two distinct phases. The first early events include viral attachment, entry into the cytoplasm, reverse transcription, entry into the nucleus, and integration of the double-stranded DNA. In virus attachment, gp120 Env surface protein binds to the CD4 molecule found on some T cells, macrophages, and microglial cells. Binding to CD4 triggers a conformational change in gp120 that allows it to then bind to CCR5 or CXCR4. This second binding event exposes the fusion domain of the gp41 transmembrane protein, allowing fusion of the viral and cellular membranes, followed by viral entry into the target cell. While the second phase occurs over the lifetime of the infected cell as viral and, cellular proteins regulate the production of viral proteins and new infectious virus. (8).

1.4 Risk of transmission

The risk of transmission HIV is 27 times higher among men who have sex with men, 23 times higher among people who inject drugs, 13 times higher for female sex workers and 13 times higher for transgender women (1).

HIV infected patient who keep undetectable viral load by antiretroviral therapy (ART), will effectively no risk to transmit HIV through sex, less transmission throughout

pregnancy and delivery, reduced risk while sharing syringes or other drug-injection equipment and substantially reduce the risk of transmission via breastfeeding (9).

1.5 Clinical presentation

There are three stages of HIV infection; acute infection, clinically latency, and acquired immunodeficiency syndrome (AIDS). In acute infection, within 2 to 4 weeks after acquiring HIV infection, the patient may experience a flu-like illness, which may last for a few weeks. In this phase, they have a very high viral load and are very contagious.

In the clinical latency stage, HIV is still active but reproduces at low levels of viral, however still able to transmit the virus. At the end of this phase, the viral load starts to go up, and the CD4 T-lymphocyte count begins to go down.

As this happens, the patient may begin to have symptoms as the virus levels increase in the body, and the person moves into the AIDS stage. Given of badly damaged immune systems that they get an increasing number of severe opportunistic illnesses and their CD4 T-lymphocyte count drops below 200 cells/mm .People with AIDS can have a high viral load and be very infectious (10).

1.6 Treatment

Infected patients should take antiretroviral therapy to treat HIV as soon as possible. If taken as prescribed, antiretroviral suppressed the viral load of HIV to a low level, which keeps the immune system working and prevents illness. Viral suppression defined as having less than 200 copies of HIV per millilitre of blood (11).

In 2017, 21.7 million HIV patients (59%) were accessing ART globally HIV treatment access is key to the global effort to end AIDS as a public health threat. (1) There are lists of HIV medicines recommended for the treatment of HIV infection which include; Nucleoside Reverse Transcriptase Inhibitor (NRTI) class (e.g. Lamivudine, Zidovudine, and Tenofovir disoproxil fumarate), Non Nucleoside Reverse Transcriptase Inhibitor (NNRTI) class (e.g. Efavirenz, Nevirapine, and Doravirine), Protease Inhibitor (PI) class (e.g. Ritonavir and Fosamprenavir), Fusion inhibitor class (such as Enfuvirtide), C-C chemokine receptor type 5 antagonist class (such as Maraviroc), Integrase inhibitor (such as Raltegravir), Post attachment inhibitor (e.g. Ibalizumab), and Pharmacokinetic enhancer (e.g. Cobicistat). There are lists of the combination of HIV medicine containing two or more drug from one or more drug classes, such as Truvada, Combivir, and Kaletra (12).

Many commonly used antiretroviral drugs are active against HIV infection. However, NNRTI like Nevirapine and Efavirenz do not work against the HIV-2 virus. The best way to treat HIV-2 less clearly defined than HIV-1 (13).

The first line of highly active antiretroviral therapy is provided for free in Malaysia by the Ministry of Health since 2006. However, in 2017 out of 72,399 total number of HIV patients in Malaysia, only 54% receiving ART (2).

1.7 Prevention

There are many strategies to reduce the risk of acquiring or transmitting HIV. Besides used ART as treatment, ART can be used as pre prophylaxis exposure (PrEP) and post prophylaxis exposure. Furthermore, using condoms, having only low-risk sex, only

having partnered with the same HIV status, and not having sex can all effectively reduce the risk of acquiring and transmitting HIV. Combining prevention strategies can be even more effective. However, it must be used correctly and consistently (9).

In PrEP, when taking daily with adherence indicated by laboratory-detected presence of drug, the risk of acquiring HIV is reduced by 70% for HIV-negative intravenous drug users (IVDU) (14). While the risk of acquiring HIV is reduced by 90% for HIV-negative heterosexual men or women, (15) and the risk of acquiring HIV is reduced by 92% for HIV-negative men who have sex with men (MSM) (16). Missed doses result in a lower effectiveness of PrEP.

Besides PrEP, by using a condom, insertive anal sex with an HIV-positive partner reduces the risk of HIV transmission by 63% among MSM, while receptive anal sex with an HIV-positive partner reduces the risk of HIV acquisition by 72% among MSM. Among heterosexual men and women, sex with an HIV-positive partner reduces the risk of HIV transmission by 80% (9).

1.8 Prognosis

During clinical latency phase, the patient may not experience any clinical symptoms. For people who aren't diagnosed to have HIV and not taking ART, the phase can last a decade or longer, but some may progress through this phase faster. However, patients who are taking ART as prescribed may stay in this stage for several decades. Whereas, without treatment, people with AIDS phase typically survive for about three years (10).

Although clinical symptoms are similar in both type of HIV, HIV-2 is less pathogenic. HIV-2 the latency period is longer than ten years; and case fatality is estimated to be

one half to one third that of HIV-1, possibly due to lower viral load. However, without treatment, most people living with HIV-2 will eventually progress to AIDS and die from the disease.(13) (17). However, a study found HIV-2 mono-infection was associated with severe multi-organ cytomegalovirus (CMV) infection, encephalitis, cholangitis and chronic diarrhoea compared to HIV-1 mono-infection (18).

A prospective follow-up study among tuberculosis patients by reported that HIV type associated risk of tuberculosis (TB) was 7-fold higher for HIV-1&2 dual infection, 6-fold higher for HIV-1, and 2-fold higher for HIV-2, compared with the HIV-uninfected. Furthermore, the mortality was 4–5-fold higher for HIV-1 and HIV-1&2 patients and two-fold higher for HIV-2-infected patients (19).

1.9 Laboratory diagnosis

1.9.1 Laboratory diagnosis of HIV

Laboratories should conduct HIV test screening with US FDA approved antigen/antibody immunoassay that detects HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen to test for HIV-1 and HIV-2 infection and acute HIV infection. Nonreactive test on the screening immunoassay No further testing is required for nonreactive test on initial screening test. In case of possibility of early infection with nonreactive antigen/ antibody immunoassay, HIV-1 nucleic acid test (NAT) should be conducted, or request for new specimen to repeat the screening test. For reactive test on screening immunoassay test, further discriminatory type of HIV antibody test is required, either by Immunoblot Line immunoassay (INNOLIA), Western blot (WB) or Particle agglutination (PA) methods. The methods were

able to differentiate the HIV-1 and HIV-2 antibody. However, indeterminate cases or suspected of early HIV infection, NAT for HIV-1 could be used to confirm the diagnosis of HIV (21).To date; there was no commercially PCR test for HIV-2 (20).

1.9.2 Laboratory diagnosis of Haematological parameters

A study reported 74.3% HIV patient had CD4 T-lymphocyte level ≥200/ml at diagnosis with mean hemoglobin (g/dl):male: 14.4 (6.4–20) and female:12.6 (6.4–15.9), neutrophil count (× 109/l):2.800 (4.00–17.500) and platelet count (× 109/l):227.000 (16.000–615.000) (21). CD4 level is an indicator of disease progression, CD4 count at AIDS diagnosis and near the time of death of HIV-1 infection was lower compared to HIV-2 infection. While another study reported, normal CD4 T-lymphocyte counts in HIV-2-infected individuals were three times greater than in HIV-1-infected individuals with comparable levels of CD4 T-lymphocyte (22).

1.9.3 Laboratory diagnosis of HIV co-infection

There were many studies on HIV co-infection with hepatitis B(HBV), hepatitis C (HCV), syphilis and TB did worldwide with different socio demographically background and risk factors. A cross-sectional study reported that out of 1944 HIV-positive patients in China, only 9.5% were HBV co-infected. HBV prevalence was 14.5% in Eastern China (23).

There were many studies on HIV co-infection with HBV, HCV, TB and syphilis did worldwide with different socio demographically background and risk factors. A cross-sectional study reported that out of 1944 HIV-positive patients in China, only 9.5% were HBV co-infected. HBV prevalence was 14.5% in Eastern China (23).

In comparison to a study done in Italy, they found that 15.4% of HIV patient had HBV co-infection. HBV prevalence is high in HIV-positive worldwide, but it differs by geographic region (24). A descriptive study conducted in 2014 in the city of Kashan, Iran reported 85% of HIV patient had HCV co-infection (25). However, a study found that only 8.3% of HIV patient had HCV co-infection in China (23). A study reported 16.5% HIV patient had syphilis in Shanghai, China (26).

A hospital-based retrospective study conducted among adult HIV-positive patients attending HIV clinic between June 2006 and January 2014 in Amhara Region, Ethiopia. reported 27.7% HIV patient had TB co-infection (27). However, a cross-sectional study of single-recentre population in Isfahan, Iran only found 8% HIV patient had TB co-infection (28).

1.10 Problem statement and Justification of study

In managing patients with HIV infection, there are several factors, that are considered to predict failure of treatment which include; haematological parameters including haemoglobin ,total white blood cell, platelet and cluster of differentiation 4 (CD4) T-lypmphocyte count level, co-infection (e.g HBV, HCV, TB and syphilis) and also patient's insights regarding HIV infection and treatment. Besides that, the type of HIV need to be considered when choosing ART because it has implication in the progression of disease and emerging resistance of ART as HIV-2 is known to be intrinsically resistant to NNRTI agents, which are commonly used as the first line of HIV treatment worldwide including Malaysia.

In Malaysia, even though HIV infection is a big health issue, there is no published epidemiological data regarding the prevalence of HIV-1, HIV-2 and HIV-1&2 infection. Furthermore, Malaysia is endemic for diseases such as TB, HBV and HCV which may also contribute to the failure of ART. Up to date, there are no published epidemiological data in Malaysia that differentiate between the type of HIV infection and co-infection especially in HBV, HCV, TB, and syphilis infection. As the high prevalence of HIV in Kelantan and Terengganu (2) it is important to know the epidemiology and clinical status of HIV-, HIV-2 and HIV-1&2 in Kelantan and Terengganu. It is hoped that by knowing the epidemiology and clinical status of HIV-1, HIV-2 and HIV-1&2, we can predict the development of severe HIV infection in our population and effective measures can be implemented to reduce such occurrences.

1.11 Research Question

1. What are the proportions of HIV-1 and HIV-1&2 patients in the tertiary hospitals of Eastern Peninsular, Malaysia?

2. Is there any association between haematological parameters (haemoglobin, total white blood cell, platelet, CD4 T-lymphocytes level) with HIV-1 and HIV-1&2 patients in the tertiary hospitals of Eastern Peninsular, Malaysia?
3. Is there any risk factor associated with the type of HIV among HIV patients in the tertiary hospitals of Eastern Peninsular, Malaysia?

1.12 Objective

General Objective

To study the epidemiology and determinants of serologically diagnosed of HIV-1 and HIV-1&2 among HIV patients in tertiary hospitals of Eastern Peninsular,Malaysia.

Specific Objectives

1. To describe the proportion of serologically diagnosed of HIV-1 and HIV-1&2.
2. To compare the differences in haematological parameters (haemoglobin, total white blood cell, platelet, CD4 T-lymphocyte level) between serologically diagnosed of HIV-1 and HIV-1&2 patients.
3. To determine factors (sociodemographic, mode of transmission, haematological parameters, and co-infection) associated with the type of HIV.

1.13 Methodology

Study Design

This was a cross-sectional study conducted in tertiary hospital of eastern Peninsular Malaysia.

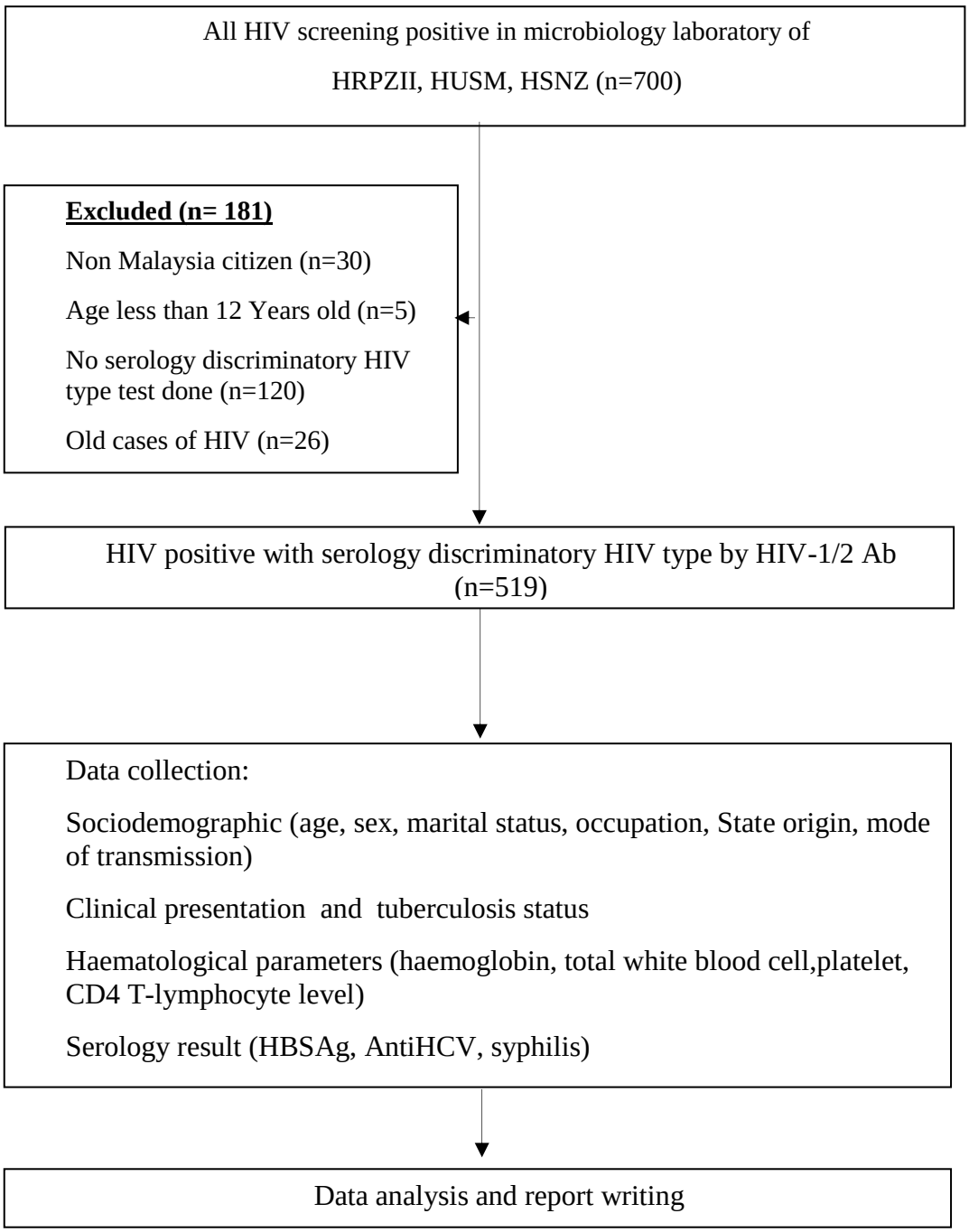

Figure 1: Study flow chart

Study Location and Duration

The study was conducted in Hospital Raja Perempuan Zainab II (HRPZ II), Hospital Universiti Sains Malaysia (HUSM), Kelantan and Hospital Sultanah Nur Zahirah (HSNZ), Terengganu from January 2016 until December 2018. All three hospital are tertiary hospital in Eastern Peninsular, Malaysia, in which HRPZ (II) and HUSM are located in Kota Bharu, Kelantan whereas HSNZ is located in Kuala Terengganu, Terengganu. Microbiology laboratory in that hospital act as a HIV testing referral centre of Eastern Peninsular region for all HIV test for patients from all hospitals in 10 districts in Kelantan and 8 districts in Terengganu. All three laboratories were supervised by Clinical Microbiologist and Infectious disease physician.

Study population and sample

All HIV positive patients tested in microbiology laboratory of HRPZ (II), HUSM and HSNZ from January 2016 until December 2018.

Sampling method

A convenience sampling was applied to select the sample from the list results of HIV patients tested in microbiology laboratory of HRPZ (II), HUSM and HSNZ from January 2016 until December 2018 that fulfils the inclusion criteria. The reason for choosing this sampling method was a scarcity of the potential number of study subjects.

Inclusion criteria

Newly diagnosed HIV positive patients who were aged more than 12 ,Malaysian citizen, positive screening test of p24 Ag/HIV-1&2 Ab either by Antibody Enzyme Linked Immunosorbent assay (ELISA) or Electrochemilucent Immunoaasay Ab (ECLIA) method and positive discriminatory test of HIV-1/HIV1&2 either by Particle agglutination (PA) or Western blot (WB) or Line Immunoassay (INNOLIA) method were included in this study.

Exclusion criteria

HIV patients who were pregnant and those shown negative result by discriminatory **test** of HIV-1/HIV1&2 either by Particle agglutination (PA) or Western blot (WB) or Line Immunoassay (INNO LIA) method were excluded in this study.

Sample size calculation

For the first objective, no samples size was calculated because it only involved descriptive statistic to look on the proportion of serological diagnosis of HIV-1 and HIV-1&2 among HIV patients.

For second objective , in order to identify the differences between Haematological parameters (haemoglobin, total white blood cell, platelet and CD4 T-lymphocyte count) with HIV-1 and HIV-1&2, the sample size (n) was calculated using two proportions formula using Power and Sample Size software as : α=0.05, Power = 0.8, p0 = X, p1 = Y, m = 1

Alpha (α) defined as the probability of detecting a significant difference between study group . Typically, α is set at 0.05 or 0.01.

Power defined as the probability of correctly rejecting the null hypothesis that sample estimates does not statistically differ between study groups in the underlying population. At least 80%, large values of power are desirable, is desirable given the available resources and ethical considerations. Power proportionately increases as the sample size for study increases.

p0=X and p1=Y were defined as the proportion for the study based on previous studied or expert opinion.

m=1 defined as the ratio of sample sizes in group 1 and group 2.

Parameter	**p0**	**p1**	**n**	**Adjusted n after 20% missing data**	**Reference**
CD4 T- lymphocyte	0.12	0.19	418	502	29
	p0=Proportion of CD4 T-lymphocyte level less than 200 /ml among HIV-1 patient based on study (1) p1=Estimated proportion of CD4 level less than 200/ml among HIV-1&2 patient				
Haemoglobin	0.12	0.19	418	502	30
	p0=Proportion of Haemoglobin level less than 10 g/dl among HIV-1 patient based on study(2) p1=Estimated proportion of Haemoglobin level less than 10 g/dl among HIV-1&2 patient				
White Blood cell	0.14	0.22	361	433	30
	p0=Proportion of neutropenia (absolute neutrophil count $\leq 1.3\times10^{9}$/l) among HIV-1 patient(2) p1=Estimated proportion of neutropenia (absolute neutrophil count $\leq 1.3\times10^{9}$/l among HIV-1&2 patient				

For third objective , to determine the factors associated with type of HIV among HIV patients, sample size was calculated using two proportions formula using Power and Sample Size software as

$\alpha = 0.05$, Power = 0.8, p0 = X, p1 = Y, m = 1

Parameter	**p0**	**p1**	**n**	**Adjusted n after 20% missing data**	**Reference**
Tuberculosis	0.18	0.26	420	504	31
	p0=Proportion of tuberculosis co infection among HIV-1 patient based on study (3) p1=Estimated proportion of Tuberculosis co infection among HIV- 1&2 patient				
Syphilis	0.16	0.24	391	469	32
	p0=Proportion of syphilis co infection among HIV-1 patient based on study (4) p1=Estimated proportion of Syphilis co infection among HIV-1&2 patient				
Hepatitis B	0.15	0.23	376	451	24
	p0=Proportion of HBV co infection among HIV-1 patient based on study (5) p1=Estimated proportion of HBV co infection among HIV-1&2 patient				
Hepatitis C	0.15	0.23	376	451	33
	p0=Proportion of HCV co infection among HIV-1 patient based on study (6) p1=Estimated proportion of HCV co infection among HIV-1&2 patient				

Thus, 504 was the largest sample size calculated and used in this study.

Research tools

This study used research proforma form to gather the interested variables from different sources. The primary source of data was from the HIV positive folder of each hospital. The other sources were from the laboratory request form, Laboratory Information System (LIS), medical record folder, and Hospital Information System (HIS).

The information gathered from HIV positive folder were result of screening positive HIV by p24 Ag/HIV -1 &2 Ab by ELISA or ECLIA method and result of serology discriminatory HIV type by HIV-1/2 Ab by PA, WB or INNOLIA method.

Whereas, from Laboratory Information System (LIS),result of full blood count (haemoglobin, total white blood cell and platelet), CD4-T-lymphocytes count and result of hepatitis B surface antigen (HBS Ag), hepatitis C antibody (Ab HCV), and syphilis were gathered.

Then, from laboratory request form, medical record folder, and Hospital Information System (HIS), information regarding sociodemographic data of age, sex, marital status, state and occupation; clinical diagnosis at the presentation of HIV diagnosis, status for mode of HIV transmission and status of tuberculosis were collected.

Data collection

List of HIV positive patients by screening and discriminatory HIV test were collected from the HIV positive folder from year 2016 until 2018 from all three laboratories. Laboratory request form of the patients were reviewed to obtained the sociodemographic data (age, sex, marital status, state of origin, mode of transmission and clinical presentation) at HIV diagnosis. In case of tuberculosis, status at presentation or any enquiry of sociodemographic data, medical record folder or HIS will be further reviewed.

Result of full blood count (haemoglobin, total white blood cell and platelet) at HIV diagnosis were reviewed. Whereas result of CD4 T-lymphocytes level and hepatitis B surface antigen (HBS Ag), hepatitis C antibody (Anti HCV), and syphilis within 2 weeks of HIV diagnosis done were collected.

Statistical analysis

The gathered data were analysed using the Statistical Package for the Social Science (SPSS) software version 24 (SPSS,Inc, Chicago,IL). The sociodemographic data were analysed using descriptive statistic and reported as frequencies and percentage for categorical data, whereas numerical data were shown in mean and SD. The association between haemotological parameters (haemoglobin, total white blood cell, platelet and CD4 level) and serological diagnosis of HIV-1 and HIV-1&2 were analysed by using Independent T-test. This study used the Pearson Chi-square test to determine the association between serological evidence of HBV, HCV, TB and syphilis. Further advanced data analysis were conducted by using Simple Logistic Regression followed by Multiple Logistic Regression to identify the significant risk factors to develop HIV-1&2 in HIV patients. A p-value <0.05 was considered as statistically significant.

1.14 Confidentiality and privacy

The patients' information was identified by using the study number. All data coded to protect privacy.

1.15 Ethical consideration

This study was approved by Human Research Ethics Committee (JEPeM-USM) Centre for Research Initiatives Clinical and Health USM Health Campus, Kubang Kerian,16150, Kota Bharu, Kelantan Darul Naim.Date of approval on 7th June 2018. The approval number was USM/JEPeM/18030162. The Medical Research Ethics Committee, Ministry of Health Malaysia, also approved this study. Date of approval on 7th May 2018. Approval number was NMRR-18-862-39602 (IIR).

1.16 References

1. The Joint United Nations Programme on HIV/AIDS(UNAIDS). Global HIV & AIDS statistics 2018 [cited 2019 July 15]. Available from:https://www.unaids.org/en/resources/fact-sheet.

2. Country Progress Report in HIV/AIDS,2018. Ministry of Health, Malaysia. 2018.

3. Malaysia Global AIDS Response Progress Report, 2015. HIV/STI Section of Ministry of Health, Malaysia; 2015.

4. Hemelaar J. The origin and diversity of the HIV-1 pandemic. Trends Mol Med, 2012 Mar;18(3):182-92.

5. AVERT. Global information and education on HIV and AIDS, Last updated 26 February 2019 [cited 2019 15 July]. Available from: https://www.avert.org.

6. Ds Nsagha AN, Hlf Kamga, Jcn Assob, Ea Bongkem. HIV-1/HIV-2 co-infection among voluntary counselling and testing subjects at a regional hospital in Cameroon. African Health Sciences. 2012 Sep; 12(3): 276–281.

7. Auld AF, Ekra K , Shiraishi RW, Tuho MZ, Kouakou JS, Mohamed F, et al. Temporal Trends in Treatment Outcomes for HIV-1 and HIV-2-Infected Adults Enrolled in Côte d'Ivoire's National Antiretroviral Therapy Program . PLOS ONE. 2014;9 (5): e98183

8. Vidya Vijayan KK, Karthigeyan KP, Tripathi SP, Hanna LE. Pathophysiology of CD4+ T-Cell Depletion in HIV-1 and HIV-2 Infections. Front Immunol. 2017;8:580.

9. Centre of Disease Control and Prevention. HIV Risk and Prevention 2019 [cited 2019 July 2019]. Available from: https://www.cdc.gov/hiv/risk/index.html.

10. Centre of Disease Control and Prevention. Stages of HIV infection 2019 [cited 2019 July 15]. Available from: https://wwwn.cdc.gov/hivrisk/what_is/stages_hiv_infection.html.

11. Centre of Disease Control and Perevention. HIV Treatment as Prevention 2019 [cited 2019 July 15]Available from: https://www.cdc.gov/hiv/risk/art/index.html.

12. Food and Drug Administration US. 2018 [cited 2019 July 15] Available from: https://www.fda.gov/patients/hiv-treatment/antiretroviral-drugs-used-treatment-hiv-infection.

13. Campbell-Yesufu OT, Gandhi RT. Update on Human Immunodeficiency Virus (HIV)-2 Infection. Clinical Infectious Diseases. 2011 Mar 15;52(6):780-787.

14. Choopanya K MM, Suntharasamai P, et al. Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok,Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2013;381(9883): 2083-2090.

15. Baeten JM DD, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N Engl J Med. 2012;367(5): 399-410.

16. Grant RM LJ, Anderson PL, et al. Pre-exposure chemoprophylaxis for HIV prevention in men who have sex with men. The New England Journal of Medicine 2010;363(27): 2587–2599.

17. Joakim Esbjörnsson, Fredrik Månsson, Anders Kvist, Zacarias J da Silva, Prof Sören Andersson, Prof Eva Maria Fenyö, et al. Long-term follow-up of HIV-2-related AIDS and mortality in Guinea-Bissau: a prospective open cohort study. The lancet HIV. 2019;6 (4): e214-215.

18. Nyamweya S, Hegedus A, Jaye A, Rowland-Jones S, Flanagan KL, Macallan DC. Comparing HIV-1 and HIV-2 infection: Lessons for viral immunopathogenesis. Reviews in Medical Virology. 2013 Jul;23(4):221-240.

19. Wejse C, Patsche CB, Kuhle A, Bamba FJ, Mendes MS, Lemvik G, et al. Impact of HIV-1, HIV-2, and HIV-1+2 dual infection on the outcome of tuberculosis. Int J Infect Dis. 2015;32:128-34.

20. Centre of Disease Control and Prevention. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations 2018 [cited 2019 July 15]Available from: https://www.cdc.gov.

21. Santis GCD, Brunetta DM, Vilar FC, Branda RA, Muniz RZdA, Lima GMNd, et al. Hematological abnormalities in HIV-infected patients. International Journal of Infectious Diseases 2011;15 (12): e808-e811.

22. Duvall MG JA, Dong T, Brenchley JM, Alabi AS, Jeffries DJ. Maintenance of HIV-specific CD4+ T cell help distinguishes HIV-2 from HIV-1 infection. J Immunol 2006;176(11) :6973-6981.

23. Xie J, Han Y, Qiu Z, Li Y, Li Y, Song X. Prevalence of hepatitis B and C viruses in HIV-positive patients in China: a cross-sectional study. Journal of the International AIDS Society. 2016;19(1): 20659.

24. Morsica G AF, Bagaglio S, Maracci M, Cicconi P, Cozzi Lepri A. Occult hepatitis B virus infection in a cohort of HIV-positive patients: correlation with hepatitis C virus co-infection, virological and immunological features. Infection. 2009;37(5): 445-449.

25. Afzali H, Momen-Heravi M, Farokhzad A. Epidemiological Distribution and Genotype Characterization of the Hepatitis C Virus Among HIV Patients in Kashan, Iran. Hepat Mon. 2016;16(7): e30459.

26. He H WM, Zaller N, Wang J, Song D, Qu Y, Zhang H. Prevalence of syphilis infection and associations with sexual risk behaviours among HIV-positive men who have sex with men in Shanghai, China. International Journal of STD & AIDS. 2014;25(6):410–9.

27. Mitku AA, Dessie, Z. G., Muluneh, E. K. & Workie, D. L. . Prevalence and associated factors of TB/HIV co-infection among HIV Infected patients in Amhara region, Ethiopia. African Health Sciences. 2016 Jun;16(2):588-595.

28. Meidani M, Rezaei F, Maracy MR., Avijgan M, Tayeri, K. Prevalence, severity, and related factors of anemia in HIV/AIDS patients. Journal of Research in Medical Sciences :The Official Journal of Isfahan University of Medical Sciences. 2012; 17(2):138-142.

29. Nascimento FG, Tanaka PY. Thrombocytopenia in HIV-Infected Patients. Indian J Hematol Blood Transfus. 2012; 28(2): 109–111.

30. Firnhaber C, Smeaton L, Saukila N, Flanigan T, Gangakhedkar R, Kumwenda J, et al. Comparisons of anemia, thrombocytopenia, and neutropenia at initiation of HIV antiretroviral therapy in Africa, Asia, and the Americas. Int J Infect Dis. 2010; 14 (12): e1088-e1092.

31. Sintayehu Fekadu, W. T., Getnet Alemu & 2, P. O. A. 2015. Prevalence and determinants of Tuberculosis among HIV infected patients in south Ethiopia. Jo Infect Dev Ctries. 2015 Aug 29; 9 (8):898-904.

32. Zhou Y, Li D, Lu D, Ruan Y, Qi X, Gao G. Prevalence of HIV and syphilis infection among men who have sex with men in China: a meta-analysis. BioMed research international. 2014; 2014: 620431.

33. Chen M, Wong W-W, Law MG, Kiertiburanakul S, Yunihastuti E, Merati TP, et al. Hepatitis B and C Co-Infection in HIV Patients from the TREAT Asia HIV Observational Database: Analysis of Risk Factors and Survival. PloS one. 2016; 11(3): e0150512.

SECTION B

STUDY PROTOCOL

PROPOSAL SUBMITTED FOR ETHICAL APPROVAL

RESEARCH TITTLE

Epidemiology and determinants of HIV-1 and HIV-1&2 in East Coast of Peninsular Malaysia.

Principle Investigator:

Dr Siti Aishah Bt Muhadi (MMC No: 58932)

Co-Researchers:

Prof Dr. Habsah Bt Hasan (MMC No:9657)

Dr. Nurahan Bt Maning (MMC No: 27170)

Dr. Fatimah Haslina Bt Abdullah (MMC No: 34265)

INTRODUCTION

Background of the study

Human immunodeficiency virus (HIV) is known as the leading cause of mortality and morbidity worldwide. In 2010, according to Global AIDS Response Progress Report Malaysia 2015 there were 28.8 people was diagnosed to have HIV infection for every 100,000 in Kelantan. In comparison to national ratio, it is only 12.9 people for every 100,000 population.

In 2014, about 24,711 new tuberculosis (TB) cases were registered in Malaysia with 5.9% had TB with HIV co infection. Indeed, in 2015, according to Global Tuberculosis report 2016, about 10.4 million people estimated to have TB worldwide with 1.2 million

had TB co infection. Furthermore, there were an estimation of about 1.4 million TB deaths with 0.4 million had TB with HIV co infection, and it shows TB co infection with HIV is a leading cause of death worldwide among HIV patients.

In view of that, Micheal Sidibe, who is the Executive Director of United Nations Programme on HIV and AIDS UNAIDS 2017 comments that it is unacceptable that so many people living with HIV die from tuberculosis, and that most are undiagnosed or untreated. He suggest that, we should be stepping up the collaboration between HIV and tuberculosis programmes to prevent the increased death among HIV patients due to TB co infection.

Globally, most HIV infections are caused by HIV-1. However, HIV-2, initially restricted to West Africa, has spread to all continents including Malaysia. Both infections of HIV-1 and HIV-2 can cause acquired immunodeficiency syndrome (AIDS). Although clinical symptoms are similar in both cases, HIV-2 is less pathogenic; the latency period is longer than ten years; and case fatality is estimated to be one half to one third that of HIV-1, possibly due to lower viral load.

As HIV-1 and HIV-2 infections differ in prognosis and require different treatment strategies, identification of the types of HIV is the better step to understand the epidemiology and risk factor of HIV infection in geographical areas where both viruses circulate especially in Malaysia. Currently, for HIV screening test in most of laboratory in Malaysia used 4th generation assay of HIV-1/2 Ag /Ab which simultaneous detect both antibodies and antigen in HIV either by enzyme linked immunosorbent assays (ELISA) or Electrochemiluminescence immunoassay (ECLIA) method