ORIGINAL ARTICLE

Epidemiology and Determinants of Serologically Diagnosed HIV-1 and HIV-1&2 in Tertiary Hospitals of Eastern Peninsular Malaysia

Siti Aishah Muhadi[1,5], Habsah Hasan[1,5], Nurahan Maning[2], Nik Rosmawati Nik Husain[3,5], Fatimah Haslina Abdullah[4]

[1] Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kubang Kerian, Kota Bharu, Kelantan, Malaysia.
[2] Hospital Raja Perempuan Zainab II, 15586, Kota Bharu, Kelantan, Malaysia.
[3] Department of Community Medicine, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kubang Kerian, Kota Bharu, Kelantan, Malaysia.
[4] Hospital Sultanah Nur Zahirah, 20400, Kuala Terengganu, Terengganu, Malaysia.
[5] Hospital Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan, Malaysia.

ABSTRACT

Introduction: HIV is the leading cause of mortality and morbidity worldwide. There are two types of HIV, 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 in the three tertiary hospitals of Eastern Peninsular Malaysia. **Method:** A cross-sectional study of confirmed serologically HIV-1 and HIV-1&2 from January 2016 until December 2018. SPSS analysed all collected data, descriptive statistics for sociodemographic data and Pearson chi-square for the association between type of HIV with HCV, HBV, syphilis, and tuberculosis. In identifying the risk factor associated with HIV-1&2, several variables were tested by the Multiple Logistic Regression Model. A P-value of <0.05 was considered statistically significant. **Results:** Out of 519 serologically diagnosed HIV, 344 (66.28%) were HIV-1, and 175 (33.72%) were HIV-1&2. HIV positive were highly distributed in Malay male in both groups. Most HIV-1 patients were single, unemployed, and presented with tuberculosis. HIV-1&2 were single and employed, mostly asymptomatic at diagnosis. The commonest mode of transmission for HIV-1 was by sexual contact (31.87%), whereas IVDU (13.63%) 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:** HIV-1&2 was high in this study. Patients with HIV-1&2 present in less severe symptoms compared to the patient with HIV-1. A further molecular diagnostic study should be tested to confirm the type of HIV.

Keywords: HIV-1, HIV-2, Serology

Corresponding Author:
Habsah Hasan, MPath (Pathology)
Email: drhabsah@usm.my
Tel: +6019 9179609

INTRODUCTION

Human immunodeficiency virus (HIV) is known as the leading cause of mortality and morbidity worldwide (1). Worldwide, there are HIV-1 and HIV-2 infections. Genetically, HIV-2 is to be more than 55% distinct from HIV-1 (1). 95% of HIV infection caused by HIV-1(2). Initially, HIV-2 only restricted to West Africa but eventually has spread to all countries with links to West Africa. Today HIV-2 has spread to all continents (1).

Immunoblot Line immunoassay (INNO-LIA), western blot (WB), and particle agglutination (PA) methods were able to differentiate the type of HIV by detection of antibodies of the HIV-1 and HIV-2, respectively. However, in indeterminate cases or suspected of early HIV infection, Nucleic acid amplification test (NAT) for HIV-1 could be used to confirm the diagnosis of HIV (3). To date, there was no commercially PCR test for HIV-2 approved by USA FDA.

Clinical symptoms are similar in both types of HIV infection, HIV-2 is less pathogenic, and the latency period is longer than ten years. However, people living with HIV-2 without ART will also eventually progress to AIDS and die from the illness (4-5). There were

many studies on HIV co-infection with hepatitis B (HBV), hepatitis C (HCV), syphilis, and tuberculosis (TB) done worldwide with different socio demographically background and risk factors (6-12). In Malaysia, there were high populations of HBV, HCV, syphilis, and TB among HIV patients (14). However, no data regarding the association between HIV co-infection with the type of HIV.

In general, among men who have sex with men (MSM), the risk of acquiring HIV is 27 times, while among intravenous drug users (IVDU) people who the risk of acquiring HIV is 23 times followed by 13 times risk in female sex workers and transgender women (13). In Malaysia, there were high populations of IVDU and MSM in HIV patients (14). However, no data regarding the association between the risks of acquiring HIV with the type of HIV.

The aim of this study is to determine the epidemiology and determinants, mode of transmission and co-infection (i.e. HBV, HCV, TB, and syphilis) of HIV-1 and HIV-1&2 patients in Eastern Peninsular Malaysia.

It is hoped that by knowing the epidemiology and clinical status of HIV and its type, we can predict the development of severe HIV infection in our population, and effective measures can be implemented to reduce the development of severe HIV (15).

MATERIALS AND METHODS

Study design, patient population, inclusion and exclusion criteria

A cross-sectional study from retrospective data was conducted in three tertiary hospitals, namely Hospital Raja Perempuan Zainab II, Kelantan, Hospital Sultanah Nur Zahirah, Terengganu, Hospital Universiti Sains Malaysia, Kelantan in Eastern Peninsular Malaysia from January 2016 until December 2018. The sampling method was convenience sampling from the list results of HIV positive patients tested in three microbiology laboratories of the tertiary hospitals in Eastern Peninsular Malaysia. All patients aged more than 12 years old Malaysian that fulfill the inclusion criteria were included; all newly diagnosed HIV patients by fourth-generation p24 Ag/HIV-1&2 Ab assay either by AxSYM HIV Ag/Ab Combo assay® (Abbott Laboratories, Abbott Park, Ill.) or Elecsys HIV Combi PT assay® (Roche Diagnostics, GmbH, Germany) method and positive discriminatory test of HIV-1/2 antibodies either by particle agglutination Serodia® (Fujirebio Inc., Japan) or HIV Blot 2.2 Western Blot assay® (MP Biomedicals) or INNO-LIA HIV I/ II Score® (Fujirebio, Ghent, Belgium) method were included in this study. Whereas HIV patients who were pregnant and those shown negative result by discriminatory test of HIV-1/HIV-2 antibodies either by particle agglutination Serodia® (Fujirebio Inc., Japan) or HIV Western Blot 2.2 assay® (MP Biomedicals) or INNO-LIA HIV I/II Score® (Fujirebio, Ghent, Belgium) method were excluded in this study. Based on HIV clinical stage, the clinical presentation can be asymptomatic, mild, moderate, or severe symptoms, stage 1 to 4, respectively (15).

Sample Size calculation

The sample size was calculated by the Power and Sample Size calculation programme (PS) based on the objectives of the study and from the literature review of previously studied. No sample size was calculated to determine the proportion of serologically diagnosed HIV-1 and HIV-1&2 among HIV patients because it only involved a descriptive statistic. Whereas to identify the determinants of HIV-1 and HIV-1&2, sample size was calculated using two proportion formula: $\alpha = 0.05$, Power = 0.8, $P_0 = 0.18$, $P_1 = 0.26$, m = 1, P_0 based on study proportion of tuberculosis co-infection among HIV-1 patient (16) and P_1 based on estimated proportion of tuberculosis co-infection among HIV-1&2 patients, the calculated sample size with dropped out rate 20% was 504 patients.

Research tool

All results tested positive for HIV by p24 Ag/ HIV-1&2 Ab and positive HIV-1 or HIV-1&2 on discriminatory HIV type tests were collected from the microbiology laboratory. Based on the list of HIV positive patients, sociodemographic data of age, sex, marital status, state, occupation, clinical diagnosis at the presentation of HIV, and mode of HIV transmission were reviewed from laboratory request form. In the case of tuberculosis status at HIV diagnosis and any enquiry of insufficient data collection from the laboratory request form, the medical record folder and Hospital Information System (HIS) were also further reviewed. Haematological parameter (haemoglobin, total white blood cell, platelet, and CD4 T lymphocyte count) result taken within two weeks from the date of HIV diagnosis done. Whereas, serology of Hepatitis B surface antigen (HBs Ag), Hepatitis C antibody (Anti-HCV), and syphilis result has also taken within two weeks from the date of HIV diagnosis. All the data were reviewed by the Laboratory Information System (LIS). All data were entered into the SPSS and kept confidential.

Statistical analysis

Data collected from this study were analysed using the Statistical Package for the Social Science (SPSS) version 24 (SPSS, Inc, Chicago, IL). All variables under study were analysed by using the descriptive statistic, comparing between HIV-1 and HIV-1&2 patients. The baseline characteristics of the study were described

in table form; categorical data were reported as frequencies, whereas numerical data were shown in mean (SD). The association between categorical data with the type of HIV were analysed by Pearson chi-square, whereas Independent T-test analysed the association between numerical data with the type of HIV. Further advanced data analysis was examined by Simple Logistic Regression Model to determine the risk factor of HIV-1&2; variables with P-value < 0.25 were followed by the Multiple Logistic Regression Model test in identified the determinant to develop HIV-1&2 in HIV patients. A P-value of <0.05 was considered statistically significant.

RESULTS

A total of data from 700 patients was diagnosed to have HIV positive from three microbiology laboratory which were covering all patients seek medical treatment as out-patient or in-patient from all 10 districts of Kelantan state and 8 districts of Terengganu state in 3 years duration, as the laboratories were the HIV reference laboratory in both state and 519 patients that met the inclusion criteria were included in this study. As shown in Table I, 344 (66.28%) were HIV-1, and 175 (33.72%) were HIV-1&2. HIV positive were highly distributed in Malay male in both groups. 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 was by sexual activities (31.87%) in HIV-1, and IVDU (13.63%) in HIV-1&2.

As shown in Table I and Table II, most HIV-1 patients were single, unemployed, and presented with tuberculosis co-infection. While in HIV-1&2, mostly employed and asymptomatic at diagnosis. However, there were some missing data as not all data on marital status, occupation, mode of transmission, and clinical diagnosis at the presentation from 519 patients were obtained.

HIV with tuberculosis co-infection (P=0.005) and HCV co-infection (P<0.001) were significantly higher in HIV-1 as compared to HIV-1&2, as shown in Table III. There were also some missing data in the serologically diagnosed with HBV, HCV, and tuberculosis status among HIV patients. About 50% missing data of serological syphilis status of HIV patients as the test only requested by a clinician if indicated.

There was no association between the haematological parameter (haemoglobin, total white blood cell, platelet, and CD4 T-lymphocyte count) at the time of diagnosis with the type of HIV, as shown in Table IV. There were also missing data in the haematological parameter.

As shown in Table V, IVDU was a significant determinant to develop HIV-1&2 (Adjusted OR: 3.5, 95% CI=1.875-5.227, P<0.001) while other parameters were not statistically significant.

Table I : Sociodemographic of serologically diagnosed HIV-1 and HIV-1&2 patients (n=519)

Sociodemographic	HIV-1 n(%)	HIV-1&2 n(%)
Race		
Malay	320(93.0)	169(96.6)
Chinese	16(4.6)	2(1.1)
Indian	3(0.9)	1(0.6)
Others	5(1.5)	3(1.7)
Sex		
Female	53(15.4)	21(12)
Male	291(84.6)	154(88)
Age mean(SD)	38(11)	39(9)
Marital status (n=319) [a*]		
Single	125(57.3)	51(50.5)
Married	66(30.3)	31(30.7))
Divorced	27(12.4)	19(18.8)
Occupation (n=310) [b*]		
Unemployed	41(18.8)	18(19.6)
Employed (Unprofessional)	117(53.6)	48(52.2)
Employed (Professional)	20(9.2)	11(12.0)
Student	18(8.3)	4(4.3)
Prisoner	5(2.3)	5(5.4)
Housewife	17(7.8)	6(6.5)
Mode of transmission (n=411) [c*]		
IVDU only	73(25.4)	56(45.2)
Homosexual only	58(20.2)	16(12.9)
Heterosexual only	63(22.0)	21(16.9)
Bisexual only	10(3.5)	1(0.8)
IVDU & Homosexual	1(0.3)	1(0.8)
IVDU & Heterosexual	8(2.8)	3(2.4)
IVDU & Bisexual	1(0.3)	1(0.8)
Blood recipient	4(1.4)	1(0.8)
Needle Injury	4(1.4)	0(0.0)
Mother to child	3(1.1)	0(0.0)
Not revealed	62(21.6)	24(19.4)
State		
Kelantan	249(72.4)	91(52.0)
Terengganu	84(24.4)	80(45.7)
Others	11(3.2)	4(2.3)

Missing data [a,b,c*]

DISCUSSION

The proportion of HIV-1&2 was high (33%) in this study, in contrast to a study in India, they reported only 17% of HIV-1&2 (17). Similar to our study, they utilized serological method to detect HIV 1 and HIV 2 and there was no further confirmation test by the PCR method to determine the molecular status of HIV. The lower proportion in the study is most likely due to the selected groups which include patients from sexually transmitted disease (STD) clinic and in female prostitutes from two different states of India. Whereas, in our study, we involved all HIV-positive people being screened by serological method for diagnosis or as part of national screening programme in the three states of Malaysia.

Table II : Clinical diagnosis at presentation in serologically diagnosed HIV-1 and HIV-1&2 patients (n=328)*

Cinical diagnosis	HIV-1 (n=231)	HIV-1&2 (n=97)
Bacteria only		
Pulmonary Tuberculosis only	39	12
Extrapulmonary Tuberculosis only	7	3
Disseminated Tuberculosis	7	0
Salmonellosis	8	4
Staphylococcus aureus septicaemia	4	6
Beta Haemolytic Streptococcus group B	0	1
Pseudomonas aeruginosa septicaemia	1	0
Klebsiella pneumoniae septicaemia	0	1
Rhodococcus spp. Septicaemia	1	0
Acute Treponema Pallidum infection	0	3
Proteus mirabilis septicaemia	1	0
Fungi only		
PCP(Pneumocystis pneumonia)	12	4
Candidiasis	10	4
Cryptocococcus neofarmans	3	2
Tinea magnum	1	0
Tinea cruris	0	1
Nocardiosis	0	1
Saccharomycosis	1	0
Talaromyces marneffei	2	0
Virus only		
Cytomegalovirus	4	1
Epstein Barr Virus	1	0
Acute Hepatitis (HBV and HCV)	6	1
Parasitology only		
Cerebral Toxoplasmosis	18	11
Combination (Bacteria, fungi, parasite and Virus)		
Pulmonary Tuberculosis and Cerebral Toxoplasmosis	3	0
Pulmonary Tuberculosis and Candidiasis	1	0
PCP and Talaromyces marneffei	1	0
PCP and Extrapulmonary Tuberculosis	1	0
PCP and Candidiasis	3	2
Pulmonary Tuberculosis and *Cryptococcus neoformans*	0	1
Disseminated Tuberculosis and *Cryptosporidium* spp.	3	2
Atypical pneumonia and Candidiasis	1	0
Salmonellosis and Cerebral Toxoplasmosis	1	1
Candidiasis and Cerebral Toxoplasmosis	1	0
Candidiasis and Chronic diarrhea	2	1
Candidiasis and *Staphylococcus aureus*	1	0
Candidiasis and Perianal abscess	1	0
Others (No Microbiology laboratory evidence of infection)		
Asymptomatic	26	16
Prolonged fever unknown origin	18	2
Chronic diarrhea	4	3
Atypical pneumonia	14	5
Infected surgical site infection	1	1
Ovarian cyst	1	0
Epididymoorchitis	1	0
Sialolithisis	0	1
Perianal abscess	2	1
Psoas abscess	1	0
Chest wall abscess	1	0
Prostate abscess	1	0
Thigh abscess	1	0
Recurrent liver abscess	0	1
Mycotic aneurysm	1	0
Ischaemic heart disease	1	0
Acute Lymphoblastic leukaemia	1	1
Diffuse Large B cell lymphoma	1	0
Severe septicaemia (Unspecific)	1	0
Substance induce psychosis, altered Sensorium	7	1

Missing data*

Table III : Association between the serological evidence of syphilis, HBV, HCV and Clinical Tuberculosis in serologically diagnosed HIV-1 and HIV-1&2 patients by Pearson Chi-square test

Serological evidence	HIV-1 n(%)	HIV-1&2 n(%)	P-value
Syphilis (n=219) [a*]			
Yes	30(17.9)	8(15.7)	0.720
No	138(82.1)	43(84.3)	
HBV (n=447) [b*]			
Yes	24(8.1)	15(9.9)	0.539
No	271(91.9)	137(90.1)	
HCV (n=455) [c*]			
Yes	120(40)	91(58.7)	<0.001
No	180(60)	64(41.3)	
Tuberculosis (n=453) [d*]			
Yes	67(22.3)	17(11.1)	0.005
No	239(79.7)	136(88.9)	

Missing data [a,b,c,d*]

Table IV : Association between haematological parameter in serologically diagnosed HIV-1 and HIV-1&2 patients by Independent T-test

Haematological parameters	HIV-1 mean(SD)	HIV-1&2 mean (SD)	P-value
Haemoglobin (n=376) [a*]	10.9(2.9)	10.5(2.9)	0.699
Total white blood cell (n=375) [b*]	7.9(6.1)	8.0(5.9)	0.887
Platelet(n=375) [c*]	237.6(122.9)	210.0(116.8)	0.749
CD4 T-lymphocyte (n=367) [d*]	214.6(281.4)	226.8(253.6)	0.749

Missing data [a,b,c,d*]

Table V : Determinants of serologically diagnosed HIV-1&2 in HIV patients by Multiple Logistic Regression Model

Factors		Crude OR (95% CI)	p value	Adjusted OR (95% CI)	P-value
Age (Year)*		1.010 (0.993-1.028)	0.248		
IVDU*	No	1.00			
	Yes	2.685(1.765-4.086)	<0.001	3.5 (1.875-5.227)	<0.001
Homosexual	No	1.00			
	Yes	0.755(0.439-1.301)	0.312		
Heterosexual	No	1.00			
	Yes	0.912(0.575-1.447)	0.697		
Bisexual	No	1.00			
	Yes	1.388(0.580-3.323)	0.462		
TB*	No	1.00			
	Yes	0.374(0.183-0.764)	0.007		
Syphilis	No	1.00			
	Yes	0.856(0.365-2.006)	0.720		
HBV	No	1.00			
	Yes	1.236(0.628-2.433)	0.539		
HCV*	No	1.00	<0.001		
	Yes	2.133(1.438-3.164)			

*P-value <0.25 by Simple Linear Regression Mode

Worldwide studies by the molecular confirmation methods in serologically diagnosed HIV-1&2 showed various results. A study in India showed that more than half of patients who were serologically diagnosed HIV-1&2 were only infected with HIV-1 by the PCR method (23). Another study in Senegal found that nearly half (40.4%) serologically diagnosed HIV-1&2, had an infection with both HIV-1 and HIV-2 confirmed by genetic sequence analysis as shown by the presence of HIV-1 and HIV-2 gag and env in peripheral blood mononuclear cells (PBMC) of the viral Deoxyribonucleotide (DNA) sequences (24). However, a study in Malaysia, out of 27 from 29 seropositive HIV-2 by Western blot was negative by the HIV-2 RNA method (25). HIV-2 surveillance has been carried out since 1995, in Malaysia.

Currently, there are no commercially FDA-approved HIV 2 molecular testing. The rate of molecular detection of HIV-2 varies depending on the specimen and method used. The viral load of HIV-2 in plasma could be very low and not detected by the HIV-2 plasma RNA method. Different specimens such as a serum, plasma, whole blood, or peripheral blood mononuclear cell will influence the positivity rate. The detection will also depend on whether Total Nucleic acid (TNA), DNA, or RNA being the target. Recently, a study recommends, for patients with serologically uncertain or undifferentiated HIV-2 status and undetectable HIV-2 plasma RNA, HIV-2 TNA qualitative assay can be used for the detection of HIV-2 DNA/RNA in PBMC specimens (26).

In our study, 2. 26% were asymptomatic HIV and 87.2% were symptomatic HIV patients. We found that the commonest clinical presentation among HIV-1 was tuberculosis, while in HIV-1&2, most were asymptomatic, at the time of serologically diagnosed HIV. The asymptomatic HIV patients included those who volunteers for HIV screening test, including people with high-risk behaviours (e.g. MSM, IVDU, a sexual partner who is known HIV positive) and a requirement for premarital HIV status and Methadone replacement therapy programme (27). Asymptomatic HIV patient who is an IVDU is most likely to have HIV-1&2, therefore the clinical presentation is less severe than HIV-1. In dual infection, HIV-2 infection may influence HIV-1disease progression due to the vigorous production of chemokines and robust humoral and cellular immune response of HIV-2 (21). This findings corresponds to a study that found dually infected individuals had two-fold lower mortality risk during follow up compared to HIV-1 single infected individual (18).

In this study, IVDU was a significant determinant of serologically diagnosed HIV-1&2. In contrast to another study, in which they found that HIV-1&2 patients acquired HIV from sexual transmission, and there was no dual infection among IVDU (19-20). Our findings are most likely due to high populations of IVDU in Malaysia (14).

The limitation of this study was that not all data were obtained from 519 patients, due to missing data and limited time in data collection. Thus, the results where there are a high percentage of missing data affects the findings for this study. It could be the main reason that some of the parameters were not statistically significant. In the future, to determine the correct odds ratio or relative risk, a prospective cohort study should be conducted.

Furthermore, molecular study to determine the type of HIV was not performed in this study because of limited funding. The serologically diagnosed HIV-1&2 can be due to real HIV-1 and HIV-2 infections, a broad immune response against infection with a single strain of HIV-1 or HIV-2, infection with other third virus-containing epitopes common to either HIV-1 or HIV-2 or exposure to both HIV-1 and HIV-2 but established disease with only one type of virus (22).

In the clinical setting, in the case of indeterminate and undifferentiated serologically diagnosed HIV-1&2, further tested by the molecular method is needed to determine the type of HIV, either HIV-1, HIV-2, or both. The ability of a test to confirm and distinguish HIV-1, HIV-2, and HIV-1/HIV-2 dual infections has important implications for clinical management and antiretroviral therapy. However, up to date, there is no molecular study for HIV-2 approved by the USA FDA for in vitro diagnostic use in the United States.

CONCLUSION

Serologically diagnosed HIV-1&2 was high in this study. Those who are diagnosed serologically positive for HIV-1&2 were asymptomatic or less severe at presentation compared to HIV-1. HIV-1&2 patients are most likely acquired HIV infection from sharing needle among IVDU. However, to confirm the positivity of HIV-2 infection in the serologically positive HIV-1&2 a further molecular diagnostic study should be tested to confirm HIV-2 infections.

ACKNOWLEDGEMENT

We would like to thank the Director General of Health, Ministry of Health Malaysia and Director General of Hospital Universiti Sains Malaysia for their permission to publish this article.

This study was approved by the 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. The Approval number was NMRR-18-862-39602 (IIR).

REFERENCES

1. AVERT. Global information and education on HIV and AIDS, Last updated 26 February 2019 [cited 2019 15 July]. Available from: https://www.avert.org.
2. Hemelaar J. The origin and diversity of the HIV-1 pandemic. Trends Mol Med, 2012 Mar;18(3):182-92.
3. Centre of Disease Control and Perevention. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations 2018 [cited 2019 15 July].Available from: https://www.cdc.gov.
4. Joakim Esbjurnsson, Fredrik Mensson, Anders Kvist, Zacarias J da Silva, Prof Suren Andersson, Prof Eva Maria Fenyu, 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.
5. Campbell-Yesufu OT, Gandhi RT.Update on Human Immunodeficiency Virus (HIV)-2 Infection. Clinical Infectious Diseases. 2011 Mar 15;52(6):780-7.
6. Xie J, Han Y, Qiu Z, Li Y, Li Y, Song X, et al. 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.
7. Morsica G AF, Bagaglio S, Maracci M, Cicconi P, Cozzi Lepri A, et al. . 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.
8. 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.
9. 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.
10. Khana S, Antony G, MenezesbRahul, DhodapkarcBelgode, NarasimhaHarishc Seroprevalence of syphilis in patients attending a tertiary care hospital in Southern India. 2014;4(12):995.
11. 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.
12. 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.
13. UNAIDS. Global HIV & AIDS statistics 2018 [cited 2019 July 15]. Available from: https://www.unaids.org/en/resources/fact-sheet
14. Country Progress Report in HIV/AIDS,2018. Ministry of Health, Malaysia. 2018.
15. Campbell-Yesufu OT, and Rajesh T. Gandhi. . Update on human immunodeficiency virus (HIV)-2 infection. Clinical infectious diseases. 2011 Mar 15; 52(6): 780–787.
16. 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.
17. Janak M, Susanne G,Helga Rubsamen W High proportion of HIV-1 2 and HIV-1&2 double reactive sera in two Indian states. Maharashtra and Goa: First appearance of and HIV-2 epidemic along with an HIV-1 Epidemic, outside of Africa International Journal of Medical Microbiology. 1994 Jan; 280 (3):398-402.
18. Esbjornsson J, Mansson F,Kvist A,Isberg PE, Biague AJ, da Silva ZJ. Increased survival among HIV-1 and HIV-2 dual infected individuals compared to HIV-1 single infected individuals. AIDS.2014: 28 (7):949-957.
19. Kokkotou EG, Sankale Jl,Mani. In Vitro correlates of HIV-2 mediated HIV-1 protection.Proc Natl Acad SCI USA.2000; 97(12):6797–6802.
20. John N. Nkengasong LK, Peter D. Ghys, Stйphania Koblavi-Dиme, Ronald A. Otten, Cйlestin Bilй, Chantal Maurice, et al. Dual Infection with Human Immunodeficiency Virus Type 1 and Type 2: Impact on HIV Type 1 Viral Load and Immune Activation Markers in HIV-Seropositive Female Sex Workers in Abidjan, Ivory Coast.AIDS Research and Human Retroviruses. 2004: 16 (14):1371-1378.
21. Kannangai R, David S, Sridharan G. Human immunodeficiency virus type-2-A milder, kinder virus: An update. Indian J Med Microbiol. 2012;

30 (1):6-15.
22. Peeters M, Gershy-Damet GM, Fransen K, Koffi K, Coulibaly M, Delaporte E, et al. Virological and polymerase chain reaction studies of HIV-1/HIV-2 dual infection in Cфte d'Ivoire. Lancet. 1992 August; 340 (8815): 339-340.
23. Ingole NA, Sarkate PP, Paranjpe SM, Shinde SD, Lall SS, Mehta PR. HIV-2 Infection: Where Are We Today? J Glob Infect Dis. 2013;5(3):110-113.
24. Geoffrey S. Gottlieb PSS, Stephen E. Hawes, Ibra Ndoye, Awa M. Coll-Seck, Marcel E. Curlin, Cathy W. Critchlow, et al. Nancy. Molecular Epidemiology of Dual HIV-1/HIV-2 Seropositive Adults from Senegal, West Africa. AIDS Research and Human Retroviruses. 2003;19(7):575-584.
25. Mohd Zain R, Ismail S, Ellan EK, Wan Mahmood NAN, Md Kassim F, Thayan R. HIV-2 Infection in Malaysia: Current situation and the use of in-house real-time reverse transcription PCR for HIV-2. Tropical Biomedicine 2018; 35(3): 769-774.
26. M Changa AW, DN Raugib, RA Smithb, AM Seiliea, J Ortegaa, K Bogusza, et al. Clinical Validation of a Novel Diagnostic HIV-2 Total Nucleic Acid Qualitative Assay Using the Abbott m2000 Platform: Implicationsfor Complementary HIV-2 Nucleic Acid Testing for the CDC 4th Generation HIV Diagnostic Testing Algorithm. J Clin Virol. 2017 Jan;86:56-61.