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Research Report

## THE PREVALENCE OF HUMAN IMMUNODEFICIENCY VIRUS-1 (HIV-1) SUBTYPES AND TRANSMISSION METHOD AMONG HIV/AIDS INFECTION PATIENT IN TULUNGAGUNG, EAST JAVA INDONESIA

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### ABSTRACT

*The rapid epidemic growth of HIV is continuing in Indonesia. There are some factors which have influenced the spreading of this epidemic in Indonesia, such as the poor awareness to avoid unsafe free sex attitude and the sharing of needles and syringes among intravenous drug users (IDUs). The sexual transmission of HIV has also apparently increased in Tulungagung. Commercial sex workers play a significant role in the spread of HIV in Tulungagung. People in Tulungagung have worked at other countries as Indonesian migrants. This condition can cause the increase number of HIV-1 case and the possibility of genetic variation (subtype) HIV-1 in Tulungagung. This research is aimed to analyze the subtype and to determine estimation of transmission mode on infected patient of HIV-1 and AIDS who came to Seruni clinic Dr. Iskak hospital in Tulungagung. 40 HIV/AIDS patients were interviewed to determine the subtype and the transmission mode. The results showed that 14 of 40 plasma samples (35%) were successfully to amplified and sequenced. Overall CRF01-AE were identified as predominant subtype among HIV/AIDS patients in Tulungagung. Based on individual information, 31 of 40 subjects (77%) were heterosexual transmission.*

**Key words:** prevalence, subtype, transmission mode, HIV-1, Tulungagung

### ABSTRAK

*Pertumbuhan epidemi HIV di Indonesia berkembang sangat pesat. Ada beberapa faktor yang mempengaruhi penyebaran epidemi di Indonesia, diantaranya kesadaran masyarakat dalam melakukan hubungan seksual secara aman dan berbagi jarum suntik di kalangan pengguna narkoba suntikan (penasun). Pola penyebaran HIV melalui hubungan seksual meningkat di daerah Tulungagung. Pekerja seks komersial memiliki peranan penting dalam penyebaran HIV di Tulungagung. Banyak warga di Tulungagung yang bekerja di luar negeri sebagai imigran Indonesia. Kondisi ini dapat mempengaruhi jumlah kasus HIV/AIDS dan kemungkinan munculnya variasi genetik (subtipe) HIV-1 baru di Tulungagung. Penelitian ini bertujuan untuk menganalisis subtipe dan menentukan estimasi transmisi pada pasien yang terinfeksi HIV-1/AIDS yang datang ke klinik Seruni RS Dr Iskak Tulungagung. Dalam penelitian ini, sebanyak 40 pasien HIV/AIDS yang diwawancarai untuk mengetahui subtipe dan pola penyebaran HIV/AIDS. Hasil menunjukkan bahwa dari 40 sampel plasma terdeteksi 14 (35%) sampel positif secara kualitatif. Hasil analisa RIP dan pohon filogenetik menunjukkan bahwa 14 sampel tersebut termasuk dalam kelompok rekombinan CRF01-AE. Berdasarkan informasi pasien, pola penyebaran HIV/AIDS melalui heteroseksual sebanyak 31 sampel (77%).*

**Kata kunci:** prevalensi, subtipe, pola transmisi, HIV-1, Tulungagung

## INTRODUCTION

Human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) causes a serious health problem and has a big impact on Indonesian economics. In addition, rapidly growing epidemic of HIV-1 is a serious public health problem in Indonesia.

In Indonesia, the number of people living with HIV was estimated to be 591,823 in 2012 and 735,256 in 2015. Whereas newly infected with HIV were estimated to be 71,879 in 2012 and 85,523 in 2015<sup>1</sup>.

Several factors affect the rate and magnitude of growth of HIV prevalence, but two of the most important are injecting drug use and the commercial sex workers; transmission between men who have sex with men usually has a secondary role<sup>2,3</sup>.

In Indonesia, CRF01\_AE continually dominates HIV epidemic, although HIV subtype B is responsible for a large amount of infection sexually transmitted. Furthermore, Circulation Recombinant Form (CRF) can occur from one subtype to another one and nowadays it has been found 43 CRF. It is stated that different HIV subtype can have different effect on its transmission, causing drug resistance and disease progressivity<sup>4</sup>.

The growth and finding of HIV and AIDS cases in Tulungagung are increasing rapidly and its transmission is getting wider. Supposedly, it is triggered by hidden prostitution activities in two ex-brothels which have been closed down since 2012<sup>5,6</sup>. This research is intended to identify subtype HIV-1 and to know the method of HIV-1 infection transmission in Dr. Iskak General Hospital, Tulungagung.

By doing so, it is very essential to get data analysis on subtype HIV-1 and its infection transmission of HIV-1 at the newest and more representative hospital, Dr. Iskak General Hospital.

## METHODS

### Study Population and Data Collection

A total of 40 peripheral blood samples from HIV/AIDS patients in Dr. Iskak General Hospital, Tulungagung were collected. A cross-sectional survey, the study population were interviewed in Dr. Iskak General Hospital, Tulungagung using a questionnaire that collected information on socio-demographic characteristics, and transmission.

5-8 ml whole blood samples were collected from 40 HIV/AIDS patients by using sputum 5 cc. Whole blood sample were added into a EDTA tube. Plasma was then

isolated from peripheral blood samples by centrifugation for 10 min at 2,000 rpm. Plasma were analyzed at HIV/AIDS laboratory, Institute of Tropical Disease (ITD), Universitas Airlangga, Surabaya.

### The Examination of Polymerase Chain Reaction (PCR) HIV

The examination of Polymerase Chain Reaction (PCR) HIV was done to identify cDNA, as the following steps: RNA HIV was extracted from plasma and synthesis of cDNA HIV, amplification reaction with PCR, gel electrophoresis, and photo development using gel electrophoresis.

RNA virus was changed into cDNA using Super Script III First-Strand Synthesis Kit (Invitrogen, Carlsbad, CA, USA) with reverse primer, K-env- RI, 5'-CCAATCAGG-GAAGAAGCCTGG-3'.

Fragment HIV-1 gene pol 33 base pair encoding fragment partial integrase was amplified with nested PCR using Ex Taq (Takara Bio, Shiga, Japan) and primary set as follows:

For amplification of fragment gene pol virus, UNIPOL5; 5' -TGGGTACCAGCACACAAAGGAATAGGAG GAAA -3' (nt 4152 to 4183) and UNIPOL6; 5' -CCA CAGCTGATCTCTGCCTTCTCTGTAATSGACC-3' (nt 4934 to 4901) is used for *first PCR*. UNIPOL1; 5' -AGTGGATTCATAGAAGCAGAAGT-3' (nt 4470 to 4492) and UNIPOL2; 5' -CCCCTATTCCTCCCCTTCTT TAAAA-3' (nt 4806 to 4781) is used for *nested PCR*.

**The condition of first PCR:** 94°C for 3 minutes for denaturation (one cycle), 94°C for 1 minute for denaturation (35 cycles), 72°C for one minute for extension (35 cycles) and 72°C for 5 minutes for the last extension.

**The condition of second PCR:** 94°C for 3 minutes (1 cycle), 94°C for 1 minute (35 cycles), 50°C for 1 minute (35 cycles), 72°C for 1 minute (35 cycles), 72°C for 5 minutes. PCR products amplified at the end-point dilution of DNA templates were subjected to sequencing analysis to examine the genomic fragment of the major viral population in a sample.

### The Analysis of Sequencing Results

Sequencing analysis on fragment genomic HIV-1 which has been amplified is done by using sequencing cycle kit BigDye Terminator V1.1 with ABI PRISM310 genetic analyzer, and data is analyzed by Genetyx ver10 software. HIV-1 subtyping was carried out using the Recombinant Identification Program (RIP) available on the HIV sequence database website (<http://www.hiv.lanl.gov/>). In addition, neighbor-joining (NJ) trees with the Kimura two-parameter model were constructed using MEGA6.2 software<sup>7,8</sup>.

## RESULTS

### Demographic and behavioural characteristics

We interviewed 40 HIV/AIDS infected patients who came to Seruni Clinic, Dr. Iskak General Hospital, Tulungagung on June 2014.

**Table 1.** Sample Characteristic based on Age and Sex

Age (in years)	Gender		Total
	Male	Female	
21-29	7	7	13
30-39	4	9	13
>40	8	6	14
Total	19 (47.5%)	21 (52.5%)	40 (100%)

Based on questionnaires, it indicates that the number of female patients is 21 (52.5%), age ranges from 30-39 while there are 19 male patients (47.5%). Most of them were over 40.

**Table 2.** Sample Characteristic based on Age and Transmission Methods

Age (in years)	Transmission way					Total
	Heterosexual	Homosexual	IDUs	Transfusion	Vertical	
21-29	7	4	2	-	-	13
30-39	13	-	-	-	-	13
>40	11	1	1	1	-	14
Total	31 (77.5%)	5 (12.5%)	3 (7.5%)	1 (2.5%)	-	40 (100%)

There were 31 (77.5%) heterosexual patients infected by HIV-1, their ages are various, 30-39 and only 5 persons (12.5%) homosexual patients got infected by HIV-1 as well. They were 20-29 years of age.

**Table 3.** Sample Characteristic based on Age and Marital Status

Age (in years)	Marital Status				Total
	Married	Unmarried	Widower	Widow	
21-29	9	4	-	-	13
30-39	9	2	1	1	13
>40	12	1	1	-	14
Total	30 (75%)	7 (17.5%)	2 (5%)	1 (2.5%)	40 (100%)

The data gives us information that there were 30 persons (75%), ranges of age are mostly over 40 and 7 persons (17.5%) of 20-29 are not married yet.

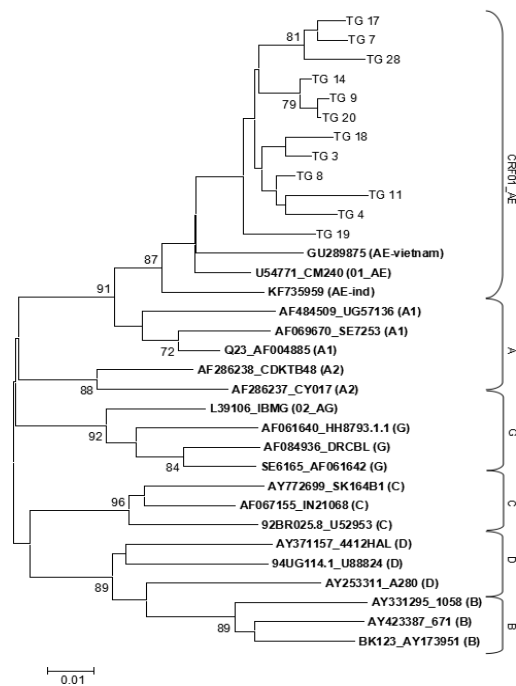
**Table 4.** Sample Characteristic based on Age and The Length of ART Usage

Age (in years)	ART Duration				Total
	Haven't taken ART	< 6 months	6 months - 2 years	> 2 years	
21-29	2	7	2	2	13
30-39	3	1	3	6	13
>40	1	3	4	6	14
Total	6 (15%)	1 (27.5%)	9 (22.5%)	14 (35%)	40 (100%)

Data obtained from ART indicate that patients who have got ART treatment for over 2 years are 14 (35%). They are mostly 30-39 years old, and over 40 years old. In addition, those 6 persons (15%) of 30-39 did not receive ART treatment.

**HIV-1 Subtyping**

HIV subtyping has been an important molecular tool for monitoring geographic changes in the worldwide AIDS epidemic<sup>9</sup>. Of the 40 samples derived from HIV patients, 12 *pol* genes were successfully sequenced. Based on the results of RIP and phylogenetic tree analyses for *pol* genes, overall CRF01\_AE has been identified as the predominant HIV-1 subtype, similar to the epidemics in Malaysia, Thailand, and Taiwan<sup>10,11</sup> and no other unique recombinant forms. Our results were consistent with previous findings.



**Figure 2.** Phylogenetic Tree (Dendrogram) Subtype HIV-1

Phylogenetic analysis is done to determine gene sequence pol HIV-1 with strain reference virus gen HIV-1 which shows the plasma sample. Those samples are: CRF01\_AE (01\_AE). *Accession Number* GU289975 (AE-vietnam) from Vietnam, KF735959 (AE-ind) from Indonesia, AF484509 (A1) from Uganda, AF069670 (A1) from Somalia, AF286238 (A2) from Republic of Congo, AF286237 (A2) from Cyprus, AY423387 (BY) from Netherlands, AY331295 (B) from USA, AY772699 (C) from South Africa, AF067155 (C) from India, AY371157 (D) from Cameroon, AY253311 (D) from Tanzania, AF084936 (G) from Republic of Congo, AF061640 (G) from Finland, L39106 (02\_AG) from Nigeria, U54771 (01\_AE) from Thailand.

A molecular analysis is done toward nucleotide as a DNA HIV sequence result taken from patient's plasma sample and compared with nucleotide sequences from HIV subtype which has been published before. In later analysis, from HIV-1 pol gene, the all gained sample are CRF, especially one branch with HIV CRF01\_AE which comes from Asia. Those are Thailand, Japan, Malaysia, China, and Hongkong.

## DISCUSSION

The characteristic of research sample based on age and gender is taken that women are the greatest number who suffer from HIV-1 (52.5%) age ranges from 30-39. This case may happen because the women can easily infected by HIV-1. The greatest number of spreading way is sexual activity with men who are infected. Biologically, the women are easier to be infected with HIV because the flatten areas for HIV are mostly in women (vagina, cervix, and uterus) than in the men (gland penis and urethra). The women are also greater in number who roll out with ejaculate than the men (vagina liquid contains less HIV than semen). The perimenopausal and postmenopausal women who undergo increasing of mucosa genital secondary fragility toward the changing of hormones on its cycle so that it increases the spreading risk of HIV<sup>12</sup>.

The characteristic of research sample based on age and assumption of transmission way is reported that heterosexual is the greatest number of transmission way (77.5%) in age between 30-39 years old. Heterosexual is the transmission way which has greatest factor to infect HIV-1 in the world. It becomes worse because of risky sex behavior, for example, having sex with someone with HIV-1 infected risk (a prostitute) or without using condoms.

The second way of transmission is probably homosexual (12.5%) age ranges from 20-mostly 29. It shows that homosexual is risky behavior which can infect HIV-1. There is a trauma in rectum causing wound which can be an easier way of HIV to human body. Rectum consists of lymphoid tissue which can make HIV-1 get into fragile lymphosit cell easily<sup>13</sup>.

The characteristic of research sample based on age and marital status is reported that the biggest number is marital status (75%) age ranges from 20-29. The second biggest sample is 17.5% unmarried people with age ranging from 20-29. It has been stated that the biggest sample is married people more than 40 years old when at these age most people have been married and had children. In the previous research, 76% from HIV sufferers are married women. This fact supports the dynamics of HIV-1 infection where in many cases, the first found HIV-1 infections are men who infected their wives.

The characteristic of research sample based on age and assumption of ART duration is reported that the longest ART duration is more than 2 years (35%) ranging from 30-39 years old and more than 40 years old. Most respondent state that they have been diagnosed to be infected by HIV-1 since 2 years ago and at that time, the CD4 examination was done and they continue to take ARV regularly. Medicine drinking obedience is always informed by the medical officer and also by ODHA fellows to others which take the medicine regularly in SeruniClinin Dr. Iskak General Hospital in Tulungagung. The role of counselor, KPA and every element in society has helped medicine drinking obedience and routine control for ODHA community in Tulungagung. The meeting of ODHA community is filled by informing from medical officers (doctors) and also giving the moral supports from ODH fellows to enrich the quality of their lives.

This research explains that the result of blood sample PCR of HIV-infected patients is that the percentage of positive RNA HIV-1 (35%) is lesser from the percentage of negative RNA HIV-1 (65%). The negative PCR HIV-1 result which is quite much is possibly caused by the research subjects who have been given ARV therapy. The research subjects which have not been given ARV therapy are reported to have no many significant differences in the PCR result between negative RNA HIV and positive RNA HIV. On the negative RNA HIV samples in the research, there is possibly a changing or mutation of the nucleotide sequences in the annealing primer. So that the primer cannot attach then it causes the PCR result to be negative.

HIV-1 is marked by the large heterogeneity and divided into 4 groups: M (*major*), O (*outlying*), N (*new or non-M, non-O*) and P (*pending*). The viruses in group M, which are responsible for the HIV pandemic in the world, are further classified into many subtypes and *circulating recombinant forms (CRFs)*. HIV-1 subtype B is the most apparent subtype in USA, Europe, and Australia whereas the subtype non-B is the subtype which causes an epidemic in Africa and Asia. Lately, new CRFs, CRF33\_01B and CFRF34\_01B, have been isolated in Indonesia<sup>14,15</sup>. In the previous research, it has been found the genome virus fragment CRF01\_AE, a CRF is which is dominant in Southeast Asia. That CRF is predominantly found in HIV-positive prostitutes in Surabaya, Indonesia and still a dominant virus strain<sup>16</sup>. In order to know the unidentified subtype,

we need to examine the virus subtype in this research. The genomic fragment of virus CRF01\_AE which was found in this research is still the most apparent HIV-1 subtype in Tulungagung, East Java, Indonesia.

## CONCLUSION

Based on the research result, it can be concluded that the most found subtype in HIV-1-and-AIDS-infected patients in Dr. Iskak General Hospital, Tulungagung is CREF01\_AE. It is relevant with the subtype which develops in Southeast Asia. The greatest number of transmission way in HIV-1-and-AIDS-infected patients in Dr. Iskak General Hospital, Tulungagung is heterosexual.

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