UNIVERSITI PUTRA MALAYSIA

ASSOCIATION OF BETA-DEFENSIN GENE COPY NUMBER VARIATION WITH CD4 COUNT AND VIRAL LOAD AMONG MALAYSIAN ETHNICS HIV PATIENTS AFTER HAART INITIATION

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NURFARAHIN HANINI BINTI ACHIM

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HIV is a global health concern, including in Malaysia, where more than a hundred thousand cases has been reported since 1986 until 2016. After the introduction of highly active antiretroviral therapy (HAART) regimen, HIV- related mortality and morbidity has greatly declined. Despite that, HIV clinical course were identified to be variable across population. Host genetic variation has been long recognized to have a part in influencing the susceptibility and prognosis of HIV infection. Beta-defensins gene (*DEFB*) encoded beta-defensins peptide, which has anti-HIV and chemoattractant properties. Interestingly, *DEFB* is characterized as copy number variables (CNV), a structural variation that presents in variable copy number between individuals. CNV may alter the expression of the gene and subsequently, will affect the susceptibility and progression of diseases. The CNV of *DEFB* has been associated with susceptibility towards a few diseases, including HIV. So far, there is still lack in information on distribution of *DEFB* copy number, especially among Malaysian HIV-infected patients. Moreover, the investigation on the association of *DEFB* CNV with CD4 count and viral load of Malaysian HIV patients are also lacking. Therefore, this study aimed to investigate the relationship of *DEFB* gene copy number variability with CD4 count and viral load in Malaysian HIV patients. A total of 182 HIV-infected patients and 156 controls were recruited in this study. *DEFB* copy number of all the participants was quantified using Triplex PRTs and validated with microsatellite analysis. *DEFB* copy number in HIV patients were found from 2 and 8 copies, and the modal copy number was 4. No significant difference was identified in *DEFB* distribution among Malay, Chinese, and Indian HIV patients. A comparison between HIV and control group found that individuals with a high copy number (> 4) are significantly higher among HIV patients as compared to controls (p = 0.039). However, no significant association was identified between *DEFB* copy number with the recovery of CD4 count to a normal level and viral load suppression. In conclusion, while individuals with high

DEFB copy number is suggested to have higher susceptibility towards HIV, *DEFB* is not a significant factor in influencing the disease prognosis.

Keywords: HIV, *DEFB*, CNV, CD4 count, viral load

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PERKAITAN DIANTARA KEPELBAGAIAN SALINAN GEN BETA-DEFENSIN DENGAN BILANGAN CD4 DAN BEBAN VIRUS DALAM PESAKIT HIV ETNIK MALAYSIA SELEPAS INISIASI HAART

Oleh

NURFARAHIN HANINI BINTI ACHIM

Januarl 2020

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Penyakit HIV merupakan suatu kebimbangan global, termasuk di Malaysia, di mana lebih daripada seratus ribu kes telah dilaporkan sejak tahun 1986 hingga 2016. Selepas pengenalan rejimen terapi *highly active antiretroviral therapy* (HAART), kematian dan morbiditi berkaitan dengan HIV telah banyak merosot. Namun begitu, prognosis penyakit HIV dikenalpasti sebagai berbeza- beza diantara populasi. Variasi genetik telah lama diiktiraf sebagai sebahagian daripada penyebab-penyebab yang mempengaruhi kecenderungan dan prognosis jangkitan HIV. Gen beta-defensins (*DEFB*) mengkodkan beta-defensins peptida, yang mempunyai sifat anti-HIV dan chemoattractant. Menariknya, *DEFB* mempunyai ciri nombor salinan bervariasi (CNV), dimana ia adalah variasi struktur yang mampu hadir dalam bilangan salinan berbeza-beza antara individu. CNV boleh mengubah ekspresi gen dan seterusnya, akan memberi kesan kepada kecenderungan dan perkembangan penyakit. Sifat CNV *DEFB* has telah dikaitkan dengan kecenderungan ke arah beberapa penyakit, termasuk HIV. Setakat ini, terdapat kekurangan maklumat mengenai taburan bilangan salinan *DEFB*, terutamanya dalam kalangan pesakit HIV di Malaysia. Tambahan pula, siasatan mengenai hubungan diantara sifat CNV *DEFB* dengan bilangan CD4 dan beban virus dalam pesakit HIV Malaysia juga kurang. Oleh itu, kajian ini bertujuan untuk mengkaji hubungan diantara jumlah salinan gen *DEFB* dengan bilangan CD4 dan beban virus pada pesakit HIV Malaysia. Seramai 182 orang pesakit HIV dan 156 orang kawalan telah direkrut untuk kajian ini. Bilangan salinan *DEFB* bagi semua peserta telah diukur menggunakan Triplex PRTs dan disahkan dengan analisis mikrosatelit. Didapati bilangan salinan *DEFB* pada pesakit HIV adalah daripada 2 hingga 8 salinan, dan bilangan salinan modal adalah 4. Walau bagaimanapun, tiada perbezaan signifikan dalam taburan *DEFB* diantara pesakit HIV Melayu, Cina, dan India. Perbandingan diantara pesakit HIV dan kumpulan kawalan mendapati bahawa individu yang mempunyai bilangan salinan yang banyak (> 4) adalah lebih tinggi

dalam kalangan pesakit HIV berbanding dengan kumpulan kawalan (p = 0.039). Namun begitu, tiada hubungan yang signifikan dapat dikenalpasti diantara bilangan salinan *DEFB* dengan pemulihan bilangan CD4 ke tahap normal dan penahanan beban virus. Kesimpulannya, kajian ini berpendapat bahawa individu dengan bilangan salinan *DEFB* yang banyak mempunyai kecenderungan yang lebih tinggi untuk dijangkiti HIV. Namun begitu, bilangan salinan *DEFB* bukan lah faktor penting yang mempengaruhi prognosis penyakit.

Kata kunci: HIV, *DEFB*, CNV, bilangan CD4, beban virus

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Declaration by Members of Supervisory Committee

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABREVIATIONS

AIDS	Acquired immunodeficiency syndrome
AMP	Antimicrobial peptide
AMY	Salivary amylase gene
ART	Antiretroviral treatment
bp	Base pair
C4	Complement component 4
CCR5	C-C chemokine receptor type 5
cM	Centimorgan
CNV	Copy number variation
CXCR4	C-X-C chemokine receptor type 4
DEFB	Beta-defensins gene
DEFT	Theta-defensins gene
DNA	Deoxyribonucleic acid
EBV	Epstein Barr Virus
ECACC	European Collection of Cell Culture
Gp41	Glycoprotein 41
Gp120	Glycoprotein 120
HAART	Highly active antiretroviral treatment
HESN	HIV-exposed seronegative
HBD	Human beta-defensins
HD	Human defensins
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HNP	Human neutrophil proteins

HRC	Human Random Control
HSV	Herpes simplex virus
Indel	Insertion and deletion
kb	Kilobase
KIR	Killer cell immunoglobulin-like receptors
LPG	Lysyl-phosphatidylglycerol
LPS	Lipopolysaccharides
LTA	Lipoteichoic acids
M	Mean
mBD	Murine beta-defensins
MHC	Major histocompatibility complex
ml	Milliliter
mM	Millimolar
mRNA	Messenger RNA
NAHR	Nonallelic homologous recombination
NK	Natural killer
ng	Nanogram
nm	Nanometer
p	P- value
PCR	Polymerase chain reaction
PRT	Paralogue ratio test
REPD	Repeat distal
REPP	Repeat proximal
RNA	Ribonucleic acid
rpm	Revolutions per minute
RSV	Respiratory syncytial virus

SD	Standard deviation
SLE	Systemic lupus erythematosus
TA	Teichoic acids
TLR	Toll-like receptor
UPM	Universiti Putra Malaysia
VL	Viral load
µl	Microliter

CHAPTER 1

INTRODUCTION

1.1 Background of study

Human Immunodeficiency Virus (HIV) infection in Malaysia was first reported back in 1986 (Global AIDS Response Progress Report Malaysia, 2015). Since then, a cumulative of 111,916 HIV cases have been reported with 18,827 AIDS-related deaths, giving a total of 93,089 people living with HIV in 2016 (Malaysian AIDS Council, 2016). Moreover, HIV susceptibility and progression has been long observed to vary among individuals. Data published by the Malaysian AIDS Council in 2016 showed a remarkable difference in the ethnic proportion of total HIV cases reported in Malaysia since 1986 (Figure 1.1).

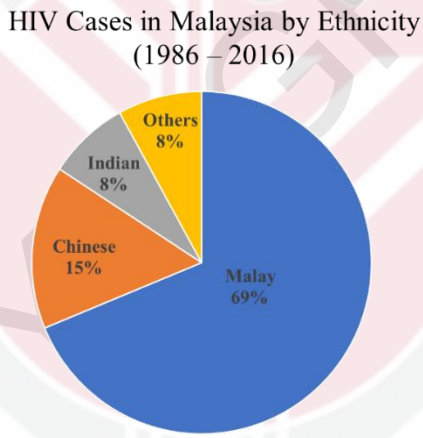

Figure 1.1: Percentage of HIV infections by three major ethnics in Malaysia from 1986 to 2016 (Malaysian AIDS Council, 2016)

The difference in susceptibility of HIV is, in part, due to host genetic variability (Mehlotra et al., 2016). Therefore, it is crucial to investigate how human genetic variation influences the difference in HIV risk and prognosis (Shea et al., 2012).

Copy number variation (CNV) is a type of structural variation. CNV, to put it simply, is the same DNA sequence that present in different numbers of copies between individuals (Hollox & Hoh, 2014). Changes in copy number may potentially affect the gene expression, alter the organization of chromatin, and influence the regulation of neighboring genes (Eichler, 2008). Therefore, CNV may be associated with susceptibility to certain diseases such as autoimmune

disease, cancer, genomic disorders, and more (Smith et al., 2008). One of the most widely studied CNV regions is within chromosome 8p23.1, which locates genes encoding for beta-defensins peptide, *DEFB.*

Beta-defensins are important antimicrobial peptides in the immune system. Aside from their anti-microbial properties, beta-defensins also induce chemoattraction of CD4+ memory T cells and may stimulate its proliferation and survival (Yang, 1999; Machado & Ottolini, 2015). Moreover, beta-defensins has been shown to directly inhibit HIV replication and induce internalization of receptor CXCR4 in CD4+ cells, which is one of the co-receptor used by the virus to infect CD4+ cells (Quin˜ones-Mateu et al., 2003; Sun et al., 2005; Feng et al., 2006).

Considering the importance of beta-defensins in the immune system and as anti-HIV, the copy number variable locus of *DEFB* is a strong candidate in affecting the immune response towards HIV (Hardwick et al., 2012). Therefore, highlighting the importance of quantifying *DEFB* copy number and subsequently, determining its relationship with HIV

1.2 Research questions

DEFB genes are commonly found in 2 to 8 copies per diploid genome, with 4 copies as the modal copy number (Hollox et al., 2003). The distribution of *DEFB* has been reported in various population, including Nigeria, Japan, Germany, and more. (Hollox, 2008). However, there is limited information regarding the *DEFB* copy number of Malaysian populations, especially among HIV patients. Moreover, data of *DEFB* copy number distribution among three major ethnics in Malaysia has yet to be published.

Besides, a few studies have shown a difference in *DEFB* copy number distribution among control and disease groups and thus, may be an important component of susceptibility towards diseases (Hollox et al., 2003; Hollox, Huffmeier, et al., 2008). HIV-positive children were shown to have lower *DEFB* copy number compared to HIV-exposed uninfected children in the Brazilian population (Milanese et al., 2009). Therefore, it is important to identify the difference of *DEFB* copy number pattern among the control group and HIV patients, especially in the Malaysian population, to establish the association between *DEFB* copy number with HIV susceptibility.

Expression of beta-defensins is positively correlated with *DEFB* copy (Hollox et al., 2003; Groth et al., 2010). Given the fact that beta-defensins is antimicrobial, anti- HIV, and able to chemoattract peripheral CD4+ cells (Yang, 1999; Quin˜ones-Mateu et al., 2003; Feng et al., 2006), it is expected that having higher copy number of *DEFB* gene will reduce the susceptibility towards HIV and to

some extent, good prognosis after infected. However, among sub-Saharan African population, researchers found that individuals with high copy number of *DEFB* is associated with higher HIV viral load before Highly active antiretroviral therapy (HAART) initiation (Hardwick et al., 2012). In addition, they also reported that that high *DEFB* copy number is related to low immune reconstitution in HIV patients. Meanwhile, another study by Abujaber et al. (2017) has discovered no association between *DEFB* copy number variation with viral load in European and African HIV patients. These findings were in contrast to the functional studies that suggested an anti-HIV effect of beta-defensins. Also, there is a lack of information regarding the association between *DEFB* copy number with CD4 count and viral load in Malaysia. Therefore, a replicated study should be executed to investigate the association between *DEFB* copy number variation with CD4 cells and the viral load of HIV patients, in another population, particularly among Malaysian HIV-infected patients.

This study will not only establish the *DEFB* copy number variation in Malaysian population but also, may contribute to a further understanding of how genetic variation may influence predisposition and prognosis of HIV. This study may also serve as insight into the identification of new targets for drug or vaccine development for possibly personalized HIV treatment in the future.

1.3 Research Objectives

1.3.1 General objectives

To investigate the relationship of *DEFB* copy number variability with CD4 count and viral load in Malaysian HIV patients.

1.3.2 Specific objectives

1. To determine and compare the copy number of *DEFB* in Malaysian HIV patients (Malay, Chinese, and Indian) and non-HIV controls.
2. To establish the association of *DEFB* copy number with the CD4 count.
3. To identify the relationship between *DEFB* copy number with viral load concentration.

1.4 Hypothesis

The distribution of *DEFB* copy number is predicted to be different among the three major ethnics in Malaysian HIV patients, and also dissimilar between HIV and control group. Variation in *DEFB* copy number is expected to influence the CD4 cell count and viral load level in Malaysian HIV-infected patients.

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