The Impact of Genetic Profile Diversity of *Mycobacterium Tuberculosis* In Tb-HIV Compared to TB Non HIV Patients

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Abstract

Introduction: Tuberculosis (TB) remains as ignificant global health problem,but it is possibly curable when TB is detected and effectively treated. Drug resistant become the hardest challenge to cure this disease. The Multidrug resistance TB is of ten linked to the diversity of Mycobacterium tuberculosis genotype.This study aimed to investigate whether diversity of genetic profile M.tuberculosis influenced the drug resistance.

Methods: A literature search was conducted in several electronic databases. PubMed, Proquest, and Scopus were used to find articles which investigate the association between diversity of MTB genotype and drug resistant. After applying the inclusion and exclusion criteria,there were six eligible articles. However, after restricting more thoroughly, there were only one case control study was used for appraisal.

Results: One case control study identified the impacts of genetic profile diversity and drug resistant was found. It also compared HIV infected and non HIV patients.A total of 158 samples were used. The association between drug resistance mutation and genetic strain background was strongly established (P<0.0001). The most frequent mutations in Switzerland were *poB* (*S531L*) resistance to rifampin and *katG* (*S315T*) which was resistant to isoniazid. The study found that genetic background, especially lineage 2 (Beijing strain) of the M.TB had a high association with drug resistance.This association was stronger formulation drug resistance and in HIV positive patients. (OR19,70;95%CI,1,30to298,19;P=<0,0001).

Conclusion: Current evidence showes there was a clear evidence that the diversity of genetic give an impact to resistant of the drug. It is recommended that future studies in Indonesia is needed to demonstrate consistent effects in clinical settings since Indonesia is an endemic area for Tuberculosis

Keywords: M.Tuberculosis, genome, genotype, genetic Profile, TB-HIV, TB-Natïve, drug resistance

ABSTRAK

Latar Belakang: Tuberkulosis (TB) masih merupakan masalah kesehatan yang signifikan di seluruh dunia. Akan tetapi, penyakit ini dapat ditangani dengan baik apabila TB dapat dideteksi dan ditatalaksana dengan efektif. Resistensi obat menjadi tantangan terbesar dalam menyembuhkan penyakit TB. Resistensi Obat Ganda terhadap TB sering kali dihubungkan dengan keberagaman atau diversitas genotip dari kuman *Mycobacterium tuberculosis*. Telaah kritis ini bertujuan untuk mencari tahu pengaruh dari diversitas profil genetik kuman *Mycobacterium tuberculosis* terhadap Resistensi Obat Ganda.
Metode: Dilakukan pencarian artikel ilmiah pada beberapa database elektronik yaitu PubMed, Proquest serta Scopus dengan menggunakan kata kunci yang sesuai. Setelah dilakukan seleksi dengan memasukkan kriteria inklusi dan eksklusi, terdapat 6 artikel yang lolos seleksi. Namun, setelah melakukan seleksi lebih detail, hanya tersisa 1 studi *case-control* yang akan digunakan untuk penilaian.
Hasil: Terdapat 1 studi *case-control* yang mengidentifikasi dampak dari keberagaman profil genetik *Mycobacterium tuberculosis* dan Resistensi Obat Ganda. Artikel ini juga membandingkan populasi di pasien TB HIV dengan TB Non-HIV. Dari total 158 sampel digunakan pada studi ini, menunjukan adanya hubungan kuat antara Resistensi Obat dan mutasi genetik dengan latar belakang *strain* genetik kuman TB tersebut ($P < 0.0001$). Mutasi yang paling sering terjadi di Switzerland adalah *poB* (*S531L*) resisten terhadap rifampin dan *katG* (*S315T*) resisten terhadap isoniazid. Studi ini menemukan latar belakang genetik, khususnya *lineage* 2 (Beijing strain) dari M.TB mempunyai asosiasi yang kuat terhadap resistensi Obat Anti Tuberkulosis (OAT). Asosiasi ini lebih kuat untuk Resistensi Obat Ganda pada pasien TB dengan HIV positif. (OR19,70; 95% CI, 1,30 to 298,19; p = <0,0001).
Kesimpulan: Bukti terbaru menunjukan adanya bukti yang jelas bahwa keberagaman profil genetik kuman TB memberikan dampak terhadap resistensi OAT. Sangat disarankan untuk melakukan penelitian lanjut di Indonesia untuk mendemonstrasikan efek yang konsisten secara klinis, dikarenakan Indonesia merupakan daerah endemis untuk penyakit Tuberkulosis.
Kata Kunci: M.Tuberculosis genom, genotipe,profil genetik, TB-HIV, TB-Natïve, resistensi obat

Case Illustration

A Male patient, Mr. S, 31 years old, with the chief complaint of chronic coughing since 2 months prior hospital admission. Patient also had night sweat and decrease body weight 6 kg since the last two months. Patient has a risk factor of HIV, he had been using an injected drug and had multiple partner. Patient had been diagnosed with of lung Tuberculosis, meningitis TB and co-infection of HIV. Patient were under treatment of anti-tuberculosis drug but he had stopped the medication in the middle of treatment. Patient had developed drug resistant to antituberculosis drug.

Regarding the incident of drug resistant happened in this patient and during my clinical year, I have met TB patients with drug resistant.Then it triggers my curiosity. The clinical question was raised “Does the diversity of genotyping profile in *Mycobacterium*

Tuberculosis influence the resistance of the drug?"

Clinical Question

Does the diversity of genetic profile of *Mycobacterium* Tuberculosis has an impact on drug resistancy?

Table1–Formulation of Clinical Question

Patient/Problem	Intervention	Comparison	Outcome
Patients with TB HIV	Diversity of TB Genotype profile	Patient TB Non-HIV	Drug Resistant
Type of Question	Etiology		
Study design	Meta-analysis, systematic review, randomized controlled trials, cohort studies		

Introduction

Tuberculosis (TB) is a major global health problem. It causes morbidity among millions of people each year and ranks along side the human immunodeficiency virus (HIV) as a leading cause of death world wide. According to WHO Global Report 2011, it is possibly curable when TB is detected and effectively treated.[1] In 2010 there were 8,8 million people developed TB for the first time, and in 2014 an estimated 1,2 million (12%) of the 9,6 million people who developed TB world wide were HIV-positive.[2] The number of people dying from HIV-associated TB peaked at 570000 in 2004 and had fallen to 390000 in 2014.[2] The effective treatment is the key to reduce the prevalence of the disease. However, the appearance of drug-resistant TB influence the morbidity and mortality rate in this disease.

Drug resistant not only become a problem for the treatment, but also for the control of TB in populations. It has developed into a serious threat to the global TB control plans. Standard methods to diagnose drug resistant TB rely on culture and phenotypic drug susceptibility testing (DST). Multidrug-resistance TB (MDR-TB) is resistant to the two most commonly used drugs (isoniazid and rifampicin) in the common four drug regimen.[3] The Multidrug resistance TB is often linked to the diversity of *Mycobacterium tuberculosis* genotype. Molecular characterization of tuberculosis has revealed the presence of 4 lineages of *Mycobacterium tuberculosis* and 2 of *Mycobacterium africanum*, with differential geographic distribution worldwide.[4] The clinical relevance of this genetic diversity is not known important differences have been found in experimental models, the relevance of different genetic sub-groups of M.tuberculosis to clinical disease in humans remains inconclusive, with potential implications for vaccine development, treatment,and diagnosis.

The aim of this study was to understand the genetic diversity of *Mycobacterium tuberculosis* isolates from HIV-infected and non-HIV patient and its association to drug-resistant. It is expected that this Evidence Case Based Report may contribute and help to estimate which type of *Mycobacterium tuberculosis* genotype appears to have more resistant to anti-tuberculosis drugs.

Methods

The search for literatures was conducted on January 16th, 2016 in PubMed, Cochrane, and Proquest databases by using key words of “tuberculosis” AND “Genome” AND “HIV” AND “Naive” AND“ drug resistance” including their synonyms (Table1). Strategy of the search, consisting of selection criteria and results of each selection step is presented in Figure1. Articles, which did not fulfill the criteria, such as not written in English, not clinical trials or cohort studies or systematic review or meta-analysis, not using adult subjects, would be excluded.

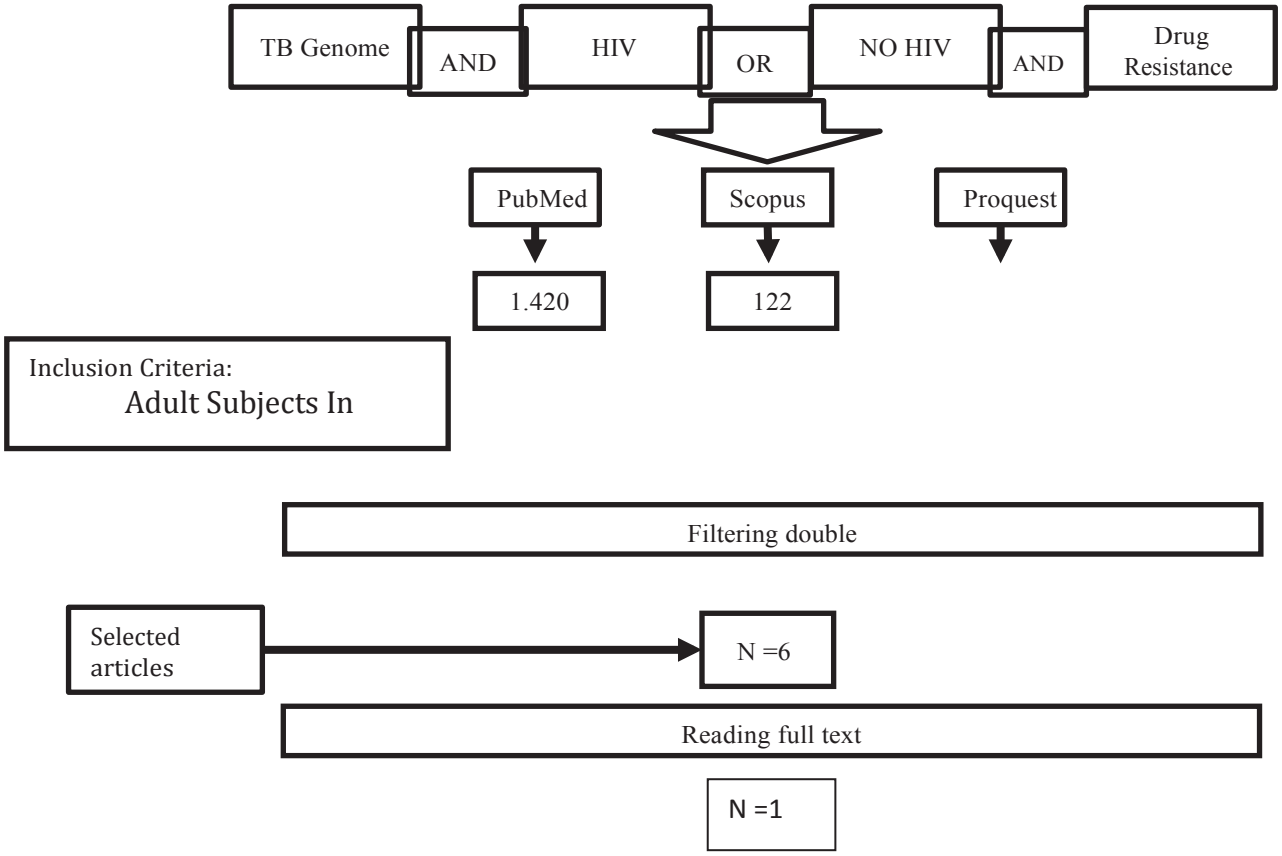
Initial selection was done by reading title and abstract, then exclude the same journal found in different database. There were six eligible articles

but two of them could not be accessed. Four articles were selected, one of them is review article so it must be excluded. Three articles were cohort and case control studies. After reading the whole article, finally, there were two studies that could be included for this present study. Selected article is case control study, which used adult participants in their studies and reported in English. Critical appraisal of the selected studies was performed by consensus of all authors and those were appraised according to adjusted essential criteria from Center of Evidence Medicine, Oxford University.

Table1. Search Strategy on January 16th 2016

Queries	Hits	Relevant Articles
(((TB Genome or (Genotype) OR MTB)) AND (((HIV) OR Non HIV (Naive)) AND (Drug resistance)))	1420	11
(((TB Genome or (Genotype) OR MTB)) AND(((HIV) OR Non HIV (Naive)) AND (Drug resistance)))	42	4
((TBGenotypeAND(((HIV)ORNonHIV(Naive))AND (Drugresistance))	122	0

Figure1.*Flow Chart* Search Strategy



Results

After the selection process there is only one journal which isused to assess the eligibility through the appraisal tools. This study objective was to examine the genetic structure of *M.tuberculosis* and its association between strain variations, patient's geographic origins, and clinical characteristics in HIV infected compared to HIV negative TB Patients.

Table2. Critical Appraisal of Fenner *et al*. Articles Based on Validity, Importance, and Applicability(5)

1. Were there clearly defined groups of patients, similar in all important ways other than exposure to the treatment or other cause?
This paper: Examine the genetic population structure of *M.tuberculosis* and the associations between strain variation, patients'geographic origins, and clinical characteristics in HIV-infected compared to HIV-negative TB patients Comment:The intervention, comparison, and the outcome of interest are expressed clearly.
2.Were treatment exposures and clinical outcomes measured the same ways in both groups (e.g., was the assessment of outcomes either objective (e.g.,death) or blinded to exposure)?
This paper:Yes, it is measured the same ways for both group. Comment:When comparing lineagere presentation among the drug-resistant strains with a group of 353 pansusceptible isolates recovered during the same study.
3.Was the follow-up of study patients complete and long enough?
This paper: No Comment: The study did not use a design that allows for follow up, however the study collects data in patients with already established drug resistance between the year 2000 and 2008.
Is it clear that the exposure preceded the on set of the outcome?
This paper: Yes. Comment: The samples were isolates from TB patient with HIV positive and non-HIV to determine the drug resistance genotypes
Is there a dose-response gradient?
This paper: No Comment: The dose is not needed to produce any effect towards the sample.
Is there positive evidence from a "de challenge-rechallenge" study?
No, the data collection were from patients who have developed TB, so it is unethical to cure the TB and causing the diseases for the purpose of experimental study.
Is the association consistent from study to study?

Yes, the findings were consistent and have significant effects of interactions between the effects of drug resistance-conferring mutations and strain genetic backgrounds on the level of drug resistance.
Does the association make biological sense? Yes it does.
Can the study results be extrapolated to your patient?
Yes, it can be apply in the region where I live. Since TB is one of the endemic burdened in my country.
What are your patient's risks of the adverse outcome?
NNH= $\frac{\text{PEER(OR–1)+1}}{\text{PEER(OR–1)x(1–PEER)}}$
What are your patient's preferences, concerns and expectations from this treatment?
To determine the genetic background of M.Tuberculosis that is more susceptible in developing drug resistance.
What alternative treatments are available?
Using different types of Antituberculosis drugs regimen in patients with TB phenotype that is more susceptible to mutation.

Table3. Importance of the Study Based on Critical Appraisal

		Adverse Outcome		
		Present(Case =Resistant)	**Absent (Control)**	**Totals**
Exposed to the Treatment	**Yes(HIV+)**	**21**	**92**	**113**
	No(HIV-)	**137**	**261**	**398**
		158	**353**	**511**

OR = ad/bc

21x 261 /92 x137 = 5481/ 12.604 =0.43

Fenner et al observed the differences of various genetic profile structure of M. Tuberculosis and whether iti scor related to the drug resistance. There are several aspects measured in this study in order to establish the conclusion of the study. Those aspects included phenotypic

drug resistance, drug resistance-conferring mutations, phenotypic effects of drug resistance-conferring mutations, association between main *M.tuberculosis* line ages and drug resistance,and interaction between drug resistance mutations and strain genetic background. A total of 158 clinical isolates were obtained by the Swiss Molecular Epidemiology of Tuberculosis (SMET) study between 2000 and 2008.

Discussion

The study that discuss strain diversity in *M.tuberculosis* and drug resistance by HIV status is still considered as a novel study. It is proven by the published journal that appears in the search engine are mostly conducted on the last five to ten years. To date, only a limited number of papers have reported in comparing the population of TB HIV patients and Non-HIV patients.

From one study included, it was a case control study. With the main objective is to assess the interactions between the strain genetic background could influence the drug resistance level of isoniazid in *M.tuberculosis*. From the study, it was suggested that there was a clear interaction between drug resistance mutations and strain genetic background. After being tested using logistic regressions with interaction terms between drug resistance mutation and genetic strain background(P<0,0001)

The multidrug-resistance TB (MDR-TB) is the condition where resistant to the two most commonly used drugs, which are isoniazid and rifampicin in the antituberculosis drug regimen. Based on the study finding, the most frequent mutations in Switzerland were *po B*(*S531L*) that was conferring resistance to rifampinand *katG* (*S315T*) which was conferring resistance to isoniazid (10).

This paper also provides data about the association of drug resistance with the main *Mycobacterium tuberculosis* line ages by HIV status. There are 4 lineages, which are Lineage 1, Indo-Oceanic lineage; lineage 2, East-Asian lineage (includes Beijing strains); lineage3, Delhi/CAS; lineage4, Euro-American lineage; other, West African lineages. In the study, genotype background was investigated to determine if the genetic background had a degree of influence towards drug resistance. This study was also investigated inpatient with positive HIV infection. The study found that genetic background, more specifically lineage 2 of the M.tuberculosis had a high association of with drug resistance. This association was stronger formulation drug resistance and in HIV positive patients. (OR, 19,70; 95 % CI,1,30 to 298,19; P = <0,0001). The reason

why Beijing strains are often associated with drug resistance is remain unknown (8,9). Beijing strains could have a higher overall mutation rate, which could lead to an accelerated acquisition of drug resistance mutations. It is most often isolated in East and South east Asia, in countries of the former Soviet Union and in South Africa.

Conclusion and Recommendations

In summary, there is a clear evidence that the diversity of genetic give an impact to resistant of the drug. The kat G mutations were associated with high level drug resistance for isoniazid. The genetic background, especially Lineage2 (includes Beijing strain) of the MTB was associated with drug resistance.

However, more work is needed to demonstrate consistent effects in clinical settings. Because not every person can develop drug resistant. Other contributing factors should be investigated more depth. The strain genetic diversity in MTB is more significantly pronounced than was traditionally believed, because the number of drug resistant is increasing through the time. Moreover, the study regarding genotype background of MTB has implications for the development of new TB diagnostics, drugs and vaccines.

In the future, study type like this will be necessary to make more accurate predictions about the future path of the global epidemics of TB drug resistance. Further more Indonesia is an endemic area for Tuberculosis diseases it may be useful in our country.

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