An Association of *Cryptococcus neoformans/ C. gattii* Genotype and HIV Status in Asia: A Systematic Review

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ABSTRACT

Objective: It has been known that VNI molecular type of *Cryptococcus neoformans/C. gattii* is strongly associated with HIV patients. However, this paradigm has recently been challenged because of the high prevalence of VNI molecular type among non-HIV patients with cryptococcosis in East Asia. The purpose of this study was to answer the question: "Among cryptococcosis in Asia, is there an association between the genotype and the patient's HIV status?"

Methods: Using a systematic review and meta-analysis study design, we included all relevant published data, which were any type of study designs, mainly studied clinical *Cryptococcus neoformans/C. gattii* strains isolated in Asia and had available molecular typing data. The primary study variables were *Cryptococcus neoformans* molecular type (VNI/non-VNI or ST5/non-ST5) and the HIV status of the patients at the time of diagnosis. We used a random-effects meta-analysis model to estimate the prevalence of HIV infection.

Results: Sixteen retrospective descriptive studies during 2005 – 2018 (1,584 isolates) were included. Most of the cryptococcosis cases in East Asian countries were in non-HIV patients (72.4-81.8%), which differed from non-East Asian countries (2.6-28.3% associated with non-HIV patients). In East Asia, the HIV prevalence among VNI and ST5 infected patients ranged from 7.5% - 46.7% with the pooled prevalence of 19.8% (95% CI, 12.2% - 30.4%) and 5.3% - 52.4% with the pooled prevalence of 19.9% (95% CI, 6.9% - 45.3%), respectively. In non-East Asia, the HIV prevalence among VNI and ST5 infected patients ranged from 48.3% - 98.8% with the pooled prevalence of 81.9% (95% CI, 73.3% - 88.2%) and 52.3% - 88.0% with the pooled prevalence of 74.9% (95% CI, 40.7% - 92.8%), respectively. Statistical heterogeneity was high in both analyses with the I^2 of 79-89% in all analyses.

Conclusion: Our results confirmed the low prevalence of HIV prevalence among VNI and ST5 strains in East Asian countries. The emergence of high virulence genotype causing disease in non-HIV patient is highly unlikely, because the VNI and ST5 were associated with HIV patients in other Asian countries. It can be hypothesized that the low HIV prevalence among VNI and ST5 strains in East Asian is due to the high susceptibility to cryptococcosis of people living in this region. This requires further investigation.

Keywords: Cryptococcosis; VNI; *Cryptococcus neoformans*; HIV; Asia (Siriraj Med J 2019;71: 158-164)

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Received 21 August 2018 Revised 12 November 2018 Accepted 4 February 2019
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http://dx.doi.org/10.33192/Smj.2019.24

INTRODUCTION

Cryptococcus neoformans and *C. gattii* play an important role in today's medical practice and have received intensive attention from researchers/clinicians because they cause not only meningitis but also pneumonia in human worldwide.[1] The two species are different in many aspects. This includes environmental niche, molecular epidemiology, pathogenesis and clinical manifestations once they cause the disease, the so-called "cryptococcosis".[1]

It is known that *C. neoformans* is associated mainly with immunosuppression, especially among HIV-infected patients, while *C. gattii* generally causes cryptococcosis in immunocompetent patients.[2] However, some reports have demonstrated contradictory results. Recent studies from China and South Korea showed that most of cryptococcosis patients caused by *C. neoformans* were HIV-negative patients without other immunocompromised conditions.[3,4] Especially in China, cryptococcosis frequently occurred in immunocompetent patients.[4,5]

C. neoformans is composed of 4 major molecular types, VNI, VNII, VNIII, and VNIV and *C. gattii* is composed of 4 major molecular types, VGI, VGII, VGIII and VGIV.[6] The VNI-molecular type of *C. neoformans* is the most common cryptococcal molecular type and typically associated with HIV patients.[7] However, VNI in China was associated with non-HIV patients.[4] Further molecular typing among the VNI strains by the Multi Locus Sequence Typing (MLST)[8] revealed the non-HIV-associated VNI strains was almost exclusively belonged to the sequence type 5 (ST5) genotype.[4] This phenomenon has also been found in Korea[3] and Japan[9] Therefore, the association of this VNI-ST5 genotype and non-HIV status may be possible. However, although Thais are a close sibling of Chinese, there was no association between VNI-ST5 and non-HIV status.[10] The East Asian race is highly susceptible to VNI-ST5 or is a high virulent genotype capable of causing infection in immunocompetent individuals.

A search of the published data revealed no evidence of any randomized trials or systematic reviews addressing the association of the VNI-ST5 and HIV status in Asian patients with cryptococcosis. The purpose of the present report was to complete a systematic review and meta-analysis to answer the following research question: "Among cryptococcosis in Asia, is there an association between the genotype and the patient's HIV status?" We hypothesized that such association is limited only to patients in East Asia – China and neighboring. Our specific aims were to perform a systematic review of the published data and identify studies for analysis and to execute a meta-analysis to determine whether the VNI-ST5 causes diseases mainly in non-HIV patients across all Asian countries.

MATERIALS AND METHODS

Study design

To address our research question, we designed and implemented a systematic literature review and meta-analysis.

Samples

Our review of the published data was designed and conducted in concordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.[11] Three authors (P. N., P. K. and K. K.) searched the published studies from January 1999 (The year that cryptococcal molecular typing system was firstly established[12]) to May 2018, using the National Library of Medicine (PubMed, available from http://www.pubmed.gov), the Web of Science (available from https://webofknowledge.com), and the Cochrane database (available from http://www.cochranelibrary.com/) using specific medical subject headings and key words, including (Cryptococ*) AND (molecular typ* OR molecular epidemiology). Because each *Cryptococcus* species comprises 4 major molecular types: *C. neoformans*, VNI (AFLP1), VNII (AFLP1A of AFLP1B), VNIII (AFLP3) and VNIV (AFLP2); and *C. gattii*, VGI (AFLP4), VGII (AFLP6), VGIII (AFLP5) and VGIV (AFLP7) (REF), we also used the specific terms for all major molecular types (VNI OR VNII OR VNIII OR VNIV OR VGI OR VGII OR VGIII OR VGIV OR AFLP1 OR AFLP1A OR AFLP1B OR AFLP2 OR AFLP3 OR AFLP4 OR AFLP6 OR AFLP5 OR AFLP7) AND (Cryptococ*) to identified all publications investigating cryptococcal molecular typing. Citations in the accessed articles were further analyzed for additional published data.

In addition, the available post-meeting online materials of relevant meetings including American Society for Microbiology (ASM) 2010-2015, 48th-55th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), ASM microbe 2016-2017, International Society for Human & Animal Mycology (ISHAM) 2000-2015 and International Conference on *Cryptococcus* and Cryptococcosis (ICCC) 4th-10th were searched for additional eligible studies.

Study inclusion and exclusion criteria

Studies were included if they were mainly studied Asian isolates, provided data on statistics, patient's HIV status and major molecular type data. Searching methodology was mentioned above. We excluded studies that, selectively included only one type of immune status of patients or genotype of the strains, were case-reports or case-series, did not identify the correlation between HIV status with individual isolates or contained 100% duplicated cryptococcal isolates.

Study variables

The primary predictor variable was the genotype (i.e., major molecular type or sequence type). The main outcome variable was the patient's HIV status. The primary analysis of interest was the prevalence of the patient's HIV positivity across study groups (i.e., VNI and ST5 in East Asia [China, Japan, Taiwan, and Korea] and Non-East Asia [Thailand, Malaysia, Singapore, Vietnam, Indonesia, India, Kuwait, and Qatar] countries). The other study variables were classified as demographic, anatomic, environmental and genetic. The demographic data were total sample size, number of subjects in both genotype groups, and mean patient's age. The anatomic location of infection, environmental niche and genetic profiles were also recorded.

Data extraction and statistical analysis

Using a standardized data extraction form, two authors (P. Ng. and K. K.) extracted and tabulated all data. Discrepancies were resolved by group discussion of the investigator authors. As standard quality assessment of descriptive study for meta-analysis was not available, a quality assessment for case-control studies was used according to a previous published method.[13] A quality scale for observational study was used. A narrative overview is provided summarizing the data gathering from included published data. The pooled prevalence of HIV positivity and 95% CI for ST5 and VNI were calculated using DerSimonian-Laird random-effect model with double arcsine transformation.[14] The pooled prevalence and 95% CI were calculated for each region (East Asia and non-East Asia). Random-effect model, rather than fixed-effect, was used because of the high likelihood of between-study heterogeneity. Cochran's Q test and I^2 statistic were used to determine the between-study heterogeneity. This I^2 statistic quantifies the proportion of total variation across studies that is due to heterogeneity rather than chance. A value of I^2 of 0% to 25% represents insignificant heterogeneity, greater than 25% but less than or equal to 50% represents low heterogeneity, greater than 50% but less than or equal to 75% represents moderate heterogeneity, and greater than 75% represents high heterogeneity.[15] The software Comprehensive meta-analysis (Englewood, NJ, USA) and IBM SPSS statistics 18.0 (IBM (Thailand), Bangkok, Thailand) were used for statistical analysis.

Research ethics

The recommendations of the Helsinki declaration were thoroughly maintained during this study. Since this study did not involve human subjects or records directly, ethical approval by an ethics committee and consent from the authors of the articles studied were not required.

RESULTS

Search results

The literature searches initially yielded 1,630 potentially relevant studies; titles and abstracts were screened, and full papers were obtained for relevant articles (Fig 1). A total of 36 full-text articles were reviewed, of which 20 were excluded for various reasons. The remaining 16 studies were finally included in this meta-analysis and yielded a total of 1,584 isolates.[3-5,7,10,16-26] There was no additional published data from citations in the accessed articles.

Characteristics and quality of included studies

The characteristics of the 16 included studies are shown in Table 1. All were retrospective descriptive studies. Quality of the included studies was scored 4.5 to 8.5 out of 14.5. All included studies reported the data on HIV status in at least some of the isolates but the availability of this data varied considerably across the studies, ranging from 13.5% to 100% (median 80.2%). Only strains with HIV status data were used in this current analysis. Only 7 studies had information of ST5 genotype with HIV data (Table 1).

Prevalence of HIV and genotype across all studies of cryptococcosis

Cryptococcosis in Asia was not common in non-HIV patients (2.6-28.3%), except in East Asia (72.4-81.8%) and Northwest Asia (60-70%). Interestingly, VNI molecular type was predominant across all Asian countries, regardless of HIV status of the patients. Among the VNI strains, ST5 was more prevalent than non-ST5 only in East Asia. The results were summarized in Fig 2.

HIV prevalence in East Asian countries

The HIV prevalence among VNI-infected cryptococcosis patients from the included 8 studies ranged from 7.5% - 46.7% with the pooled prevalence of 19.8% (95% CI, 12.2% - 30.4%) (Fig 3). The HIV prevalence among ST5 infected patients from the 5 studies ranged from 5.3% - 52.4% with the pooled prevalence of 19.9% (95% CI, 6.9% - 45.3%) (Fig 4). Statistical heterogeneity was high in both analyses with the I^2 of 89% in both analyses.

HIV prevalence in non-East Asian countries

The HIV prevalence among VNI-infected cryptococcosis patients from the 9 studies ranged from 48.3% - 98.8% with the pooled prevalence of 81.9% (95% CI, 73.3% -

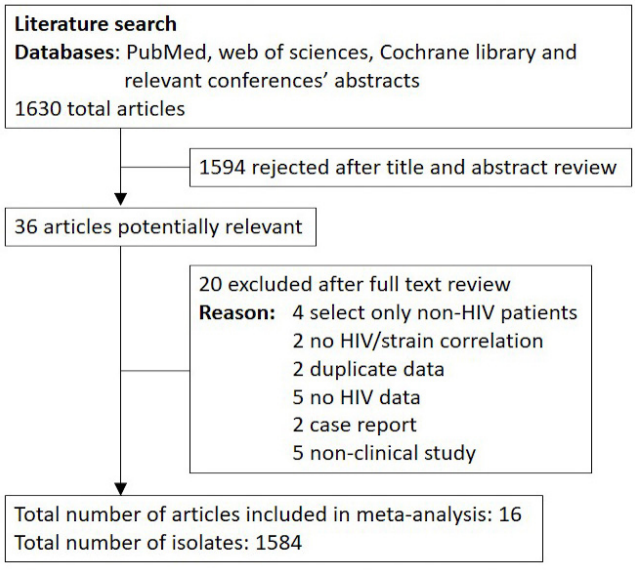

Fig 1. Study selection process.

88.2%) (Fig 3). The HIV prevalence among ST5-infected patients from the 3 studies ranged from 52.3% - 88.0% with the pooled prevalence of 74.9% (95% CI, 40.7% - 92.8%) (Fig 4). Statistical heterogeneity was high in both analyses with the I^2 of 79% in both analyses.

DISCUSSION

The study's principal aim was to identify the particular association between the cryptococcosis genotypes VNI/ST5 and the non-HIV status of patients in Asia. We hypothesized that this association would be seen only in East Asia countries. Our specific aims were to conduct a systematic review and meta-analysis to identify the relationship between the VNI/ST5 strain and the patient's HIV status.

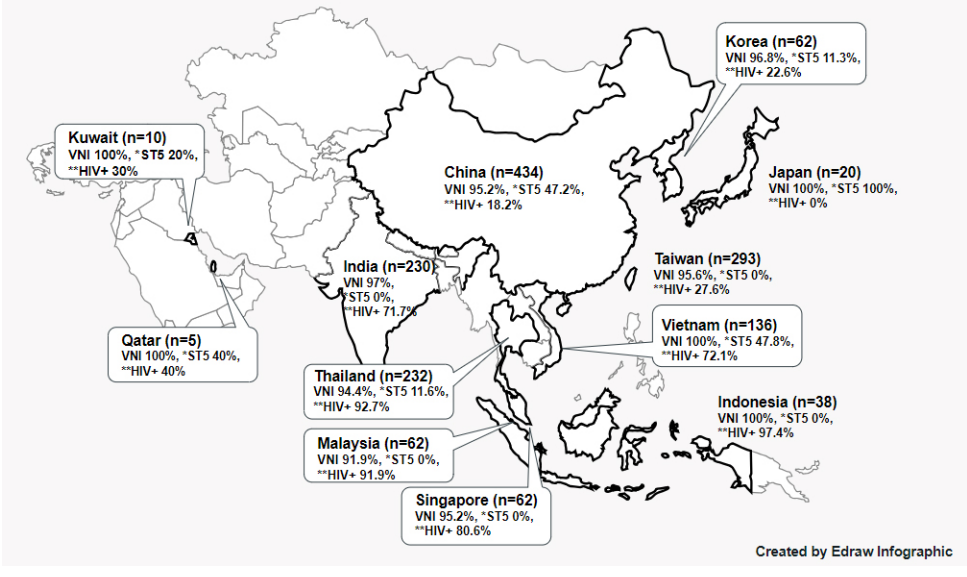

Fig 2. Summary of the collected data; n=a total number of strains with the major molecular type information (*Availability of the ST data was varied: China = 234, Korea = 13, Japan = 20, Taiwan = 0, Thailand = 195, Malaysia = 0, Singapore = 0, Vietnam = 136, Indonesia = 38, India = 58, Kuwait = 10, and Qatar = 5; **Availability of HIV data was varied: China = 349, Korea = 59, Japan = 20, Taiwan = 293, Thailand = 87, Malaysia = 62, Singapore = 62, Vietnam = 136, Indonesia = 38, India = 172, Kuwait = 10, and Qatar = 5).

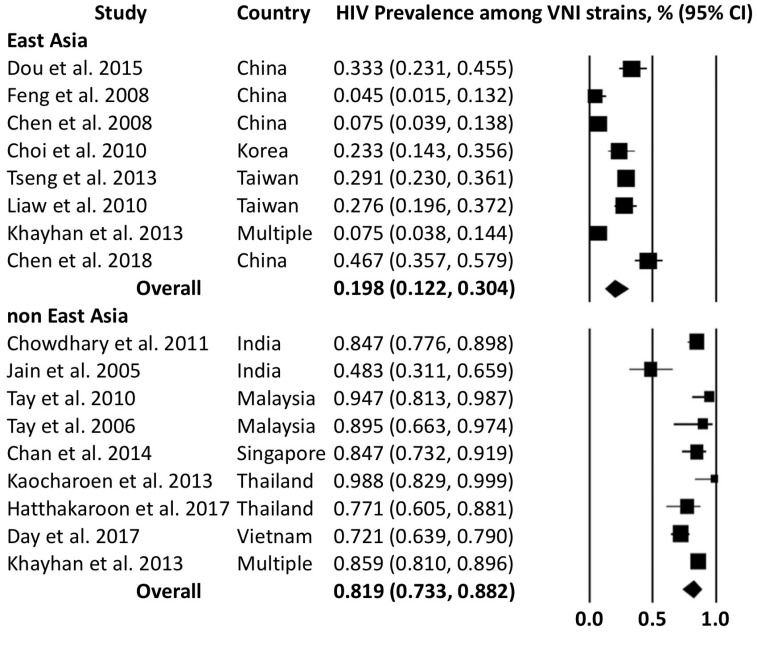

Fig 3. Meta-analysis of HIV prevalence in VNI infected patients. Khayhan *et al.* contained data of China and Japan for East Asia, and India, Indonesia, Kuwait, Qatar and Thailand for non-East Asia.

TABLE 1. Characteristics of the included studies.

Study	Country of origin	No. of isolates	Study design	Years	Study center types	HIV data coverage (%)	HIV prevalence (%)	Primary typing method
Dou *et al*. 2015*	China	83	Descriptive, retrospective	2007-2013	Multicenter	80.7	32.8	MLST
Feng *et al*. 2008	China	115	Descriptive, retrospective	1976-2007	Multicenter	67.8	3.8	M13
Chen *et al*. 2008*	China	129	Descriptive, retrospective	1980-2006	Multicenter	100.0	8.6	M13
Chowdhary *et al*. 2011	India	160	Descriptive, retrospective	2002-2009	Multicenter	85.6	84.7	M13
Jain *et al*. 2005	India	57	Descriptive, retrospective	before 2005	Single center	61.4	40	URA5
Choi *et al*. 2010*	Korea	78	Descriptive, retrospective	1990-2008	Multicenter	79.5	22.6	M13
Tay *et al*. 2010	Malaysia	96	Descriptive, retrospective	2003-2004	Multicenter	39.6	94.7	URA5
Tay *et al*. 2006	Malaysia	78	Descriptive, retrospective	1980-2003	Multicenter	30.8	87.5	URA5
Chan *et al*. 2014	Singapore	62	Descriptive, retrospective	1999-2007	Single center	100.0	80.6	URA5
Tseng *et al*. 2013	Taiwan	219	Descriptive, retrospective	1997-2010	Multicenter	89.0	27.7	M13
Liaw *et al*. 2010	Taiwan	100	Descriptive, retrospective	1994-2004	Single center	98.0	27.6	M13
Kaocharoen *et al*. 2013	Thailand	386	Descriptive, retrospective	1993-2005	Multicenter	13.5	88.5	M13
Hatthakaroon *et al*. 2017*	Thailand	51	Descriptive, retrospective	2012-2014	Single center	68.6	77.1	MLST
Day *et al*. 2017*	Vietnam	151	Descriptive, retrospective	1996-2010	Single center	90.1	72.1	MLST
Khayhan *et al*. 2013*	Multiple**	476	Descriptive, retrospective	1983-2009	Multicenter	76.1	62.7	MLST
Chen *et al*. 2018*	China	86	Descriptive, retrospective	2016-2017	Single center	87.2	46.7	MLST

*ST5/HIV data available, **China, India, Indonesia, Japan, Kuwait, Qatar, Thailand

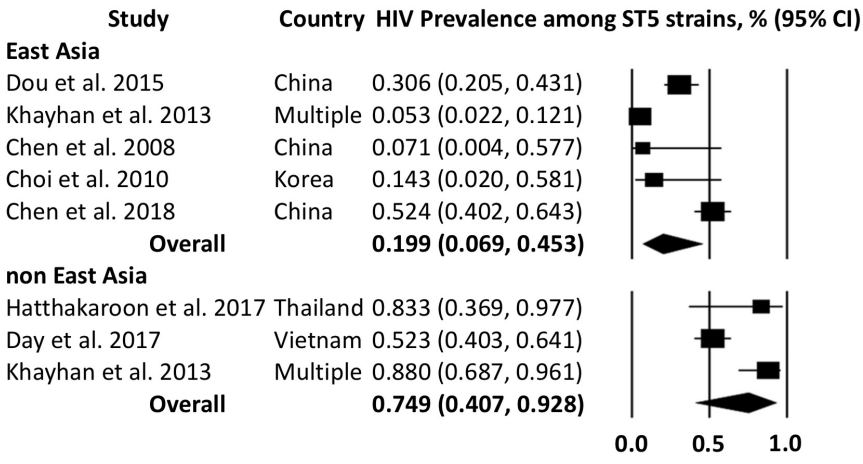

Fig 4. Meta-analysis of HIV prevalence in ST5 infected patients. Khayhan *et al.* contained data of China for East Asia, and Qatar and Thailand for non-East Asia.

Cryptococcosis, especially from *C. neoformans* VNI molecular type, has been found to be linked to HIV infection since the HIV pandemic in 1980s.[1,6] Our study confirmed this association. The pooled HIV prevalence among non-East Asia patients with VNI infection was >80%. However, contradictory observations from China and other East Asian countries have been reported since 2008.[3,4,21] Our analysis included the results of those studies and demonstrated a strong link between the VNI molecular type and non-HIV patients in this region with the low pooled prevalence of HIV among VNI-infected cryptococcosis patients (only 19.8%). Although the exact mechanisms behind this observation are not known, few possible explanations include the highly virulent VNI strain of *C. neoformans* and/or the high susceptibility of individuals living in those areas.

The MLST has been introduced and used to study the association between the VNI molecular type and non-HIV patient's status.[8] This technique provides more genotypic details of microorganisms. With this particular technique, ST5 was found to be highly prevalent (86.9%) among the VNI molecular type strains in East Asia.[4,5] This might be implied that ST5 is a highly virulent genotype among VNI molecular type strains and specifically infects non-HIV individuals. In fact, our analysis demonstrated the similar finding that the pooled HIV prevalence among ST5 strains was low in East Asia (19.9%). High pooled HIV prevalence was found in other Asia countries only (74.9%). As one would expect that if the ST5 strains is high virulent, it should be able to infect non-HIV individual regardless of their countries. Therefore, the assumption about the highly virulent genotype causing infection in non-HIV individuals should be omitted.

Another assumption is that people in East Asia might be susceptible to cryptococcosis more than those in other Asian region. A recent environmental study in China revealed that ST5 prevalence in nature was low (6.2%); thereby, one must be highly susceptible to the causal microorganism to be infected. In contrast, ST31 was commonly found in China (66.7%)[27], but rarely caused any diseases (only 1.3% among cryptococcosis in East Asian countries, data not shown). However, it is still unclear whether this high susceptibility is due to genetic background or other factors. Molecular epidemiological studies focused on patient's genetics are still warrant.

Our study suffers from many limitations. First, not all published data provided underlying diseases other than HIV. The non-HIV patients may be immunocompromised because of other conditions. This can be considered as the common pitfall and limitation in evidence-based medicine[28] and may help to explain the extraordinary relationship between the non-HIV patient and cryptococcosis in East Asia. Second, high statistical heterogeneity in this study may arise from many factors, such as difference in methodology and quality of study. Lastly, all of the included studies were descriptive. The overall numbers of isolates/patients in some countries were relatively small and may not be representative to the whole nation of the country. Taken all together, the results of this study should be interpreted with caution.

CONCLUSION

Our results revealed the low HIV prevalence in cryptococcosis patients with VNI and ST5 strains in East Asia. The emergence of high virulence genotype causing disease in non-HIV patient seems to be impossible, because the VNI and ST5 strains were mostly associated with HIV patients in other Asian regions. Therefore, the high

susceptibility to cryptococcosis of people living in East Asia may be caused by the low HIV prevalence among VNI and ST5 strains in this geographic area. However, mechanism of this possibly high susceptibility requires further investigations.

Disclosure of potential conflicts of interest

The authors indicate full freedom of manuscript preparation and no potential conflicts of interest.

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