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Use of Multicopy and Single Copy Genes for Detection of *Pneumocystis jirovecii* and Subsequent Analysis of Genotypes of Indian Isolates

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Background: *Pneumocystis carinii* Pneumonia (PCP) caused by an opportunistic agent *Pneumocystis jirovecii* is one of the most serious respiratory infections in immunocompromised patients including HIV infected individuals. Enormous gaps still exist in understanding the basic biology of the organism and epidemiology of PCP due to non-availability of an appropriate propagation system. In the present study, we carried out molecular detection of the organism using two multicopy and one single copy gene. Further genotypic study was performed on some of the isolates to assess the genotypes.

Methods: From November 2005 to October 2006, 114 different respiratory specimens were collected from 90 patients with various underlying conditions and a clinical suspicion of PCP. PCR was done for three independent gene loci including two multicopy i.e. Major Surface Glycoprotein (MSG) and mitochondrial Large subunit rRNA (mtLSUrRNA) and one single copy, internal transcribed spacer (ITS) region

Results: Single round MSG and mtLSUrRNA PCR showed 9 positive isolates. External round of ITS PCR detected 6 cases while only nested round could detect all 9 cases. On applying nested PCR for mtLSUrRNA and ITS PCR we detected 5 additional cases. We further sequenced six isolates for mtLSUrRNA gene. Different genotypes of *Pneumocystis* were distinguished by identifying polymorphisms at position 85 and 248 of mtLSUrRNA. Two genotypes i.e. type 2 (5/6) and 3, (1/6) were observed among the six isolates. Also, one of our previous isolate showed genotype 1.

Conclusion: Thus, among multicopy genes, MSG and mtLSUrRNA appears to be equally sensitive while in case of single copy ITS a nested PCR assay is required. In our present study, genotype 2 was more frequently detected (5/7), followed by type 3 and 1(1/7) and a prospective study with large number of patients is being carried out in our centre to address this issue. To our knowledge this is the first study from South Asia describing genotypes of isolates of *P. jirovecii*.

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Hearing Loss in HIV Positive Patients

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Background: Hearing loss in HIV positive patients have been reported. However, these reports were hearing loss caused by sudden hearing loss, Ramsay Hunt syndrome, and so on. We investigated hearing loss in HIV positive patients

to know hearing loss possibility other than sudden hearing loss, Ramsay Hunt syndrome, and so on.

Methods: One hundred fifty seven patients were enrolled in this study. They consulted our hospital from April in 2006 to March in 2008. Some patients suffered from sensorineural hearing loss caused by sudden deafness, Ramsay-Hunt syndrome and so on. This study also includes patients which do not complaint of hearing loss. Usual otolaryngological examination and blood tests were examined. Their ear membranes, nasal cavity and oral cavity were checked up. Pure-tone audiometry was examined in all patients. Electronystagmogram including caloric testing were done in some patients suffered from hearing loss. In addition, CD4 T-cell counts and RNA copy numbers were investigated by blood test

Results: Patients suffered from sudden deafness, Ramsay-Hunt syndrome or Meniere's disease revealed ipsilateral sensorineural hearing loss. Some patients other than these patients showed bilateral sensorineural hearing loss. Usually their high-tone hearing levels were deteriorated. We investigated the relationship between hearing levels and CD4 T-cell counts. The hearing loss was more severe in patients with low CD4 T-cell counts.

Conclusion: Severe HIV positive patients more often suffered from sensorineural hearing loss.

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HIV-TB Coinfection: The South Australian Experience

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Tuberculosis (TB) is the most common opportunistic infection in Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS). There is an estimated 11.5 million people co-infected with HIV/TB with the highest burden in Sub-Saharan Africa. The accessibility of world travel, the displacement of communities because of conflict, war and famine as well as changes to Australian Government's immigration policies have meant that Australia is not immune to this world trend. Three subjects that were HIV positive and had suspected or confirmed *Mycobacterium tuberculosis* disease on the South Australian Tuberculosis Service database were retrospectively studied. The diagnosis of TB was made after the diagnosis of HIV in all three cases. Two had typical x-ray findings consistent with TB. The possible place of acquisition differed between all three. One was treated for TB prior to HIV, the remaining had HIV treatment commenced prior to TB treatment. All three had similar triple therapy for HIV. Two had confirmed *M. tuberculosis* on culture with one showing partial resistance to isoniazid. All three were commenced on standard quadruple therapy, moxifloxacin and streptomycin was added until drug susceptibility confirmation for the third. Treatment was halted temporarily in two due to complications arising from

HIV/TB therapy. The duration of therapy ranged from six months to two years. Each of the three subjects highlighted the difficulties to adequately manage the HIV/TB patient and the infrastructure needed to support this level of care. With the HIV/AIDS pandemic threatening to destabilize control of TB we should be supporting initiatives to improve TB and HIV programs in resource poor settings with a focus on preventive measures.

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Integration of Routine HIV Testing with TB Diagnosis Among Medical Inpatients in Mulago Hospital, Uganda

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Background: TB is the most common and serious HIV related complication. It is estimated that about half of TB cases in Uganda are co-infected with HIV and about 30% of the HIV-related deaths are attributed to TB. However, HIV and TB diagnosis programs are not traditionally integrated. The recently launched national policy guidelines for TB/HIV collaborative activities are aimed at integrat-

ing diagnosis, care and treatment for TB/HIV co-infected patients. The Mulago-Mbarara Teaching Hospitals' Joint AIDS Program (MJAP) has been providing integrated routine HIV testing and counselling, and TB screening for medical inpatients in Mulago hospital since March 2005.

Method: All patients on medical wards in Mulago are routinely offered HIV testing and counselling. Patients presenting with a history of cough for more than three weeks are also screened for TB by sputum microscopy. Patients with HIV infection are started on cotrimoxazole prophylaxis, those who are co-infected with TB and HIV are referred to the TB-HIV clinic where they receive both TB and HIV care and treatment. We analysed data for 379 patients to assess the number of new TB and HIV infections identified.

Result: Among the 379 patients, 200 (53%) did not know their HIV/TB status on admission, and received both HIV testing and TB screening. Of these, 24 (11%) were co-infected with TB and HIV, 70 (35%) had HIV infection alone while 22 (11%) had TB infection alone. Out of the 159 patients who were known to be HIV-infected on admission, 40 (25%) had TB infection. Overall, 64 out of the 379 patients (17%) were co-infected with TB and HIV. Among the 253 HIV infected patients 64 (25%) had smear positive TB.

Conclusion: Integration of TB and HIV diagnosis is feasible and provides an opportunity for appropriate linkage to care for TB-HIV co-infected patients.

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