Original Article

CLINICAL ANALYSIS ON TUBERCULOSIS CASES AMONG FOREIGNERS IN OUR HOSPITAL

[1]Manabu SUZUKI, [1]Masaaki HOJO, [1]Nobuyuki KOBAYASHI, [1]Yuka SHINOHARA, [1]Jin TAKASAKI, [1]Atsuto YOSHIZAWA, [1]Haruhito SUGIYAMA, [1]Koichiro KUDO, and [2]Emiko TOYOTA

Abstract [Background] Although the incidence rate in Japan has been decreasing since the declaration of tuberculosis emergency in 1999, the reported tuberculosis cases among foreigners have been increasing year by year (from 5.1% in 2000 to 6% in 2003). As the number of foreign residents in Japan has been increasing every year, tuberculosis cases among them are also expected to increase.

[Purpose] The aim of this study is to investigate and clarify clinical features of recent tuberculosis patients among foreigners.

[Object] Fifty-two cases were analyzed, who were admitted to our hospital because of active tuberculosis from January 2004 to April 2007.

[Results] Among total 52 cases, male was 29, female 23, and the mean age (SD) of the patients was 31.8 (±8.8) years old. Their mother countries were China, Republic of Korea and so on. The cavitary lesions were found on chest X-ray in 54%, the drug resistant rate was 8.2%, and the treatment completion rate was 92%.

[Discussion & conclusion] Comparing with reports in the past, almost parameters about tuberculosis control have improved, for example the drug resistant rate was decreased and the treatment completion rate was increased. The promotion of DOTS strategy in Japan might be attributed to the improvement of these parameters. Because more immigrants from the developing countries are expected in near future, not only strengthening current DOTS strategy but also new countermeasures such as QFT-2G and Electronic-Nose Technology should be introduced into tuberculosis control of foreigners living in Japan to decrease tuberculosis incidence and improve treatment outcome by early detection and adherence to treatment.

Key words: Foreigner tuberculosis, Age of onset, Cavitary lesion, Resistance to drugs, Treatment completion rate

[1]Department of Respiratory Medicine, International Medical Center of Japan, [2]Department of Respiratory Medicine, National Hospital Organization Tokyo National Hospital

Correspondence to: Manabu Suzuki, Department of Respiratory Medicine, International Medical Center of Japan, 1−21−1, Toyama, Shinjuku-ku, Tokyo 162−8655 Japan.
(E-mail: manabu-s@umin.ac.jp)

Original Article

TREATMENT OUTCOME OF PATIENTS WITH PULMONARY TUBERCULOSIS BEFORE AND AFTER THE INDUCTION OF DIRECTLY OBSERVED THERAPY (DOT)

Kazuko MATSUNAGA, Nobuhiko NAGATA, Yasuhiro IWATA, Hiroyuki KUMAZOE, Masashi KOMORI, Kentarou WAKAMATSU, Akira KAJIKI, Yoshinari KITAHARA

Abstract [Purpose] To evaluate the treatment outcome of patients with pulmonary tuberculosis before and after the induction of DOT.

[Methods] A retrospective study of the outcome of 239 tuberculosis patients treated during January 1996 to June 2003. We reviewed clinical charts collected on all patients with positive cultures for *Mycobacterium tuberculosis*. The patients of non-DOT group had received a traditional unsupervised drug regimen, before we have undertaken DOT. The patients of DOT group received therapy under direct observation by nurses. We compared sputum smear conversion period, sputum culture conversion period, duration of admission and treatment, recurrence rate, treatment success (cure and completion of treatment) rate and incidence of adverse effects between DOT and non-DOT group.

[Results] Sputum conversion period and incidence of adverse effects were not significantly different between both groups. The duration of admission and treatment of DOT group was significantly shorter than those of non-DOT group. Rate of relapse and treatment success was not different between two groups. Over-80-year-old patients treated by DOT revealed a higher culture conversion rate after 2-month treatment than those who were not treated with DOT.

[Conclusion] We could not prove the usefulness of DOT during hospitalization for tuberculosis patients from the points of treatment success rate and relapse rate.

Key words: DOT in hospital, Pulmonary tuberculosis, DOTS (Directly Observed Therapy, Short course)

Department of Respiratory Medicine, National Hospital Organization Omuta National Hospital

Correspondence to: Kazuko Matsunaga, Department of Respiratory Medicine, National Hospital Organization Omuta National Hospital, 1044−1, Tachibana, Omuta-shi, Fukuoka 837−0911 Japan. (E-mail: matsunaga-k@oomuta-h.com)

Original Article

RAPID, SIMPLE GENOTYPING METHOD BY THE VARIABLE NUMBERS OF TANDEM REPEATS (VNTR) FOR *MYCOBACTERIUM TUBERCULOSIS* ISOLATES IN JAPAN
— Analytical Procedure of JATA (12) -VNTR —

Shinji MAEDA, Yoshiro MURASE, Satoshi MITARAI, Isamu SUGAWARA, and Seiya KATO

Abstract [Purpose] The discriminatory power of each locus in variable numbers of tandem repeats (VNTR) analyses was evaluated for development of the genotyping method of *Mycobacterium tuberculosis* (TB) in Japan.

[Method] By using 325 TB strains collected from whole Japan and 24 mass infection cases (74 isolates), IS*6110* restriction fragment length polymorphism (RFLP), spoligotyping and VNTR (35 loci) were analyzed.

[Results and discussion] We excluded 4 loci (VNTRs 2163a, 3232, 3820, and 4120) and selected in top 12 loci (VNTRs 0424, 0960, 1955, 2074, 2163b, 2372, 2996, 3155, 3192, 3336, 4052, and 4156). The cluster rate of IS*6110* RFLP was higher than that of 12-locus [Japan Anti-Tuberculosis Association (JATA)] VNTR. And in comparison of the discriminatory power of 12-locus JATA VNTR and that of Supply (15)-VNTR, the JATA (12)-VNTR was superior, even though less loci analyses. Therefore, this JATA (12)-VNTR could be used for TB genotyping in areas where Beijing strains are prevalent.

Key words: Tuberculosis, Molecular epidemiology, RFLP, VNTR, Beijing genotype

Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association

Correspondence to : Shinji Maeda, Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, 3−1−24, Matsuyama, Kiyose-shi, Tokyo 204−8533 Japan.
(E-mail: maeda@jata.or.jp)

The 83rd Annual Meeting Symposium

THE FRONTLINE OF CLINICAL MYCOBACTERIOLOGY

Chairpersons: [1]Arisu KAMADA and [2]Satoshi MITARAI

Abstract The symposium was designed to review the mycobacterial infections from the viewpoint of clinical practices, and was composed of five presentations covering both basic and clinical aspects.

Firstly, Dr. Kawamura explained the topic of pathological interrelation of *M. tuberculosis* and host cell, for the pathogen to survive in the host. The bacterium inhibits the fusion of phagosomes with lysosomes through secretion of some bacterial components and modulation of host cell intracellular signaling pathways. Dr. Okusu showed the usefulness of rapid mycobacterium identification system by the detection of some house-keeping gene by nucleic amplification method. Dr. Nakasone explained the current methodologies of drug susceptibility testing for *M. tuberculosis* and clarified the different meaning of proportion method and ordinary susceptibility testing for general bacteria. He also emphasized the importance of clonality of tested mycobacteria for the correct interpretation of test result. Therapeutic drug monitoring will be clinically important considering the appropriate and individual treatment of *M. tuberculosis* infection. In Dr. Hanada's presentation, the effect of dosage regimen of pyrazinamide on the efficacy and adverse events, and clinical significance of therapeutic drug monitoring and pharmacogenomics were discussed. Intervention of clinical pharmacology concept should be applied to pharmacotherapy of tuberculosis in order to confirm drug compliance, adjust dosage regimen in each individuals, and avoid adverse drug reactions. Finally, Dr. Seki described the detail of genetic differences among BCG sub-strains and emphasized the importance of their differentiation and confirmation of genetic stability as vaccine. He also suggested that early BCG vaccines might even be superior to the later ones.

The contents of five presentations are basically independent, but they are related to each other in the clinical practices. The symposium may provide comprehensive idea to understand the current and futue clinical strategy against tuberculosis.

1. Pharmacotherapy of tuberculosis and therapeutic drug monitoring: Kazuhiko HANADA, Hiroyasu OGATA (Department of Biopharmaceutics and Clinical Pharmacokinetics, Meiji Pharmaceutical University)

Selection of appropriate drugs, adjustment of dose and dosage regimen, and completion of their regimen without discontinuation are very important for a successful tuberculosis pharmacotherapy. In general, efficacy and adverse reactions of drugs were determined by both pharmacokinetic (PK) and pharmacodynamic (PD) factors. It is possible to determine rational individual dosing regimen by charactering the inter- and intra-patient variation of PK/PD relationship. Intervention of clinical pharmacology concept should be applied to pharmacotherapy of tuberculosis in order to confirm drug compliance, adjust dosage regimen in each individuals, and avoid adverse drug reactions. In this presentation, effect of dosage regimen of pyrazinamide on the efficacy and adverse events, and clinical significance of therapeutic drug monitoring and pharmacogenomics were discussed.

2. Molecular diagnostics in mycobacterial infections: Kiyofumi OHKUSU, Takayuki EZAKI (Department of Microbiology, Gifu University Graduate School of Medicine)

In recent years, molecular microbiology techniques have been proven to be useful supplement to conventional assays not only to characterize organisms from culture, but also to directly detect pathogens from clinical samples. PCR-based sequencing has become the gold standard for identification of mycobacterial species. The target most commonly used is the gene coding for the 16S rRNA. Although 16S rRNA gene sequences may be employed successfully to identify many mycobacterial species, they lack sufficient discrimination to differentiate certain isolates from some species (e.g., *M. kansasii* and *M. gastri*, *M. marinum* and *M. ulcerans*). In these circumstances, sequence analysis of housekeeping genes such as *rpoB*, *hsp65*, and *dnaJ*, in addition to the 16S rRNA gene increases the robustness and power of discrimination to provide a more accurate identification. When culture results remain negative despite high clinical suspicion for infection, broad-range PCR and DNA sequencing can be extremely useful. In addition, some pathogens may not be difficult to cultivate but may require special media, growth conditions, or lengthy incubation. Therefore molecular detection and identification should be considered to isolate these organisms, notably in settings where bacteria were microscopically visible in clinical samples but had not been cultured. This review is intended to explore the application of molecular diagnostic techniques for mycobacterial infections in certain clinical contexts. Finally, we should put emphasis on the point that close collaboration between physician and clinical microbiologist is required in all cases where molecular diagnostics is contemplated because assays need to be individualized according to the clinical setting.

3. Problem of the Mycobacteria sensitivity test: Isamu NAKASONE (Clinical Laboratories, University Hospital of the Ryukyus)

The laboratory test method of growth bacteria or concentrated directly inoculum, antimicrobial sensitivity test of the

rapid result report. In this method it is inoculated, when a sensitivity and resistance bacteria was mixed, a sensitivity record becomes a resistance. It is a resistance proportion in the growth group, and it doesn't mean the resistance of the bacteria strain as of the resistance the *Mycobacteria*. The standard methods of non-tuberculosis can't get the interchangeability of the data because how to examine it isn't presented. With the recommended quality control strain, there is a limit in control of quality of the one concentration method. Then, the obtainment of the quality control strains is in the difficult conditions.

4. Advances in understanding of the virulence mechanism of *Mycobacterium tuberculosis*: Ikuo KAWAMURA (Department of Microbiology, Kyoto University Graduate School of Medicine)

Mycobacterium tuberculosis (MTB) is an intracellular pathogen that has evolved strategies to enable growth in macrophages. The bacterium is able to inhibit fusion of phagosomes with lysosomes through secretion of some bacterial components and modulation of host cell intracellular signaling pathways. Furthermore, it has been shown that phagositosed MTB is killed within macrophages after treatment with IFN-γ *in vitro*. However, virulent MTB is capable of surviving in macrophages *in vivo* and persists in host even after acquired immunity has developed. These data suggest that MTB has developed a sophisticated immune evasion mechanism. In this issue, the strategies of MTB for intracellular survival and immune evasion, which have been unraveled so far are shown and the mechanisms are discussed.

5. Molecular characterization of BCG strains for vaccine production and future considerations: Masaaki SEKI, Ikuro HONDA, Isao FUJITA, Ikuya YANO, Saburo YAMAMOTO, Akira KOYAMA (Japan BCG Laboratory)

In addition to the difference of colony morphology among BCG substrains, the difference of copy number of IS*6110* and production of antigenic proteins were also known. Furthermore, many genetic differences among BCG substrains have become clearer by comparative genomic studies. Also, it has been known that BCG substrains consist of subpopulations with different genotypes. Therefore, it is necessary to develop methods for differentiation of BCG substrains and confirmation of genetic stability during vaccine production.

The complete genome sequence of BCG Pasteur was determined, and the authors suggested that early BCG vaccines may even be superior to the later ones.

Key words: Therapeutic drug monitoring, Genetic examination, Drug susceptibility testing, Intracellular infection, BCG

[1]Department of Respiratory Medicine, National Hospital Organization Sapporo Minami National Hospital, [2]Bacteriology Division, Department of Mycobacterium Reference, Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association

Correspondence to: Satoshi Mitarai, Bacteriology Division, Department of Mycobacterium Reference, Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, 3−1−24, Matsuyama, Kiyose-shi, Tokyo 204−8533 Japan.
(E-mail: mitarai@jata.or.jp)