*Others: I'll go home since I came here for sightseeing only.
        Recommendations and complaints from the place where I undergo training at.
        My contract says that I'll return to my home country if I get sick.
        Was transferred to my home country.

Fig. 11   Reasons for returning to home country mid-way through treatment (multiple answers) (including temporary returns to home country)
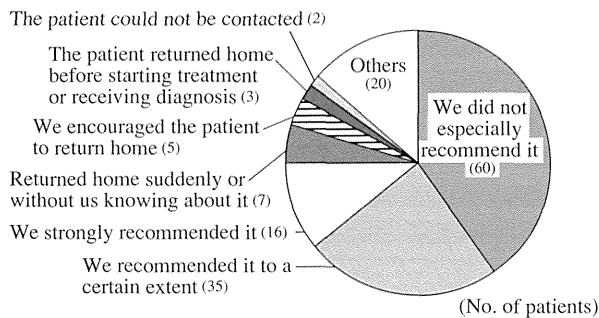

Fig. 12   Whether or not the public health center recommended the patient to stay until the treatment completed (including temporary returns)
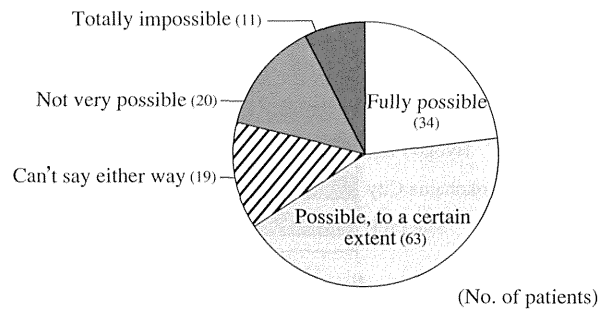

Fig. 13   Was educating the patients possible in case they returned home mid-way through treatment? (including temporary returns)
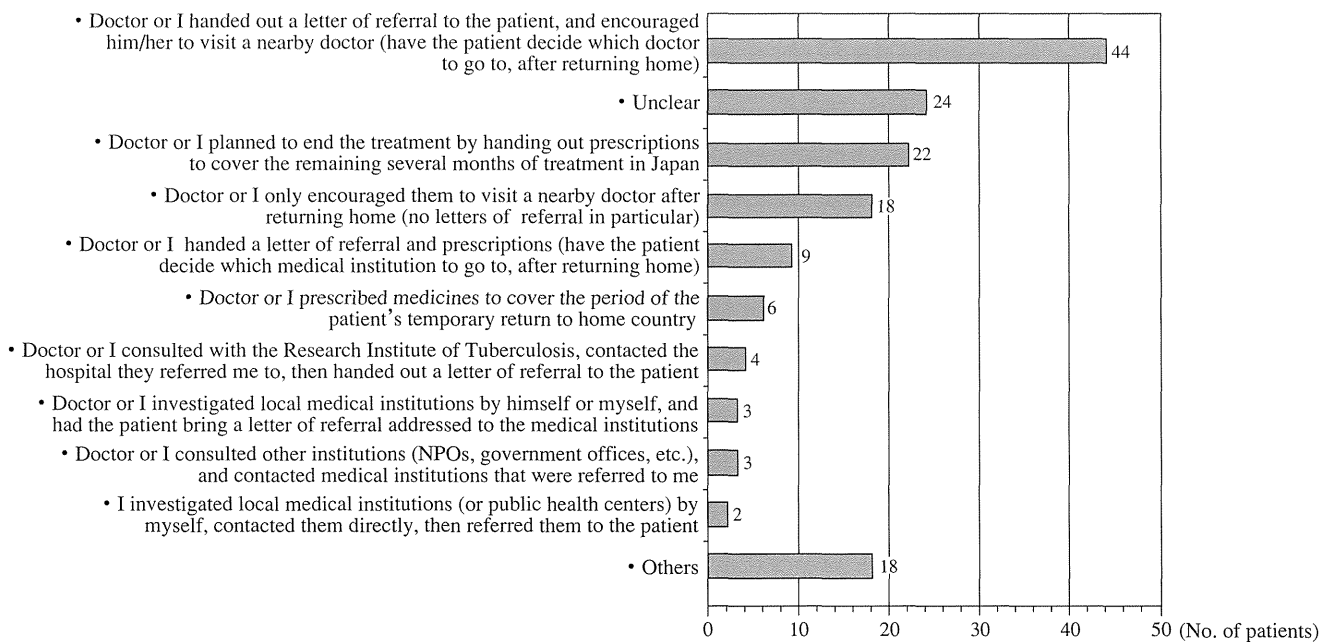

Fig. 14   Subsequent therapeutic policies in case patients returned home mid-way through treatment

Confirmed that treatment
was suspended (3)　　　　　Others (10)

Confirmed that
treatment had
succeeded (18)

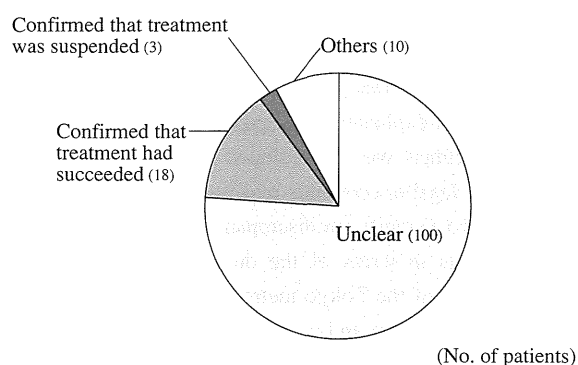Unclear (100)

(No. of patients)

Fig. 15　Grasping of the subsequent course in case
patients returned home mid-way through treatment

their local doctor in their home country, but without receiving
a referral letter (18 cases). In four cases, the facility attempted
to line up a suitable treatment facility in the patient's home
country through the Research Institute of Tuberculosis.

- Success of treatment after leaving Japan

As shown in Fig. 15, the final outcome was unknown in most
cases. Successful treatment was confirmed in only 18 cases
(13.7%).

- Key issues in treatment of foreign TB patients

Language and cultural differences were overwhelmingly the
main difficulties experienced by respondents, as illustrated in
Fig. 16. Other issues cited were expenses leading up to the
diagnosis, and difficulty grasping details of each individual
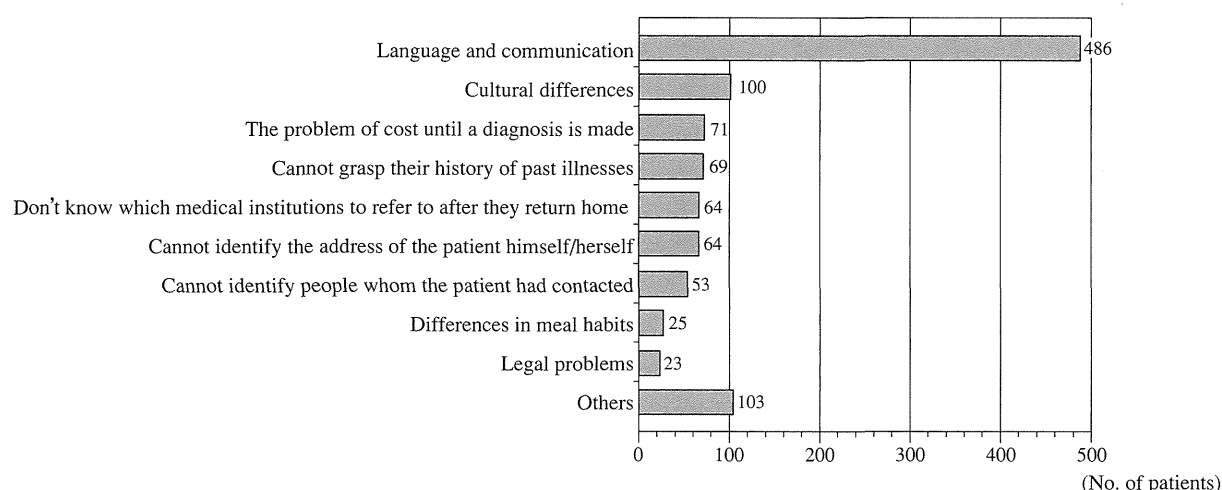case. Table lists further issues identified in the survey.

Language and communication ─ 486
Cultural differences ─ 100
The problem of cost until a diagnosis is made ─ 71
Cannot grasp their history of past illnesses ─ 69
Don't know which medical institutions to refer to after they return home ─ 64
Cannot identify the address of the patient himself/herself ─ 64
Cannot identify people whom the patient had contacted ─ 53
Differences in meal habits ─ 25
Legal problems ─ 23
Others ─ 103

0　　100　　200　　300　　400　　500
(No. of patients)

Fig. 16　Difficulties in dealing with foreign tuberculosis patients living in Japan (multiple answers accepted)

Table　Problems and other matters seen in dealing with foreign tuberculosis patients

- The problems of self-pay burdens of government-subsidized treatment and medical expenses (including transportation costs) (Article 37-2 of the Infectious Diseases Control Law)
- Less understanding and less cooperation of the employer
- Problems caused by a misunderstanding between the public health center and a Japanese man who was the key person
- They fail to come on the dates of appointment, and make repeated cancellations
- Since they replace cellphones quickly, it's difficult to reach them; they do not come on the dates of appointment
- We had trouble communicating with the immigration office
- Financial problems
- The patient was a domestic violence victim, so she was sent to a shelter, but ended up returning to her husband
- The patient lives with another trainee, so it's difficult to secure a place for him/her to live alone
- Making him/her understand the differences in treatment methods from his/her native country
- Coping with a lack of fixed address after treatment completion (follow-up with tuberculosis check-ups)
- Since we carried out a contact medical checkup at the workplace, the disease name was revealed, and the patient was fired.
- Difficulty in reaching and contacting the patient
- The patient was diagnosed with smear positive 3＋ during the early stage of her pregnancy. Since she was a foreign national and was in the early stage of pregnancy, it took time finding a medical institution.
- The patient did not readily understand, and agree to, our recommendation to be hospitalized
- We had a tough time since the patient's husband, who was the key person, was difficult to deal with
- Even if we could identify persons whom the patient had contacted, we cannot get hold of them
- The patient also had HIV
- The patient was being imprisoned in jail, so we could not grasp the details
- If the patient is a non-official resident, should we report him/her to the immigration office or not?
- There are no key persons

• Drug susceptibility of isolates

Participating public health centers were asked to make an estimate of drug susceptibility result based on the available information. Responses were received from 87 cases. As Fig. 17 shows, 27 cases were reported as exhibiting multiple drug-resistance. Of these, 11 returned home country during treatment. Including these cases, drug resistance was identified in 22 of 144 patients who returned home during treatment (see Fig. 18).

Discussion and strategies for achieving successful treatment

The results of this study indicate that TB in foreigners resident in Japan tends to be more prevalent in young adults, and among those from Asia (particularly China, South Korea and the Philippines). These findings are consistent with statistical data[1] and previous academic studies[2]-[4]. This study found that 14% of foreigners return to home country, a result almost identical to the findings of another study (Okada et al.). This figure rises to 17% if we include patients who return home temporarily for any reason during the treatment period. With respect to residency status and health insurance cover, the current study found that short-stay foreigners and illegal residents, as well as those without health insurance cover, were more likely to return to home country before completing the treatment regimen. Again, this is consistent with the findings of previous reports[3][5].

This study analyzed responses from health center officers directly engaged in TB program, with particular reference to patients who had returned to home. The questionnaire focused on reasons why patients chose to leave Japan, the advice and/or assistance provided by the facility, the ability of the facility to trace the patients' outcome after returning to home country, and the difficulties the officers encountered. A key issue was the inability of facilities to issue referral letters to appropriate

medical facilities in their home countries due to a lack of information of situation of TB treatment in other countries. Respondents reported many instances of patients departing with no warning or explanation. As expected, there were many cases where treatment was discontinued by patients having to return home for legal or economic reasons.

There was also a significant discrepancy between urban and regional residents in terms of the decision of returning to origins. Residents of the Tokyo metropolitan area were more likely to remain in Japan and complete their treatment, while those in the countryside were more likely to return home midway through treatment. It has been reported[6][7] that in the areas with large foreign populations, foreigners who contract TB are now more likely to remain in Japan to complete their treatment due to the success of concerted campaigns by local governments, health centers, medical institutions and NGOs, as well as greater knowledge in this area. The potential viability of producing a guidebook on treating TB in foreign patients and introducing medical TB checkups at Japanese language schools has also been investigated[4]. There is no doubt that initiatives to encourage completion of treatment are very much in the hands of prefectural and local government and health centers. This is illustrated by some of the key issues identified in our study: the duty of confidentiality in relation to the immigration department; difficulty accessing interpreting services at the local government level due to restrictions on time and availability; and insensitivity of local employers towards employees in need of treatment.

There is increasing support around the world for TB treatment in general and the directly observed treatment, short-course (DOTS) strategy in particular. However, TB treatment and management program can vary considerably between countries, and even between regions within a country due to political considerations. Some areas are not adequately equipped to deal with TB, and have only a limited number
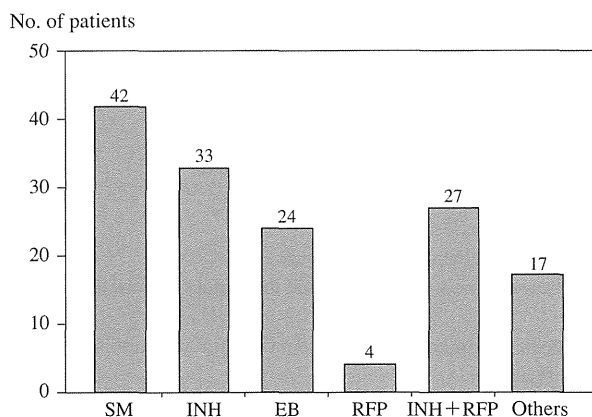

Fig. 17 Status of drug resistance of registered foreign tuberculosis patients living in Japan (87 patients who answered that they had drug resistance; some had resistance to more than two drugs)
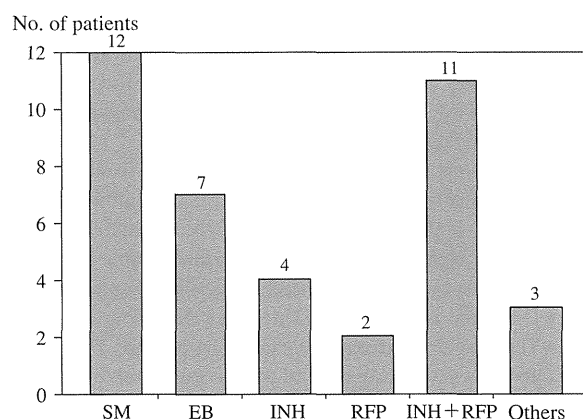

Fig. 18 Status of drug resistance of 144 foreign tuberculosis patients who returned home mid-way through treatment (including temporary returns) (22 patients had some sort of drug resistance; some had resistance to more than two drugs.)

SM: streptomycin   INH: isoniazid   EB: ethambutol   RFP: rifampicin

of hospitals and institutions capable of managing multi drug-resistant TB. Given that foreigners with TB in Japan are more likely to present with multi-drug resistant TB, the first priority should be to convince them to remain in Japan through to the end of the treatment regimen.

To this end, treating physicians should opt for a six-month treatment course, and where necessary negotiate with the immigration department to have the patient's period of stay extended to ensure that treatment can be completed. In cases where notifying the immigration department of a suspected violation of the immigration laws, as required by law, would compromise the treatment regimen to the significant detriment of the patient, our priority should be to maintain patient confidentiality[8].

In cases where returning home partway through treatment is unavoidable, the patient should normally be issued with medication to cover the remaining treatment period. In cases where the patient is obliged to leave Japan for economic or legal reasons or because of an unsympathetic employer, the treating facility should attempt to ensure continuity of treatment by forwarding the relevant patient detailed information to an appropriate medical institution in the patient's home country. The Department of Programme Support at the Research Institute of Tuberculosis (RIT) has already identified a number of reliable institutions in China, South Korea, the Philippines, Cambodia and Nepal, with whom patients can make a consultation. In addition, RIT has an extensive network of TB facilities in countries around the world, and is ready to arrange introductions and referrals for patients requiring ongoing treatment. Seeking the cooperation from them is also recommended to achieve the best outcomes for your patients.

Acknowledgements

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The International Exchanging Committee of the Japanese Society for Tuberculosis
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and Masaki FUJITA

Awareness of Disaster Reduction Frameworks and Risk Perception of Natural Disaster: A Questionnaire Survey among Philippine and Indonesian Health Care Personnel and Public Health Students

Motoki Usuzawa,[1,2] Elizabeth O. Telan,[3] Razel Kawano,[4] Carmela S. Dizon,[5] Bachti Alisjahbana,[6] Yugo Ashino,[7] Shinichi Egawa,[1] Manabu Fukumoto,[8] Takako Izumi,[9] Yuichi Ono[9] and Toshio Hattori[1,2,7]

[1]Division of Disaster Medicine, International Research Institute of Disaster Science, Tohoku University, Sendai, Miyagi, Japan
[2]International Post-Graduate Program in Human Security, Tohoku University, Sendai, Miyagi, Japan
[3]STD AIDS Cooperative Central Laboratory, San Lazaro Hospital, Manila, Metropolitan Manila, the Philippines
[4]Graduate School Program in Public Health, Angeles University Foundation, Angeles City, Pampanga, the Philippines
[5]Angeles University Foundation Graduate School, Angeles City, Pampanga, the Philippines
[6]Health Research Unit, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia
[7]Division of Emerging Infectious Diseases, Graduate School of Medicine, Tohoku University, Sendai, Miyagi, Japan
[8]Department of Pathology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Miyagi, Japan
[9]International and Regional Cooperation Office, International Research Institute of Disaster Science, Tohoku University, Sendai, Miyagi, Japan

As the impacts of natural disasters have grown more severe, the importance of education for disaster medicine gains greater recognition. We launched a project to establish an international educational program for disaster medicine. In the present study, we surveyed medical personnel and medical/public health students in the Philippines (n = 45) and Indonesia (n = 67) for their awareness of the international frameworks related to disaster medicine: the Human Security (securing individual life and health), the Sphere Project (international humanitarian response), and the Hyogo Framework for Action 2005-2015 (international strategy for disaster reduction). In both countries, more than 50% responders were aware of human security, but only 2 to 12% were aware of the latter two. The survey also contained questions about the preferred subjects in prospective educational program, and risk perception on disaster and disaster-related infections. In the Philippines, significant disasters were geophysical (31.0%), hydrological (33.3%), or meteorological (24.8%), whereas in Indonesia, geophysical (63.0%) and hydrological (25.3%) were significant. Moreover, in the Philippines, leptospirosis (27.1%), dengue (18.6%), diarrhea (15.3%), and cholera (10.2%) were recognized common disaster-related infections. In Indonesia, diarrhea (22.0%) and respiratory infection (20.3%) are major disaster-related infections. Water-related infections were the major ones in both countries, but the profiles of risk perception were different (Pearson's chi-square test, p = 1.469e-05). The responders tended to overestimate the risk of low probability and high consequence such as geophysical disaster. These results are helpful for the development of a postgraduate course for disaster medicine in Asia Pacific countries.

Introduction

The impacts of natural disasters have been growing. Leaning and Guha-Sapir (2013) described it as follows: "There were three times as many natural disasters from 2000 through 2009 as there were from 1980 through 1989." The economic damage brought by natural disasters shows that the world suffers catastrophic disasters every several years (Leaning and Guha-Sapir 2013). Disaster risk reduction has become a global issue. The United Nations promoted the 1990s as the International Decade for Natural Disaster Reduction (Housner 1989). The United Nations International Strategy for Disaster Reduction was created in 1999 (Who we are: http://www.unisdr.org/who-we-are). The international community has recognized that disaster risk reduction is a mainstream concern.

As the Hyogo Framework for Action 2005-2015 (HFA) emphasized, education is important in order to provide citizens with knowledge on disaster risk reduction and preparedness (United Nations International Strategy for Disaster Reduction 2005). There are ongoing projects and research being carried out in terms of disaster response and preparedness. In terms of humanitarian responses, the consideration of quality and accountability of aid is important. A group of non-governmental organizations and, the International Red Cross and the Red Crescent Movement initiated the Sphere Project to improve the quality of humanitarian response in disaster. They published a handbook to provide principles and core standards (Sphere Project 2011). The impact of natural disasters to public health has been growing (Keim 2011). There are educational efforts being implemented in emergency medicine and family medicine to develop capacity in disaster medicine (Franc-Law et al. 2010; Huntington and Gavagan 2011). However, the current level of knowledge of disaster medicine among medical students and health professionals is not sufficient (Su et al. 2013).

We launched a project on human security with Asian Pacific countries to develop disaster studies and education in medicine (Hattori et al. 2012). Human security is a new paradigm of security that cannot be accomplished by securing the territory from external threats, which used to be the main goal of traditional means of security (United Nations Development Programme 1994; Ogata and Sen 2003). The United Nations Development Programme (UNDP) emphasized the importance of human security. Thus, whether human security has interdependency of its components, effectiveness of early prevention, and is people-centered, should be of universal concern. There are seven categories of threats for human security: economic, food, health, environmental, personal, community, and political. The UNDP also pointed out that "the rising tide of disasters (United Nations Development Programme 1994)" would be a significant threat to human security.

Globally speaking, the burden of infectious disease is still high, especially in low or middle-income countries (Lozano et al. 2012). There are many outbreaks of infectious disease following natural disasters (Kouadio et al. 2012). Outbreaks are avoidable with proper education (de Ville de Goyet 1991). Thus, we focused on education so that we could provide adequate knowledge for disaster-related infectious diseases in low or middle-income countries with high burdens of both infectious disease and natural disasters.

In our feasibility study, we conducted a questionnaire survey in the Philippines. The purpose of our study was to collect data on opinions about disaster-related infectious diseases and education programs for disaster medicine. Our results suggested that there were relationships between risk perceptions of natural disasters and disaster-related infectious diseases (Usuzawa et al. 2013). The type of the disaster is one of the determining factors for an infectious disease outbreak following a disaster (Connolly et al. 2004).

We then conducted further surveys in the Philippines and Indonesia to investigate the awareness of frameworks for disaster preparedness and mitigation and to identify the need for education in disaster medicine, especially infectious disease. The Philippines and Indonesia are countries with a high burden of natural disasters in the number of events reported in 2012 (Guha-Sapir et al. 2013) and they are middle-income countries.

Methods

We administered an anonymous questionnaire on several issues related to disaster medicine to medical school students, public health school students, and medical personnel. The questions were on awareness of international frameworks related to disaster medicine (Human Security, Sphere Project, and HFA), and the need for prospective courses in human security and disaster medicine. To survey risk perceptions of disaster and disaster-related infectious disease, the survey also contained a question about disasters and disaster-related infectious diseases.

We distributed questionnaire sheets at lectures in universities and at conferences. They were collected either on site or via email later. R (version 2.15.0) on Windows 7 was used for statistical analysis.

Ethical consideration

We did not collect information about participants' health. Although, according to the definition of the Ethical Guidelines for Epidemiological Research by the Ministry of Health, Labor and Welfare, Japan, the survey was not an epidemiological study, we followed it. Participants were provided written documentation outlining the purpose of the survey, the data handling policy and the publication policy. They had the option not to participate by returning the questionnaire without answering any questions. We did not collect any information which could identify individuals. Those who did not involve in collecting the data processed the data to assure anonymity. The original questionnaire sheets were stored in a cabinet with a lock.

Results

The study initially included 46 Filipinos and 70 Indonesians; however, four questionnaires were returned

without all of the questions completed (one in the Philippines, three in Indonesia). In total, 45 Filipinos and 67 Indonesians participated. Of the 45 responders in the Philippines, 36% (*n* = 16) were medical students, 27% (*n* = 12) were laboratory staffs, 11% (*n* = 5) were academic staffs or managers, 9% (*n* = 4) were medical doctors, and 7% (*n* = 3) were government/non-government organization personnel working for disaster response. Of the 67 responders in Indonesia, 46% (*n* = 31) were public health school students, 36% (*n* = 24) medical school students, 12% (*n* = 8) medical doctors, and 3% (*n* = 2) local government officers. Two respondents chose not to identify their position.

The participants' awareness of international frameworks is shown in Table 1. In both countries, more than half were aware of human security. Far fewer were aware of the Sphere project and HFA (Table 1). Various subjects were listed as being preferable for prospective courses of

human security in disaster medicine. In the Philippines, infectious disease was the preferred subject, and in Indonesia, disaster studies and public health, were preferred subjects (Table 2). Table 3 shows the risk perceptions of significant disasters. In the Philippines, water-related disasters, floods (24.8%, 32/129) and typhoons (23.3%, 30/129), were recognized as significant disasters. Twenty-three percent (23.3%, 30/129) pointed out earthquakes as a significant disaster caused by natural hazards. In Indonesia, earthquakes (24.7%, 38/154) were considered the most significant disasters in this survey. Volcanic eruption (22.1%, 34/154), flood (22.1%, 34/154), and tsunami (16.2%, 25/154) followed it. To investigate the difference between the two profiles, we recounted the data and categorized it according to the criteria, which is shown in the Annual Disaster Statistics Review 2012 (Guha-Sapir et al. 2013). In the Philippines, significant disasters were geophysical (31.0%, 40/129), hydrological (33.3%, 43/129), or meteo-

Table 1. Awareness of international frameworks related disaster response.

	Human Security	The Sphere Project	HFA
Philippines (*N* = 45)	26 (58%)	1 (2%)	5 (11%)
Indonesia (*N* = 67)	35 (52%)	8 (12%)	7 (10%)

Each cell shows the number of responders who were aware of the framework and its percentage in blanket.

Table 2. Preferable subject for human security in disaster medicine.

	Community management	Disaster medicine	Disaster study	Infectious diseases	Medicine	Public health	Risk studies	Other	Total
Philippines	0 (0.0)	2 (3.4)	9 (15.3)	28 (47.5)	6 (10.2)	4 (6.8)	2 (3.4)	8 (13.6)	59 (100.0)
Indonesia	3 (5.9)	0 (0.0)	20 (39.2)	0 (0.0)	6 (11.8)	12 (23.5)	0 (0.0)	10 (19.6)	51 (100.0)

Multiple answers were allowed. Each cell shows number of those who though the subject as preferable. Participants in the Philippines were students and medical staff. Those of Indonesia were public health students. It may affect the results. Each cell shows number and its percentage in blankets.

Table 3. Awareness of significant disaster.

	Earthquake	Flood	Volcanic eruption	Typhoon	Tsunami	Landslide	Infectious disease	Storm	Other	Total
Philippines	30 (23.3)	32 (24.8)	10 (7.8)	30 (23.3)	0 (0.0)	11 (8.5)	5 (3.9)	2 (1.6)	9 (7.0)	129 (100.0)
Indonesia	38 (24.7)	34 (22.1)	34 (22.1)	0 (0.0)	25 (16.2)	5 (3.2)	8 (5.2)	3 (1.9)	7 (4.5)	154 (100.0)

Multiple answers were allowed. Each cell shows number of those who thought the disaster as significant. Earthquake was significant disaster in the two countries. Whereas typhoon was thought significant disaster in the Philippines, eruption and tsunami were regarded as significant disaster. Each cell shows number and its percentage in blankets.

Table 4. Awareness of significant disaster recollected by category.

	Biological	Climatological	Geophysical	Hydrological	Meteorological	Other	Total
Philippines	6 (4.7)	6 (4.7)	40 (31.0)	43 (33.3)	32 (24.8)	2 (1.6)	129 (100.0)
Indonesia	10 (6.5)	3 (1.9)	97 (63.0)	39 (25.3)	3 (1.9)	2 (1.3)	154 (100.0)

The data in Table 3 was categorized as appeared in Annual Disaster Statistics Review 2012. Fisher's exact test showed significant difference (*p* = 1.1156e-10) in the two countries. Each cell shows number and its percentage in blankets.

Table 5. Awareness of significant infectious disease related natural disaster.

	Diarrhea	Leptospirosis	Dengue	Respiratory infection	Influenza	Cholera	Skin infection	Tuberculosis	Other	Total
Philippines	18 (15.3)	32 (27.1)	22 (18.6)	6 (5.1)	10 (8.5)	12 (10.2)	4 (3.4)	2 (1.7)	12 (10.2)	118 (100.0)
Indonesia	27 (22.0)	5 (4.1)	11 (8.9)	25 (20.3)	11 (8.9)	2 (1.6)	8 (6.5)	6 (4.9)	28 (22.8)	123 (100.0)

Multiple answers were allowed. Each cell shows number of those who thought the infectious disease as significant and its percentage in blankets.

Table 6. Awareness of significant infectious disease recollected by route of transmission.

	Respiratory infection	Water-related infection	Other	Total
Philippines	16 (13.6)	88 (74.6)	14 (11.9)	118 (100.0)
Indonesia	36 (29.3)	55 (44.7)	32 (26.0)	123 (100.0)

The data in Table 5 was categorized by route of transmission. Respiratory infection and influenza in Table 5 were integrated in Respiratory infection in Table 5. Tuberculosis was excluded because it is chronic rather than acute in terms of disaster-related infection. Diarrhea, leptospirosis, dengue, cholera, skin infection and dysentery (Others in Table 5) were classified as water-related infection. Pearson's chi-square test showed significant difference ($p = 1.469\text{e-}05$) in the two countries. Each cell shows number and its percentage in blankets.

Table 7. Comparison of disaster risk perception and disaster statistics.

categories	a) Numbers of events				b) Numbers of people killed				c) Average numbers of people killed per one event; a/b				d) Responders' risk perception of disaster			
	Philippines		Indonesia		Philippines		Indonesia		Philippines		Indonesia		Philippines		Indonesia	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
biological	18	(3.2)	35	(8.0)	1,283	(2.1)	3,966	(1.6)	71.3	(14.9)	113.3	(5.2)	6	(4.7)	10	(6.5)
climatological	9	(1.6)	18	(4.1)	10	(0.0)	9,629	(4.0)	1.1	(0.2)	534.9	(24.6)	6	(4.7)	3	(1.9)
geophysical	55	(9.9)	165	(37.8)	13,083	(21.3)	216,887	(89.9)	237.9	(49.7)	1,314.5	(60.5)	40	(31.0)	97	(63.0)
hydrological	165	(29.6)	206	(47.2)	5,910	(9.6)	8,719	(3.6)	35.8	(7.5)	42.3	(1.9)	43	(33.3)	39	(25.3)
meteorological	311	(55.7)	12	(2.8)	41,154	(67.0)	2,013	(0.8)	132.3	(27.7)	167.8	(7.7)	32	(24.8)	3	(1.9)
other	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	NA		NA		2	(1.6)	2	(1.3)

Columns marked a) showed reported numbers of event and b) showed reported numbers of people killed in each category of disaster. The data were obtained from "EM-DAT: The OFDA/CRED International Disaster Database, www.emdat.be-Université catholique de Louvain-Brussels-Belgium". Columns c) indicate the calculated numbers of people killed per one event. In columns d), the data in the Table 4 were recounted according to ADSR classification (the Philippines, $N = 129$ and Indonesia, $N = 154$). They show risk perception of the responders of our survey. Each cell shows number and its percentage in blankets. NA, not applicable.

rological (24.8%, 32/129). In Indonesia, geophysical (63.0%, 97/154) and hydrological (25.3%, 39/154) were the top two answers (Table 4). Fisher's exact test showed that two profiles were sampled from different populations ($p = 1.1156\text{e-}10$). We concluded that risk perception of disaster differs between the Philippines and Indonesia.

In the Philippines, leptospirosis (27.1%, 32/118), dengue (18.6%, 22/118), diarrhea (15.3%, 18/118), and cholera (10.2%, 12/118), outbreaks of which occurred after water-related disasters, were recognized common disaster-related infectious diseases. In Indonesia, diarrhea (22.0%, 27/123) and respiratory infection (20.3%, 25/123) are major disaster-related infectious diseases. Dengue and influenza represented 8.9% (11/123) of the responses, respectively (Table 5). We classified the answers into three categories: respiratory infections, water-related infections, and other infections (Table 6). There was a significant difference in the

profiles of risk perception about disaster-related infectious disease (Pearson's chi-squared test, $p = 1.469\text{e-}05$).

Discussion

Disasters, which are thought of as significant, were different in the two countries. This suggests that disaster risk perception in the two countries would be different. To investigate if there are relationships between risk perceptions obtained by our survey and disaster statistics, we compared them in the two countries. Disaster statistics (Table 7) show that significant disasters are hydrological, meteorological, and geophysical and they accounted for more than 90% (96% in the numbers of events; 98% in the number killed) in the Philippines. Our survey showed that they accounted for 89% of the disasters that were viewed as significant in the Philippines.

Disaster statistics in Indonesia showed that geophysi-

cal and hydrological accounted for 85% of the number of events and 94% of the number of people killed. Our survey showed that they accounted for 88% of the disasters that were considered significant in Indonesia. Our results seem to coincide with statistics.

The psychometric approach provided a framework for risk perception. Slovic showed that the factors of risk perception consisted of two factors, controllable/uncontrollable and known/unknown (Slovic 1987). Starr found that people overestimated the risk of low probability and high consequence hazards (Starr 1969). Each country has different profiles of disaster. Hence, according to the psychometric approach of risk perception research, we will find the difference in their risk perception. In disaster risk perception, people tend to overestimate the risk of disasters, which occurs rarely but kills many people when it does occur.

In the Philippines, geophysical disaster was 10% of the numbers of events and responsible for 21% of the number of people killed (Table 7). In geophysical disasters, the average number of people killed per event was 237.9. This was more than the number of people killed in hydrological (35.8) and meteorological (132.3) disasters, combined. The same applied to geophysical disasters in Indonesia. The average number killed per one geophysical disaster was 1,314.5. This was more than the number of people killed in hydrological (42.3) and meteorological (167.8) disasters (Table 7). In terms of numbers killed, geophysical disasters in both counties are more severe than other disasters. This might affect risk perception. More precise, future research is needed if we shift our focus to risk perception research.

Researchers on risk perception are interested in subjective risk perception of people and its psychological mechanism (Slovic et al. 1979; Smith et al. 2011). Smith and his colleagues analyzed risk perception of disasters, which included both natural and technological disasters, and drew their results in a plot diagram whose axes were fear/no fear and familiar/no familiar (Smith et al. 2011). Their analysis showed comparisons between the risks perceived by people. We measured risk perceptions of disaster and disaster-related infectious disease. Our results suggested that risk perception of disasters and disaster-related infectious diseases were different in the Philippines and Indonesia and the psychometric approach to risk perception might provide a hypothetical explanation, which pointed out a connection between the disaster statistics and the risk perception of the participants. The limitation of our study was that it was not designed to verify the risk perception theory; thus, we could not show the mechanism of the risk perception that led to the obtained results.

In Table 3, we found no answers that mentioned storm surge as a significant disaster in the Philippines, although 30 out of 129 did mention typhoons, which induces a storm surge. This suggests a topic for disaster preparedness and mitigation education. If individuals were to learn that a storm surge induced by a typhoon could reach up to several meters high, they could acquire practical knowledge for disaster mitigation. In November 2013, Typhoon Haiyan struck the Philippines. The storm surge destroyed the coastal area of Tacloban, Leyte province. The International Research Institute of Disaster Science (IRIDeS), Tohoku University, organized a research project to clarify the damage done by the typhoon. According to the preliminary survey, people in Tacloban were forewarned via television or radio broadcasts that the storm surge would strike the town but they did not understand how severe it would be. This project anticipates conducting further scientific research on this topic in an effort to develop an effective early warning system.

In this article, we focused on disaster related infectious disease as a topic of education for disaster medicine in human security. Outbreaks of infectious disease are not the sole issue posed by disasters. On March 11, 2011, severe earthquakes followed by tsunamis, struck East Japan. This disaster was named the 2011 Great East Japan Earthquake and Tsunami (GEJET). At least 18,500 were killed and missing (Shibahara 2011). The tsunami, which was the largest in the past 1,000 years, destroyed a vast part of the coastal areas of Iwate, Miyagi, and Fukushima (Ishigaki et al. 2013). Although UNDP had already pointed out that a disaster would be a significant threat to human security (United Nations Development Programme 1994), the disaster was far beyond anything anticipated and demonstrated that human security was threatened in the affected areas.

In addition, there is a lot to be done before the recovery and reconstruction from the GEJET is complete. According to the Reconstruction Agency, Government of Japan, "some 470,000 people were evacuated, and over 320,000 people have been displaced on a longer-term basis" (Progress to Date. Housing Redevelopment: http://www. reconstruction.go.jp/english/topics/2013/03/housing-redevelopment.html). Although, multiple levels of government and local communities are making an effort to rebuild the lost communities, they have to cope with many difficulties (Iuchi et al. 2013). The accident in the Fukushima Dai-Ichi Nuclear Power Plant is extremely severe and not everyone has come together yet to solve it (Tanaka 2012). We cannot ignore the experience of the GEJET and the lessons learned from it when we study disaster science here in Sendai, Japan. The concept of human security is expected to be the core of education for disaster medicine.

Conclusion

In this study, we showed that risk perception of disaster and disaster-related infectious diseases are different in the Philippines and Indonesia. The results are expected to contribute to the development of a postgraduate course in disaster medicine based on the concept of human security in Asia Pacific countries.

Contributions

M.U., S.E., Y.O. and T.I. designed the questionnaire,

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CASE REPORT

A man from South Asia presenting with abdominal pain

Beata Shiratori,[1] Osamu Usami,[2] Toshio Hattori,[1] Yugo Ashino[1]

[1]Department of Emerging Infectious Diseases, Tohoku University, Sendai, Japan
[2]Department of Comprehensive Infection, Tohoku University, Sendai, Japan

Correspondence to
Dr Beata Shiratori,
beatashiratori@gmail.com

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SUMMARY

The diagnosis of abdominal tuberculosis (TB) is challenging due to the non-specific clinical presentation and frequent failure to detect the pathogen. A young Bangladeshi man presented to the Emergency Outpatient department with constipation and burning abdominal pain that was localised primarily in the epigastrium. Although the infectious agent was not detected, findings of histological examination were helpful in guiding the treatment strategy. As a good clinical practice, it is important to consider abdominal TB as a possible diagnosis in such cases, particularly when a patient has previously been residing in a high TB burden country. Thus, appropriate diagnosis and early antituberculous therapy are essential for achieving a positive outcome.

BACKGROUND

The incidence of tuberculosis (TB) has again been increasing in developed countries, due to the rise in tourism, educational exchange activities, influx of immigrants, HIV infection and frequent use of immunosuppressive therapy. In Japan, extrapulmonary TB cases account for 23% of all TB cases.[1] However, among foreigners who stay beyond their visa validity, this proportion reaches 35%, and most of these cases consist of young men from Southeast Asia.[2] Despite the efforts of public health centres to screen foreigners, most of the patients seek healthcare only when severe symptoms develop, primarily due to their illegal status, language barrier or concern regarding the high medical costs. As abdominal TB can mimic various gastrointestinal disorders, it is important to consider this condition when performing differential diagnosis in patients with acute abdomen, particularly in cases of overstaying foreigners. The failure in detecting the pathogen often delays diagnosis and appropriate treatment. Although advanced medical imaging techniques and exploratory laparoscopy facilitate the diagnosis of this condition, histological findings also appear to play a vital role in the final treatment decision.

CASE PRESENTATION

A 29-year-old Bangladeshi man presented to the emergency outpatient department with a 1-week history of constipation, and burning abdominal pain that was localised primarily in the epigastrium. The patient had developed hepatitis A 4 years previously, but had no other remarkable medical or surgical history.

On examination, the patient was alert, but had an agonised look and experienced excessive sweating. His temperature was 38.3°C, pulse rate was 93 bpm and blood pressure was 111/69 mm Hg. An abdominal examination indicated a slightly tender and distended abdomen, but no rebound tenderness was observed. The bowel sounds were slightly accelerated. An abdominal radiograph obtained in the standing position indicated the presence of small bowel gas with niveau formation and stomach expansion; however, no abdominal free gas was detected. The loss of the right psoas shadow, flank stripe sign and dog ear sign suggested the presence of acute abdomen with ascites (figure 1A). However, his chest radiograph demonstrated a raised diaphragm on both sides and basal pleural fluid accumulation (figure 1B).

Although he was administered scopolamine butylbromide and a proton pump inhibitor as a palliative treatment, he experienced continuous abdominal pain, fever, appetite loss and suspected peritonitis, and was admitted to the department of gastroenterology on the subsequent day. Thereafter, fluid replacement, antipyretics, analgesics and anti-inflammatory agents were administered.

INVESTIGATIONS

Laboratory examination indicated elevated C reactive protein and serum amyloid A levels. However, no abnormal findings were noted on gastrointestinal fibroscopy or colonoscopy. Blood, sputum, stool, urine, ascites and pleural fluid cultures yielded negative results for the presence of standard aerobic and anaerobic bacteria, acid-fast bacteria and fungi. Positive results were obtained on examination with a Quantiferon TB Gold test (QFT), but negative results were obtained for Mycobacterium tuberculosis (MTB) on PCR testing of 3-day sputum. The patient was screened for HIV, but a negative result was obtained. Pleural effusion, ascites retention and remarkable lymph node enlargement were observed around the coeliac and common hepatic arteries on chest and abdominal CT. Since the levels of inflammatory markers did not decrease and abdominal infection was suspected, the patient was administered cefozopran and biapenem. To investigate the characteristics of the enlarged lymph nodes, a whole body ^{18}F-fluorodeoxyglucose positron emission/CT scan was performed, which showed accumulation of ^{18}F-fluorodeoxyglucose along the pleura, mesenterium, small intestine, liver surface and abdominal aorta (figure 1C). In order to differentiate between the presence of malignancy and inflammatory lesions, an exploratory laparoscopy was performed on the following day. The abdominal cavity was

Figure 1 Abdominal radiograph showing small bowel gas with niveau formation, stomach expansion, loss of right psoas shadow (white arrows indicate left psoas shadow), flank stripe sign (double line arrows) and dog ear sign (black arrows) (A). Chest radiograph showing raised diaphragm on both sides and basal pleural fluid accumulation (B). ^{18}F-fluorodeoxyglucose positron emission tomography/CT image revealing accumulation of ^{18}F-fluorodeoxyglucose along the pleura, mesenterium, small intestine, liver surface and abdominal aorta (C). H&E staining of laparoscopic specimen showing epithelioid granulomas with central necrosis and multinucleate giant cells (black arrow) (D).
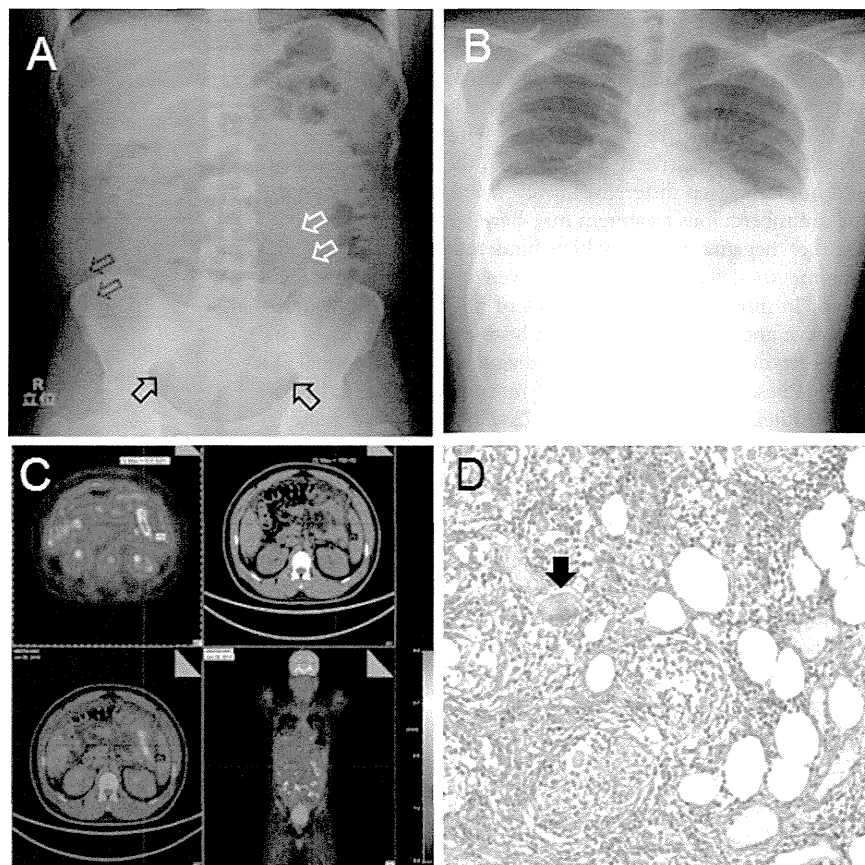
filled with ascites, and bean-sized nodules were diffusely observed in the great omentum. A peritoneal biopsy indicated epithelioid granulomas with central necrosis and multinucleate giant cells (figure 1D); however, Ziehl-Neelsen staining and PCR examination of the peritoneal tissue yielded a negative result for MTB. Although we did not have definitive evidence of TB, we suspected the presence of tuberculous peritonitis, and the patient was therefore transferred to the infectious disease department.

TREATMENT
At that department, he was administrated anti-TB treatment comprising rifampicin, isoniazid, ethambutol and pyrazinamide. On initiation of anti-TB treatment, massive left-sided pleural fluid accumulation developed, and as the patient experienced problems in breathing, pleural aspiration was repeatedly performed. The lymphocyte-predominant exudate had the following parameters: adenosine deaminase, 46.9 U/L; glucose, 110 mg/dL; carbohydrate antigen (CA)-125, 638 U/mL; CA-199, 7.9 U/mL and pleural fluid lactate dehydrogenase (LDH) to serum LDH ratio, 0.7, indicating that the exudate was a result of TB infection. The transient worsening of the patient's condition was due to paradoxical aggravation, which is occasionally observed during anti-TB therapy. Despite the temporal pleural fluid accumulation, the anti-TB treatment was continued. As the patient did not have any previous respiratory problems and chest radiograph findings were not suggestive of pulmonary TB, we assumed that the pleural effusion was caused by the passage of the ascites across the diaphragm.

OUTCOME AND FOLLOW-UP
After 5 weeks of hospitalisation in the infectious disease department, the patient exhibited clinical improvement, and a chest radiograph and abdominal CT showed extensive consolidation. The patient was discharged from the hospital, and completed the 6-month regimen of oral antimycobacterials without any re-emergence.

DISCUSSION
MTB infection of the gastrointestinal tract accounts for 2% of TB cases worldwide[3] and is usually underestimated in counties with a low TB prevalence. Peritoneal TB is caused by the haematogenous or lymphatic spread of MTB, ingestion of infected sputum or via direct extension from adjacent organs. This often results in the formation of multiple tuberculous granulomas, fibrosis, strictures, mesenteric lymph node enlargement and ascites.[4] The pathological changes may manifest as acute or chronic symptoms including fever, abdominal pain, diarrhoea or constipation, weight loss, anorexia and malaise. On admission, our patient had fever and, non-specific abdominal symptoms and the abdominal radiograph did not show acute ileus; therefore, the patient underwent conservative treatment.

A positive result in the QFT assay was an important indication in the diagnosis of MTB infection. Concomitant pulmonary TB has been detected in approximately 20% of patients with abdominal TB,[5] and immunosuppressive conditions such as HIV increase the risk of developing extrapulmonary TB.[6] In the present case, because the patient exhibited negative results on HIV testing, had no history of TB, and had no radiological evidence of TB, and since the QFT does not distinguish between

latent and active TB infection, we suspected the presence of an MTB infection, although we were unable to confirm that it was responsible for the patient's illness. Owing to the frequent false-negative results obtained, the QFT assay is mainly a supportive test of latent and active TB diagnosis. Therefore, even if our patient exhibited negative results on QFT, the diagnostic strategy would not have been different; however, the initiation of appropriate antituberculous treatment may have been delayed.

Another negative aspect, which hindered the diagnosis, was the failure to detect the pathogen even by the sensitive PCR method. In most cases, the diagnosis of abdominal TB requires an invasive approach, and obtaining histological material is vital for correct diagnosis. Finally, the diagnosis of abdominal TB was made based on the detection of histopathological indicators of TB-epithelioid granulomas with caseation and the presence of giant cells. A study conducted in India, describing the utility of PCR and histopathological examination in abdominal TB diagnosis, showed that 22% of patients with histologically verified TB granulomas had negative results on PCR testing, and the same proportion of patients with abdominal TB had negative results on PCR and histological examination.[7] However, the limitations of histological examination include obtaining an appropriate the quality and size of the specimen, since repeating the invasive laparoscopy should be avoided.

In the differential diagnosis of abdominal TB, other granulomatous diseases should be considered, such as *Histoplasma* or *Pneumocystis* infection, Crohn's disease, sarcoidosis, Whipple's disease, mesenteric panniculitis or the formation of granulomas around foreign materials. Moreover, in elderly patients, carcinomatosis should be considered.

In the present case, we decided to administer anti-TB treatment based on the histological findings, which were supported by positive results on the QFT assay and the characteristics of the pleural fluid. However, in cases of suspected abdominal TB, even in cases where the pathogen is not confirmed, empirical anti-TB treatment should be considered. In such cases, a positive clinical outcome will represent indirect evidence that TB was present.

Learning points

- ► Clinicians should consider abdominal tuberculosis (TB) as a differential diagnosis, particularly in patients with abdominal pain who have previously resided in a high TB burden country.
- ► Advanced medical imaging techniques and exploratory laparoscopy may facilitate the diagnosis; however, histological findings may provide clues towards an appropriate treatment decision.
- ► In cases of suspected abdominal TB, even in cases where the pathogen is not confirmed, empirical anti-TB treatment should be considered.

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Hindawi

Research Article
Elevated OPN, IP-10, and Neutrophilia in Loop-Mediated Isothermal Amplification Confirmed Tuberculosis Patients

Beata Shiratori,[1,2,3] Susan Leano,[4] Chie Nakajima,[5] Haorile Chagan-Yasutan,[1,2] Toshiro Niki,[6] Yugo Ashino,[1,2] Yasuhiko Suzuki,[5] Elisabeth Telan,[4] and Toshio Hattori[1,2,7]

[1] Division of Disaster-Related Infectious Diseases, International Research Institute of Disaster Science, Tohoku University, 1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan
[2] Division of Emerging Infectious Diseases, Graduate School of Medicine, Tohoku University, 1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan
[3] Japan International Corporation of Welfare Services, 2-3-20 Toranomon YHK Building 4F, Toranomon, Minato-ku, Tokyo 105-0001, Japan
[4] STD AIDS Cooperative Central Laboratory, San Lazaro Hospital, Quiricada Street, 1003 Manila, Philippines
[5] Division of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University, North 20, West 10 Kita-ku, Sapporo 001-0020, Japan
[6] Research Division, GalPharma Company, Ltd., NEXT-Kagawa 204, 2217-44 Hayashi-cho, Takamatsu-shi, Kagawa 760-0301, Japan
[7] Division of Disaster-Related Infectious Diseases, International Research Institute of Disaster Science, Tohoku University, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan

Correspondence should be addressed to Toshio Hattori; toshatto@med.tohoku.ac.jp

Tuberculosis (TB) is the second most common cause of death from infectious diseases and results in high socioeconomic losses to many countries. Proper diagnosis is the first step in TB eradication. To develop a rapid, simple, and accurate diagnostic TB test and to characterize the prevalence of *Mycobacterium tuberculosis* (MTB) genotypes and immune profiles of TB patients, a total of 37 TB patients and 30 healthy control (HC) from Metro Manila were enrolled. Loop-mediated isothermal amplification (LAMP) reliably detected MTB infection. Manila genotype was identified by spoligotyping method in all TB patients. Osteopontin (OPN), interferon-γ-induced protein 10 kDa (IP-10), and neutrophil counts were found to reflect the acute stage of MTB infection. The sensitivity and specificity were 94.6% and 93.3%, respectively, for both OPN and IP-10, and they were 83.8% and 78.6%, respectively, for neutrophils. The combination of OPN, IP-10, neutrophil count, IL-6, IL-8, TNF-α, MCP-1, platelets, galectin-9, and leukocyte count correctly identifies all the HC and 96.3% of TB patients. LAMP method may serve as a rapid, supportive method in addition to time-consuming culture methods. OPN, IP-10, and neutrophil counts are useful in detecting MTB infection and may have utility in monitoring the course of the disease.

1. Introduction

Mycobacterium tuberculosis (MTB) is the causative agent of tuberculosis (TB), the sixth leading cause of death and illness in the Philippines. Despite global trends towards decreasing incidence, prevalence, and mortality associated with MTB infection, approximately 230,000 cases were found in the Philippines alone in 2012 [1]. The elderly, urban poor, smokers, and individuals with compromised immune systems, such as those living with HIV, malnutrition, and diabetes, belong to the high-risk group [2]. Beyond the health burdens associated with MTB, serious socioeconomic losses are another consequence of this disease.

Developing a rapid, simple, and accurate test for TB diagnosis is a main focus of many investigators. In low-resource countries, TB is most often diagnosed based on clinical symptoms, radiographic features, and microscopic observation of acid fast bacillus (AFB). Bacterial culture methods require long culturing time to obtain acceptable sensitivity, which delays early initiation of anti-TB treatment.

In recent years, nucleic acid amplification (NAA) tests have shown potential as the optimal TB diagnostic approach for MTB diagnosis. Loop-mediated isothermal amplification (LAMP) is an NAA method that enables the detection of trace amounts of bacterial DNA under isothermal conditions within one hour [3].

Osteopontin (OPN) is a multifunctional phosphorylated glycoprotein that is synthesized by a variety of immune and nonimmune cells [4, 5]. Increased levels of OPN have been observed during MTB infection and other infectious pathogens [4, 6, 7], polarizing the immune response towards a T_h1 response through the enhancement of IL-12 and IFN-γ secretion [4, 8].

Galectin-9 (gal-9) is a β-galactoside-binding matricellular protein that induces cell activation, chemoattraction, and cell death. Gal-9, binding to its receptor, T-cell immunoglobulin and mucin domain-containing molecule-3 (Tim-3), stimulates bactericidal activity in mouse TB models [9, 10]. Gal-9 and Tim-3 expression in CD4+ and CD8+ T-cells may be elevated during TB infection in humans, compared to healthy individuals [11]; however, their concentrations in plasma have not been reported until now.

Interferon-γ induced protein 10 kDa (IP-10) is one of the most well studied biomarkers in TB infection and is a promising alternative marker for replacing current interferon-γ releasing assay-based methods [12]. IP-10 is involved in multiple biological functions, inducing chemotaxis, apoptosis, inhibition of cell growth, and recruiting activated T-cells, macrophages, and NK cells to sides of infection [12].

Relatively little attention has been paid to the role of neutrophils during MTB infection, compared to macrophages and other host immune response components. Neutrophils elicit strong phagocytic activity [13] and neutrophil-driven, interferon (IFN)-inducible transcript signatures in whole human blood, which were recently shown to be associated with disease severity, suggesting a direct contribution of neutrophils to pathogenesis [14]. Other studies have demonstrated that neutrophils contribute to early defense responses against MTB [15, 16], but in later stages of the disease, an opposite tendency is observed [17, 18].

In the present study, we evaluated the reliability of LAMP for detecting MTB infection and used spoligotyping to identify the most prevalent MTB genotype in Metro Manila. We also analyzed a broad spectrum of biomarkers, which reflect both cellular and humoral immune response to MTB infection. Our results confirmed the utility of the LAMP and spoligotyping methods for TB diagnostics and genotyping and showed that OPN, IP-10, and neutrophil counts reflect the acute stage of disease and are promising biomarkers to monitor the course of the disease.

2. Materials and Methods

2.1. Participants. The study population consisted of 37 HIV-negative patients randomly selected from the out-patient department of San Lazaro Hospital (SLH, Manila, Philippines) who had positive AFB staining, clinical symptoms, and chest radiographs characteristic of pulmonary TB and had no

prior history of TB treatment. The controls were healthy volunteers lacking signs of TB; however, their histories of latent tuberculosis infection (LTBI) or other pulmonary diseases are unknown. The study was approved by the ethics committee of SLH and the Tohoku University Hospital, and written informed consent was obtained from each participant.

2.2. Sample Collection. Blood was collected in 5 mL EDTA tubes and plasma was separated by centrifugation and stored at −80°C until analyzed. Sputa were decontaminated by conventional procedures using NALC-NaOH. After centrifugation, supernatants were discarded and DNA was released from cell pellets by heating at 95°C for 5 min, which was repeated three times.

2.3. Clinical Data and Biomarker Measurement. Complete blood counts (CBC), including differential counts of white blood cells and plasma levels of IgG and IgA, were performed for samples from each individual. Plasma levels of 29 cytokines and chemokines were measured using the Milliplex MAP (Millipore, Germany). The Bio-Plex Manager Software (version 6.0) was used for bead acquisition and analysis. OPN protein levels were measured using the Human Osteopontin Quantikine ELISA Kit (R&D Systems, USA) and gal-9 levels by an ELISA kit (GalPharma, Japan). Antitubercular glycolipid (TBGL) IgG and IgA antibody titers were obtained using the Determiner TBGL Antibody ELISA Kit (Kyowa Medex, Japan).

2.4. LAMP Method. LAMP reactions were performed in reaction mixture (25 μL) containing 6 primers [3], 1.4 mM deoxynucleoside triphosphates, 0.8 M betaine, 20 mM Tris/HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 8 mM $MgSO_4$, 8 U *Bst* DNA polymerase (New England Biolabs), and 5 μL of bacterial DNA. The mixture was incubated at 64°C for 60 min in a Loopamp real-time turbidimeter (LA-200, Teramecs). Reactions were considered positive when turbidity was greater than 0.1 (650 nm) within 60 min.

2.5. Spoligotyping Method. Spoligotypes of clinical MTB isolates were determined as described previously [19]. Briefly, the DR region was amplified with a primer pair, and the PCR products were hybridized to a set of 43 spacer-specific oligonucleotide probes, which were covalently bound to membranes. The spoligo-international type was determined by comparing spoligotypes against the international spoligotyping database [20].

2.6. Statistical Analysis. The Mann-Whitney U-test was used to compare the differences between the two groups, and the Spearman's rank correlation was used to analyze relationships between biomarkers and other parameters. Statistical analyses were performed using the GraphPad Prism program (version 6, GraphPad Software, USA) and the SPSS program (version 22, SPSS Inc., USA). Discriminant function analyses and Receiver operator characteristic (ROC) curve analyses were used to evaluate the predictive capacities of biomarkers. Area under the curve (AUC) values were calculated, and cut-off values were estimated based on the best proportion

TABLE 1: Characteristics of HC and TB individuals.

	HC ($n = 30$)	TB ($n = 37$)	P value
Antropometric data			
Age year; median (range)	28.5 (22–59)	40 (18–60)	ns
Gender: male; n (%)	9 (30)	23 (62.2)	**
BMI	na	20.42 (14–44.5)	
Employed (%)	100	48.4	
Had contact with TB (%)	0	48.6	
Clinical symptoms			
Cough (%)	na	94.4	
Fever (%)	na	77.8	
Sweat (%)	na	66.7	
Loss of appetite (%)	na	25	
Radiographic features			
Site of pathological finding			
One lung (%)	na	31.2	
Both lungs (%)	na	68.8	
Cavity (%)	na	46.9	
Effusion (%)	na	6.3	
Laboratory findings: median (range)			
RBC ($10^6/\mu$L)	4.8 (4.3–6.5)	4.6 (3–6.2)	*
Hemoglobin (g/dL)	13.8 (12.2–17.2)	12.5 (8.3–16)	****
Hematocrit (%)	42.2 (39.1–51.8)	37.8 (25.6–49.6)	****
WBC ($10^3/\mu$L)	6.8 (4.4–11.8)	9.1 (4.4–19.6)	****
Neutrophil (%)	52.5 (37–67)	70 (49–85)	****
Lymphocyte (%)	38.5 (24–68)	19 (6–33)	****
Monocyte (%)	5 (2–11)	8 (3–13)	****
Eosinophil (%)	3.5 (1–8)	3 (1–10)	ns
Platelet ($10^3/\mu$L)	305.5 (178–560)	467 (143–784)	****
Plasma IgG (mg/dL)	1134 (659–3266)	1618 (934–2456)	**
Plasma IgA (mg/dL)	329 (115–973)	512 (162–1126)	**

$^*P < 0.05, ^{**}P < 0.01, ^{***}P < 0.001, ^{****}P < 0.0001$.
TB group versus HC group by Mann-Whitney U-test.
ns: not significant, na: not applicable.

between sensitivity, specificity, and likelihood ratio. P values < 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of Study Participants. A total of 37 TB patients and 30 healthy controls (HC) were enrolled in this study. Basic anthropometric and clinical characteristics of study participants are shown in Table 1. Chest lung X-rays showed bilateral pathology in 68.8% of cases, and only one lung was affected in 31.2% of cases. Lung cavities and effusion were found in 47% and 6.3% of TB patients, respectively. Laboratory findings revealed lower red blood counts, hemoglobin levels, hematocrits, and relative lymphocyte counts in the TB group compared to the HC group. Absolute numbers of white blood cells (WBC) and relative counts of neutrophils, monocytes, platelets, and plasma IgG and IgA were higher in TB patients (Table 1). The absolute and

relative values of each type of WBC were strongly correlated (data not shown).

3.2. LAMP and Genotyping. We confirmed MTB infection in all 37 patients' samples by the LAMP method. All DNA samples were subjected to spoligotyping to identify the most prevalent genotype. We found that all patients were infected with a Manila type MTB.

3.3. Comparison of Plasma Biomarker Concentrations between TB and HC Individuals. Using a multiplex immunoassay, we assessed the concentrations of 29 soluble plasma biomarkers in HC, as well as TB patients before antituberculous therapy was initiated. Biomarker levels and their comparisons between groups are shown in Table 2. Significantly elevated concentrations of IL-1α, TNF-α, IL-6, eotaxin, IL-8, IP-10, and MCP-1 were observed with the TB group. IL-1β, IL-2, IL-3, IL-15, IL-17A, IL-4, IL-5, IL-13, MIP-1α, TNF-β, and IL-10 levels were not considered for statistical analyses

TABLE 2: Comparison of cellular and humoral immunity biomarkers between HC and TB individuals.

Biomarker (pg/mL)	HC	TB	P
		Median (range)	
General activation			
IL-1α	0 (0–51.7)	6.8 (0–40.7)	**
IL-1RA	39.7 (0–374.9)	34.7 (0–230.9)	ns
TNF-α	4.2 (0–15.2)	9.6 (0–60.5)	****
IFN-α	40.6 (0–128.5)	23.6 (3.9–157.9)	ns
IL-6	0 (0–23.4)	6.6 (0–41.4)	****
Th1 related			
IFN-γ	9.3 (0–35.2)	12.7 (0–60.8)	ns
IL-12p40	31.4 (0–140.6)	14 (0–249)	ns
IL-12p70	13.5 (0–73.9)	6.7 (0–58.2)	ns
Bone marrow derived			
IL-7	11 (0–34.9)	9.6 (0–25.4)	ns
GM-CSF	7.6 (0.8–23.0)	5.1 (0–49.2)	ns
G-CSF	144.1 (19.3–458.0)	129.4 (0–310.4)	ns
Stromal, angiogenic			
VEGF	128 (0–344.1)	178.4 (0–1287.3)	ns
EGF	109.8 (42.3–488.2)	109.8 (0–459.5)	ns
Chemokine			
IL-8	2.3 (0–10)	8 (0–61.1)	****
IP-10	242.6 (97.8–453.3)	1290 (0–9235)	****
MCP-1	87.1 (48.7–154.5)	128.5 (41.6–280.5)	****
MIP-1β	31.9 (0–51.9)	33.3 (0–58.8)	ns
Eotaxin	38.5 (18.1–112.6)	48 (20.7–133.8)	*
Matricellular protein			
OPN (ng/mL)	69 (40–118.9)	159 (28.9–256)	****
Gal-9	195.8 (108–507.6)	377 (0–2181)	****
Antibody			
Anti-TBGL IgG (U/mL)	2.3 (0.5–37.3)	9.3 (0–64.2)	**
Anti-TBGL IgA (U/mL)	1 (0.2–76)	2.6 (0–57.6)	*

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, ns: not significant.
TB group versus HC group by Mann-Whitney U-test.

because their median was below minimum detectable levels in both groups. We measured also the plasma levels of two matricellular proteins, OPN and gal-9, and found statistically higher levels of both proteins in TB patients compared to HC individuals. Higher titers of TBGL IgG and IgA were found in TB patients. In HC, antitubercular antibodies were correlated with age, anti-TBGL IgG ($r = 0.66$, $P < 0.00001$), and IgA and ($r = 0.327$, $P < 0.05$); however, this relationship was not observed in the TB group (data not shown). Anti-TBGL IgG correlated with anti-TBGL IgA ($r = 0.333$, $P < 0.05$) in the TB group but not in the HC group (data not shown). Anti-TBGL antibody elevation was not related to a general elevation of antibodies in the plasma (data not shown).

3.4. Correlation of OPN, IP-10, Neutrophil Count, and Gal-9 in HC and TB Groups. We performed correlation analyses of the three most predictive TB markers with other measured parameters. IP-10 expression levels positively correlated with loss of appetite, IL-1α, IL-6, IL-8, OPN, and gal-9 expression

and negatively correlated with anti-TBGL IgG antibody titer (Table 3). In addition, OPN and IL-8 expression levels were correlated (Table 3). TB patients' neutrophilia was associated with increased WBC counts, IFN-γ, and decreased hemoglobin levels, hematocrits, plasma IgG titers, and lymphocytopenia (Table 3). Finally, we studied the relationship of gal-9 to biomarkers and other laboratory parameters. High gal-9 levels correlated with higher TNF-α, IL-6, IL-5, IL-3, EGF, IL-8, and IP-10 expression and lower levels of GM-CSF (Table 3).

3.5. Discrimination Potential of Biomarkers. Biomarkers that differentiated HC and TB were analyzed by ROC analysis and cut-off values were determined based on the best proportion between sensitivity, specificity, and likelihood ratio (Table 4). The highest discriminatory property had IP-10 and OPN, followed by neutrophils, platelets, TNF-α, MCP-1, leukocyte counts, gal-9, and IL-8. Next, we performed analysis, which is a multivariate discrimination method to characterize the

TABLE 3: Correlation analysis of IP-10, neutrophil, OPN, and gal-9 with other biomarkers in TB patients.

CBC/clinical parameter/biomarker	IP-10		Neutrophil		OPN		Gal-9	
	r	P	r	P	r	P	r	P
CBC								
Hemoglobin			−0.375	*				
Hematocrit			−0.415	*				
WBC			0.684	* * **				
Lymphocyte			−0.931	* * **				
Plasma IgG			−0.455	*				
Clinical parameter								
Loss of appetite	0.466	**						
General activation								
IL-1α	0.687	* * **						
TNF-α							0.39	**
IL-6	0.367	*					0.309	*
Th1 related								
IFN-γ			0.364	*				
Bone marrow derived								
GM-CSF							−0.335	*
Chemokine								
IL-8	0.4635	**			0.44	**	0.4635	**
IP-10					0.422	**	0.295	*
Matricellular protein								
OPN	0.422	**						
Gal-9	0.393	*						
Antibody								
Anti-TBGL IgG	−0.423	**						

P values of Spearman's rank correlation are expressed as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

TABLE 4: Statistic data of ROC analysis of studied biomarkers.

Biomarker	AUC	SE	Cut-off	Sensitivity %	Specificity %	Likelihood ratio
IP-10	0.987	0.009	>342	94.6	93.3	14.1
OPN	0.966	0.027	>94	94.6	93.3	14.1
Neutrophil	0.905	0.036	>57.5	83.8	78.6	3.9
Platelet	0.799	0.055	>339	75.7	80	3.8
TNF-α	0.796	0.057	>5.99	81.1	70	2.7
MCP-1	0.786	0.056	>92	75.7	63.3	2.1
WBC	0.784	0.057	>7.5	81.1	70	2.7
Gal-9	0.766	0.063	>258	75.7	80	3.8
IL-8	0.752	0.063	>3.5	75.7	76.7	3.2
Anti-TBGL IgG	0.726	0.063	>4	67.6	70	2.3
Anti-TBGL IgA	0.667	0.069	>1.7	70.3	66.7	2.1

most discriminatory variables among groups. OPN was identified as having the highest discriminatory capacity, followed by IP-10, neutrophils, IL-6, IL-8, TNF-α, MCP-1, platelets, gal-9, and WBC. Using a combination of these markers, all healthy individuals and 96.3% of TB patients were correctly classified.

4. Discussion

Despite its low sensitivity, AFB staining is often used in attempt to differentiate mycobacterial infection from other pulmonary bacterial infections. Bacterial culture methods, the QuantiFERON TB test, and PCR have improved the

sensitivity and sensitivity of TB detection but are time-consuming and require trained laboratory workers. Recently, we developed a LAMP method for detecting MTB with excellent accuracy in one hour [3]. Here, we confirmed MTB infection in all of our samples by LAMP; bacterial cultures were not performed because previous studies showed that MTB-LAMP sensitivity in culture positive samples reached 100% and that specificity in culture negative samples was 94.2% [3]. Moreover, the research group from Thailand found that, in the clinical unknown samples test, the sensitivity of LAMP method was 98.92% and the specificity was 100% compared to those of the standard culture assay [21]. Samples were also studied by spoligotyping to determine the most prevalent circulating strains in Metro Manila. We found that all of our TB patients were infected with a Manila type of MTB and this finding of a uniformed genotype of MTB was rather surprising. The arrival of Chinese, Japanese, and Spanish groups may have influenced the acquisition of various MTB genotypes in the region; however, little information is available regarding prevalent MTB genotypes circulating in the Philippines.

The role of matricellular proteins supporting TB infection is not well studied. We found high plasma levels of two matricellular proteins, OPN, and, for the first time, gal-9 in treatment-naïve TB patients. Matricellular proteins are secreted into the extracellular matrix environment but do not play a primary structural role in this location and regulate an unusually diverse array of cellular functions, including cell adhesion, shape, migration, differentiation, proliferation, and inflammatory responses [22]. It has been proposed that matricellular proteins enter inflamed tissue and become immobilized at that site to generate signals for phagocytosis and chemotaxis of inflammatory cells [23]. OPN is highly expressed in tuberculous granuloma and supports granuloma formation via its functions as a chemoattractant cytokine [24]. OPN and gal-9 may be produced by activated circulating immune cells, but a more plausible explanation is that they are released into the circulation from tissue sites. Our unpublished data showed elevated pleural fluid/plasma ratios of gal-9 and OPN in a TB patient, and Inomata et al. showed that OPN levels increase proportionally with the extent of lung lesions [25]. We evaluate differences between patients with and without granuloma formation, factoring in the extent of lesions and other clinical parameters; however, we did not observe any statistical correlations, probably because radiological findings with most patients were not suggestive of granulomas. We found that the OPN and gal-9 correlated with IL-8 and IP-10 and we hypothesize that OPN and gal-9 activate the expression of chemokines and cytokines like IP-10, TNF-α, IL-6, and IL-8 in macrophages, helping to recruit immune cells to the site of MTB infection. This speculation is supported by observations from Bai et al., who found that matricellular protein CCN1 enhances the expression of TNF-α, IL-1β, IL-6, IFN-γ, MIP-1α, and IP-10 in murine macrophages through its receptors $\alpha_M\beta_2$ and syndecan-4 [22]. IP-10 is produced at sites of inflammation and its blood levels reportedly correlate with the extent of inflammatory process [26, 27]. In contrast with low circulating levels of IFN-γ, the high levels of IP-10 in the blood produced by antigen-stimulated cells make this chemokine a promising candidate

biomarker for MTB infection, even in HIV-infected individuals [28, 29]. We found that patients with high plasma IP-10 reported loss of appetite, but such a tendency was not observed with other biomarkers. Juffermans et al. had similar observations, where IP-10 but not IL-8, MIP-1b, or MCP-1 was associated with loss of appetite and fever [30].

Neutrophils, one of the most predictive markers of active TB infection, were found to have the largest influence on WBC elevation in TB patients. Neutrophils are generally thought to have strong activity against infectious agents. Higher neutrophil counts were observed in TB patients and were associated with poorer prognosis [31]. Neutrophils are attracted by various cytokines and chemokines, including IL-8, and quickly accumulate at sites of mycobacterial infection [32–34]. We also observed IL-8 elevation in TB patients, which may be due to hyperproduction by stimulated macrophages, by epithelial cells in lungs, or even by neutrophils themselves, although no correlation with neutrophils was observed. Previous study showed that low lymphocyte count is suggestive of extension of the disease by new tubercle formation, but switch to lymphocytosis shows tendency to healing [35]. Therefore, careful evaluation of hematological changes may have diagnostic value.

It is generally accepted that cell-mediated immunity has a critical role in the protection against MTB. However, MTB is a facultative intracellular pathogen, which has both intracellular and extracellular phases in their infectious cycle. Recent studies showed that immunization using monoclonal and polyclonal antibodies and mucosal vaccination trials demonstrate convincingly the essential interdependence and synergy between cell-mediated immunity and humoral immunity [36]. WHO warned against the utility of current serological tests in the immunodiagnosis of TB and strongly recommended that they must not be used for the diagnosis of pulmonary and extrapulmonary TB, but we believe that detection of anti-TBGL antibody titers may serve as a warrant signal of MTB infection.

In our study, TB patients represented a small group of individuals who underwent the examination due to suspected TB disease. In all of the patients, only Manila genotype was identified; therefore, it would be of interest to study the immunological profile of TB patients infected by other MTB genotypes. Future, larger sample size studies should prove the application of LAMP method and proposed biomarkers in TB research.

In conclusion, LAMP is a simple and low-cost method for the direct detection of MTB in patients' sputa. Moreover, the LAMP method together with spoligotyping method serves as sensitive, accurate tools for TB diagnosis and for monitoring prevalent lineages of TB in certain regions. Increased IP-10, OPN, and neutrophils levels best reflect the acute stage of TB infection, and measuring their fluctuations may provide a reasonable basis for determining TB severity and prognosis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Acknowledgments

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