Current Respiratory Medicine Reviews, 2018, *14*, 100-104

RESEARCH ARTICLE

Sarcoidosis or Tuberculosis? Detecting *Mycobacterium tuberculosis* Complex DNA in Sarcoidosis Granulomas

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Abstract: ***Background*:** Sarcoidosis is a granulomatous inflammatory disorder with unknown etiology and its relation with *Mycobacterium tuberculosis* (*M. tuberculosis*) has been debated for years. In this study, we have investigated the presence of mycobacterial DNA in sarcoidosis tissue samples.

***Methods*:** Formalin-Fixed Paraffin-Embedded (FFPE) tissues of 33 patients with sarcoidosis were analyzed for the presence of mycobacterial DNA. Genomic DNA extraction was done by QIAamp DNA FFPE Tissue Kit. Polymerase chain reaction using insertion element IS6110 of *M. tuberculosis* complex (MTC) was applied by commercial kits (GeneProof) for all individuals. The results were compared with 27 patients with tuberculosis and 5 other patients associated with granulomatous disease of the lung. All cases had confirmed granulomatous inflammation in their histopathological examination.

***Results*:** In this study, the IS6110 repetitive DNA element of (MTC) was not detected in any of the tissue samples from the patients with sarcoidosis. Of the 33 sarcoidosis patients, 30 (90.1%) had negative results for IS6110 and despite the repeated attempts of DNA extraction for three patients (9.1%), strong inhibitor made constant negative outcomes. In contrast, in patients with tuberculosis, 22 (81.5%) had positive results, three had (11.1%) negative results and 2 patients (7.4%) showed negative results with strong inhibitor. IS6110 was not found in any of the control group patients.

***Discussion*:** This study does not support the presence of *M. tuberculosis* in tissues of patients with sarcoidosis as a microbial pathogen or trigger of the immune response. Due to difficulties in diagnosis of sarcoidosis and different methods for diagnosis of *M. tuberculosis*, the impact of *M. tuberculosis* as a possible aetiological agent in sarcoidosis has been the point of debate.

ARTICLE HISTORY

Received: April 19, 2018
Revised: June 14, 2018
Accepted: June 22, 2018

DOI:
10.2174/1573398X14666180628124838

Keywords: Real-time PCR, sarcoidosis, tuberculosis, *Mycobacterium tuberculosis,* complex DNA, inflammatory disorder.

1. INTRODUCTION

Sarcoidosis is a systemic disorder with heterogeneous manifestation. Although pulmonary signs are predominantly seen, half of the cases show asymptomatic condition [1]. Despite the fact that more than one century has passed in describing this disease [2], diagnostic criteria, treatment algorithms, methods of follow-up and the natural course of the disease has not been properly described [3].

Although there is no single test for diagnosis of sarcoidosis, there are sets of clinical findings that can considerably be different according to organ involvement in the disease course [1]. Radiological findings such as hilar adenopathy or serologic findings including elevation of Serum Angiotensin Converting Enzyme (SACE), are also important besides the clinical and histological pattern [4, 5]. Histopathological findings consistent with sarcoidosis are granulomas that are usually non-caseating with collar lymphocyte infiltration [6, 7]; considering the fact that the other pathogens should be ruled out [8].

Despite considerable study on the pathogenesis of sarcoidosis, the etiopathogenesis is not well described. However, it is acceptable that immunopathogenesis of sarcoidosis is an interaction between extrinsic antigens, genetic factors and immune response [9, 10]. Microbial

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1875-6387/18 $58.00+.00

agents always have been suspected as important trigger antigens. Among the infectious agents, *Mycobacterium tuberculosis* (*M. tuberculosis*) infection has clinical, radiological and histological similarities with sarcoidosis, and it has been usually considered as an important differential diagnosis especially in pulmonary sarcoidosis [11, 12]. In a meta-analysis study from 1980 to 2006, of 874 sarcoidosis tissue samples that were examined for mycobacterium DNA by PCR (with their methods approved in the meta-analysis), 187 cases (21.4%) for *Mycobacterium tuberculosis* complex (MTC), 43 (4.9%) for the nontuberculous mycobacteria (NTM) and overall 231 cases (26.4%) indicated positive results for genomic DNA from Mycobacteria [13]. Meanwhile in some studies, MTC DNA was not confirmed in sarcoidosis tissue [14, 15].

So, with regard to the importance of MTC DNA in sarcoidosis tissue, differential diagnosis with Tuberculosis (TB) and immunopathogenicity of the disease, for the first time in Iran, we designed a retrospective study of sarcoidosis patients referred to an out-patient clinic of sarcoidosis in a tertiary referral hospital for pulmonary disease in Tehran. The study was conducted according to the target IS6110 for MTC on confirmed sarcoidosis patients according to clinical evaluation and follow-up data.

2. MATERIALS AND METHODS

2.1. Patient Selection

A total of 102 registered sarcoidosis patients from the outpatient clinic of Masih Daneshvari Hospital, Tehran-Iran were evaluated. These patients were confirmed sarcoidosis cases in terms of clinical presentation, radiological, histological, serological findings, and also according to clinical follow-up. Of these, 33 patients, with their Formalin-Fixed Paraffin-Embedded (FFPE) tissues available in the archive of Pathology department, entered the study. All of these selected patients had granulomatous inflammation in the histopathological analysis.

Sarcoidosis patients were compared with 27 confirmed cases of tuberculosis that had evidence of granulomatous inflammation on histopathology. Also in this study, a control group consisting of 5 patients including fungal infection (3 cases of aspergilloma), foreign bodies and hydatid cyst of the lung were registered. All 5 control cases presented definitive diagnosis and tuberculosis had been ruled out for them. Patients with sarcoidosis and tuberculosis were reviewed based on clinical, radiological, and histopathological records and the following up evaluation, by at least two pulmonary and infectious specialists. To select the patients, the following characteristics were considered for sarcoidosis patients.

The first is clinical findings consistent with sarcoidosis such as a severe pulmonary disease associated with erythema nodosum, hilar adenopathy and arthritis (Lofgren's syndrome) or slowly progressive pulmonary decompensation associated with radiology findings like hilar adenopathy, reticulonodular infiltration or pulmonary fibrosis [4]. The second is histopathological findings consistent with sarcoidosis as non-caseating granuloma, well circumscribed with surrounding tissue with varying amounts of peripheral lymphatic infiltration [5]. The third is to rule out microbial agents such as mycobacterial infection. For this reason, medical records, follow-up evaluation and treatment response of the patients were reviewed. Patients with any clinical suspicion were excluded.

Patients with tuberculosis were enrolled in this study, based on the history of positive sputum smear or culture for Koch bacillus, positive Ziehl-Neelsen staining, or positive-MTC DNA with clinical response to anti-TB drugs [16]. This study was conducted with the approval of Masih Daneshvari Hospital Ethic Committee and utilizing protocols approved by the respective institutional review boards (SBMU1.REC.1393.60).

2.2. DNA Extraction

DNA was extracted from FFPE using QIAamp DNA FFPE Tissue Kit (Qiagen Hilden Germany) according to manufacturer's structure. In summary, FFPE tissue blocks were cut into 5-micron sections and deparaffinization was done using 2 times wash of Xylene and then followed by 2 times wash of absolute ethanol. After deparaffinization, the sample was mixed with 20 µl of proteinase K and 180 µl of ATL buffer and then left in thermoblock on 56 ºC for overnight. After digestion, the sample was mixed properly with 200 µl AL buffer and 200 µl of absolute ethanol, then transferred in a Qiagen FFPE spin column. The spin column was washed two times and DNA was eluted with 50-100 µl of AE buffer.

2.3. Real-Time PCR

Mycobacterial DNA was detected by targeting specific multicopy insertion sequence, IS6110 segment and PCR products were qualified by fluorophore-labeled probes using *M. tuberculosis* Gene proof kit and finally monitored by StepOnePlus™ Real-Time PCR Systems.

This kit detects *M. tuberculosis* complex strains including *M. tuberculosis, M. bovis, M. africanum, and M. microti* and amplification can occur with vaccination strain like BCG. Amplification of IS6110 segment from M. tuberculosis was monitored by increasing fluorescence signal *in* FAM channel. To qualify DNA extraction from inhibitors, amplification of a single copy of internal standard was simultaneously detected in HEX channel (Fig. **1**). Hot start technology was used in this kit to reduce non-specific amplification and increase the sensitivity. The mix was ready to use and contained Uracil-DNA-glycosylase (UDG) to diminish contamination. Using multi-copy insertion sequence gene detection methods is 16 times more sensitive than conventional-single copy.

2.4. Results

Of total 33 tissue samples of sarcoidosis patients, 18 (54.5%) cases were female with a mean age of 45±1.04 SD. Also, 27 studied patients with tuberculosis revealed 13 (48.1%) female cases with a mean age of 37±7.09 SD. Tissue samples in 4 (12.1%) sarcoidosis cases were mediastinal lymph nodes, 2 cases (6%) of bronchial biopsy and the rest were 27 (81.8%) transbronchial lung biopsy (TBLB). Also, samples of tuberculosis patients were 4 pleural tissues (14.8%), 6 cases of lymph nodes (22.2%) and the rest were 17 cases (62.9%) of TBLB.

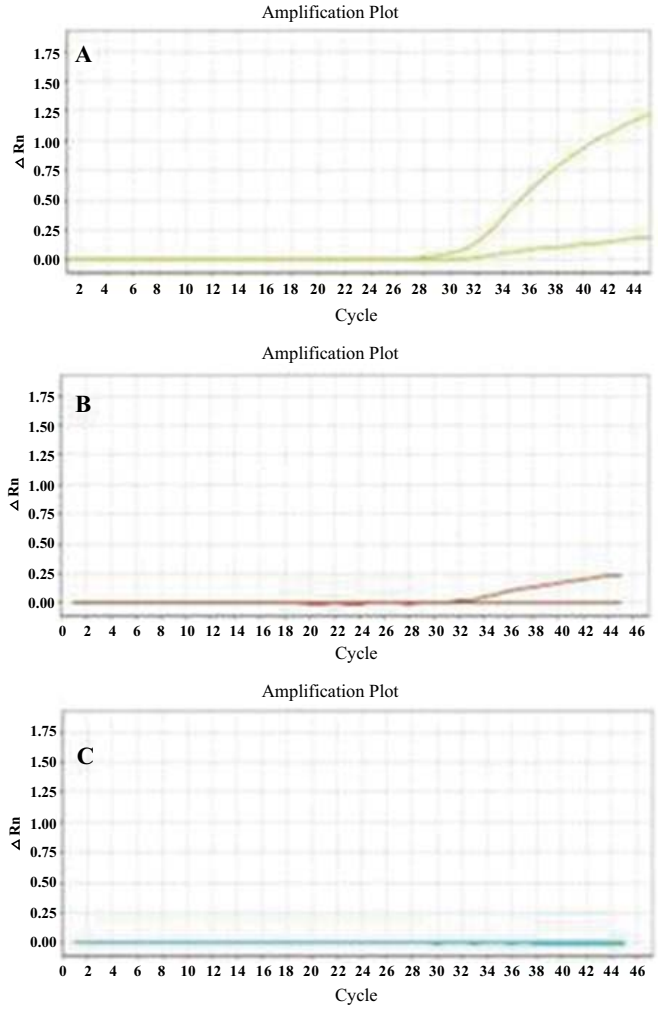

Fig. (1). Detection of MTC DNA and genomic DNA using commercial Taqman probe. **A**) Both MTC DNA and genomic DNA (Internal control) were amplified and those fluorescence emissions were simultaneously detected in FAM and HEX channels for MTC DNA and genomic DNA, respectively. **B**) MTC DNA was found and detected in FAM channel, but genomic DNA was not found in HEX, indicating negative result. **C**) Both MTC DNA and genomic DNA were negative and no amplification step was seen, as one strong PCR inhibitor exists.

All sarcoidosis patients were examined for Acid-Fast Bacilli (AFB) sputum smear and culture; with negative reports for all patients. During the admission, PCR targeting IS6110 of *M. tuberculosis* was performed for 7 (21.2%) cases of sarcoidosis. All 7 cases were negatively reported. This was also evaluated for 8 (29.6%) of tuberculosis patients with all of the TB patients positively detected by TB PCR technique. Table **1** summarizes hospital patient records.

In our evaluation, the TB test using Gene Proof kit targeting IS6110 by Real-time PCR was negative for all of the sarcoidosis patients. During the analysis for tuberculosis patients, 5 (11.1%) cases were negatively reported by Real-time PCR. Of those, an internal standard was positive for 3 cases, but it was negative for 2 cases after re-DNA extraction, showing that a strong inhibitor existed (Table **2**).

3. DISCUSSION

In the present study, *M. tuberculosis* complex (MTC) DNA was not detected in any of the tissues obtained from patients with sarcoidosis. The majority of the patients' tissues were TBLB which was prepared during bronchoscopy. All patients in the pathological examination showed a granulomatous reaction. According to previous studies, we expected to have positive MTC DNA in a few patients with sarcoidosis, but no positive MTC DNA was found in these individuals. A meta-analysis done by Gupta till 2006 showed that at least 30% of sarcoidosis patients had mycobacterial DNA [13].

According to the technology used in the test, the sensitivity of the test was acceptable, while most samples were positive for *M. tuberculosis* DNA in tuberculosis patients (81.5%). However, according to manufacturer's information, the sensitivity of this test is at the 0.06 genomes/μl with the probability of 95% [17] with DNA which is obtained from the cultured colony (that is very high), but, this sensitivity of the DNA is not measurable for FFPE tissue and comparison of these two samples is wrong.

Despite the theory for the impact of pathogens in the immunological process resulting in sarcoidosis as a granulomatous disorder, micro-organisms are not usually found in stained or cultured sarcoid granulomas [18]. Although molecular advances in the study of bacterial DNA

Table 1. Demographic characteristics of patients and controls with a review of admission investigation for identifying *Mycobacterium tuberculosis.*

Patient Group	No.	Female Sex (%)	Median Age (±Sd)	Positive Sputum Smear (%)	Positive Sputum Culture (%)	Positive Biopsy Culture (%)	Positive Mtb PCR/Total
Sarcoidosis	**33**	**18(54.5)**	*45±1.04*	**0**	**0**	**0**	**0/7**
Tuberculosis	**27**	**13(48.1)**	*37±7.09*	**4(14.8)**	**7(25.9)**	**4(14.8)**	**15/20**
Control	**5**	**3(60)**	*47±7.09*	**0**	**0**	**0**	**0/5**

Table 2. IS6110 amplification results in Sarcoidosis, Tuberculosis and control group.

Patient Group	No.	PCR Positive (IC Positive)	PCR Negative (IC Positive)	PCR Negative (IC Negative)
Sarcoidosis	**33**	**0**	**30(90.9%)**	**3(9.1%)**
Tuberculosis	**27**	**22(81.5%)**	**3(11.1%)**	**2(7.4%)**
Control	**5**	**0**	**5(100%)**	**0**

have shown the existence of MTC DNA in many cases of sarcoidosis tissues [13, 19, 20], it is still not confirmed in other cases [21-23].

Hence, the relation of tuberculosis and sarcoidosis is still debated; it should also be considered that the boundary between tuberculosis and sarcoidosis has not been properly explained. As an example, there are some reports over the last 50 years that mycobacterial bacilli are reported in sarcoidosis tissues [24, 25] which could be considered as confirmed tuberculosis case.

On the other hand, some authors believe that both sarcoidosis and tuberculosis are the opposite ends of the same disease [26], which can present as tuberculosis, tuberculosis sarcoid, sarcoid tuberculosis and finally sarcoidosis. Positive MTC DNA in the lung granuloma tissue with pulmonary symptoms can be considered as a confirmed case of tuberculosis and should be treated as in WHO 2013 revision; any method for bacteriological confirmation including molecular techniques was approved as case definition for tuberculosis [27].

Gupta *et al.* reported the detection of mycobacterial genomic DNA from zero up to 100% in various study/sample and techniques [13]. One explanation for this variety of results could be related to the epidemiology of tuberculosis. High levels of MTC DNA in sarcoidosis samples from India with a high incidence of tuberculosis [13] and evidence of Propionibacterium acnes as the pathogenic bacteria for sarcoidosis in Japan, can confirm this scenario. This epidemiological relationship has been shown in some studies [28, 29].

It seems that this association will not be able to work anywhere. In a study done by Saboor *et al*. from England in 1992, of total 20 sarcoidosis cases, 14 (70%) were positive for mycobacterial DNA and of those 10 (50%) were *M. tuberculosis* and 4 (20%) were NTM [30]. Meanwhile in a study conducted by Thakker *et al*. in the same year in UK reported that of 14 sarcoidosis patients, only one case (7.1%) was positive for *M. tuberculosis* [31].

Also, tissue type, different molecular techniques, and target used for detecting genomic DNA from Mycobacteria could strongly affect the results. For example, the quality of DNA extracted from the fresh tissue is certainly much better than FFPE tissue, particularly if the tissue is left for a while in the archive [5]. In the present study, despite repeated DNA extraction for some cases with strong inhibitors, presences of inhibitors caused a false negative in the detection of *M. tuberculosis* DNA in three sarcoidosis patients (9%) and 2 cases of tuberculosis (7%). Also, targets and molecular methods that were used could impress the analysis results. In a study by Drake *et al.*, three molecular targets including IS6110, rpoB and 16SrRNA were used for tuberculosis pathogen confirmation in sarcoidosis patients [5]. In this study, no amplification was observed in 25 cases of sarcoidosis and 25 controls using the target IS6110. This is while 6 patients (24%) using target rpoB and 12 cases (48%) of target 16SrRNA were positively reported for *M. tuberculosis* DNA and the rate reached to 60% percent by sequence analysis.

On the other hand, *M. tuberculosis* without any copy of IS6110 can exist. In some studies, *M. tuberculosis* has been reported with zero or one copy of IS6110 [32, 33]. In a survey conducted at our center in 2004 by Farnia *et al.* 129 patients were examined; five cases demonstrated 1-3 copies (3.8%), 115 cases with 6-15 copies (89%) and 7 patients (5.4%) with more than 16 copies. In two patients (1.5%) no copies of was IS6110 found [34].

In summary, once again, there is evidence of absence of mycobacterial infection in tissue samples of sarcoidosis patients, which can represent a whole range of important issues from the lack of clarity of the diagnostic criteria and guidelines for sarcoidosis and differential diagnosis from tuberculosis to a variety of molecular techniques and targets besides clinical judgments. So it seems that ruling out all pathogens in the diagnosis of sarcoidosis disease and follow-up is very important in the diagnosis and treatment of sarcoidosis. While it is still challenging to accept the impact of microbial pathogens such as *M. tuberculosis* in the

initiation of sarcoidosis, this disease remains with unknown etiology.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted with the approval of Masih Daneshvari Hospital Ethic Committee and utilizing protocols approved by the respective institutional review boards (SBMU1.REC.1393.60).

HUMAN AND ANIMAL RIGHTS

No Animals were used for studies that are base of this research. All human procedures were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Human subjects used in the study provided informed consent to participate.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Wessendorf TE, Bonella F, Costabel U. Diagnosis of sarcoidosis. Clin Rev Allergy Immunol 2015; 49(1): 54-62.

[2] Sharma OP. Sarcoidosis around the world. Clin Chest Med 2008; 29(3): 357-63.

[3] Judson MA. Advances in the diagnosis and treatment of sarcoidosis. F1000prime Rep 2014; 6: 89.

[4] Allen SS, Evans W, Carlisle J, *et al*. Superoxide dismutase A antigens derived from molecular analysis of sarcoidosis granulomas elicit systemic Th-1 immune responses. Respiratory Res 2008; 9(1): 1-12.

[5] Drake WP, Pei Z, Pride DT, Collins RD, Cover TL, Blaser MJ. Molecular analysis of sarcoidosis tissues for mycobacterium species DNA. Emerging Infect Dis 2002; 8(11): 1334-41.

[6] Rosen Y, editor Pathology of sarcoidosis. Seminars in respiratory and critical care medicine; 2007: New York: Thieme Medical Publishers, c1994.

[7] Tchernev G. Cutaneous sarcoidosis: The 'Great Imitator'. Am J Clin Dermatol 2006; 7(6): 375-82.

[8] Pierce TB, Margolis M, Razzuk MA. Sarcoidosis: Still a mystery? Proceedings (Baylor University Medical Center) 2001; 14(1): 8.

[9] Haimovic A, Sanchez M, Judson MA, Prystowsky S. Sarcoidosis: A comprehensive review and update for the dermatologist: part I. Cutaneous disease. J Am Acad Dermatol 2012; 66(5): 699. e1-. e18.

[10] Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med 2007; 357(21): 2153-65.

[11] Babu K. Sarcoidosis in tuberculosis-endemic regions: India. J Ophthalmic Inflamm Infect 2013; 3(1): 53.

[12] Gupta D. Tuberculosis and sarcoidosis: The continuing enigma. Lung India 2009; 26(1): 1.

[13] Gupta D, Agarwal R, Aggarwal AN, Jindal SK. Molecular evidence for the role of mycobacteria in sarcoidosis: A meta-analysis. Eur Respir J 2007; 30(3): 508-16.

[14] Richter E, Greinert U, Kirsten D, *et al*. Assessment of mycobacterial DNA in cells and tissues of mycobacterial and sarcoid lesions. Am J Respir Crit Care Med 1996; 153(1): 375-80.

[15] Bocart D, Lecossier D, De Lassence A, Valeyre D, Battesti J-P, Hance AJ. A search for mycobacterial DNA in granulomatous tissues from patients with sarcoidosis using the polymerase chain reaction. Am Rev Respir Dis 1992; 145(5): 1142-8.

[16] http://www.cdc.gov/tb/education/corecurr/pdf/chapter4.pdf CfDCa PCCCDoTD. (Accessed on: October 4, 2018)

[17] http://www.geneproof.com/en/products/diagnostic-kits/microbiological-dna-diagnostics/geneproof-mycobacterium-tuberculosis-pcr-kit. (Accessed on: October 4, 2018)

[18] Brownell I, Ramírez-Valle F, Sanchez M, Prystowsky S. Evidence for mycobacteria in sarcoidosis. Am J Respir Cell Mol Biol 2011; 45(5): 899-905.

[19] Fite E, Fernandez-Figueras M, Prats R, Vaquero M, Morera J. High prevalence of Mycobacterium tuberculosis DNA in biopsies from sarcoidosis patients from Catalonia, Spain. Respiration 2006; 73(1): 20-6.

[20] Xu M, Zhong H, Heng W, Wu B. Detection of *Mycobacterium tuberculosis* DNA in sarcoidosis samples using real-time fluorescence polymerase chain reaction. Zhonghua Bing Li Xue Za Zhi 2006; 35(12): 735-7.

[21] Wilsher M, Menzies R, Croxson M. *Mycobacterium tuberculosis* DNA in tissues affected by sarcoidosis. Thorax 1998; 53(10): 871-4.

[22] Marcoval J, Benítez MA, Alcaide F, Mañá J. Absence of ribosomal RNA of *Mycobacterium tuberculosis* complex in sarcoidosis. Arch Dermatol 2005; 141(1): 57-9.

[23] Milman N, Lisby G, Friis S, Kemp L. Prolonged culture for mycobacteria in mediastinal lymph nodes from patients with pulmonary sarcoidosis. A negative study. Sarcoidosis Vasc Diffuse Lung Dis 2004; 21(1): 25-8.

[24] Scadding J. *Mycobacterium tuberculosis* in the aetiology of sarcoidosis. BMJ 1960; 2(5213): 1617.

[25] Ustvedt J. Necropsies in cases of sarcoidosis. Tubercle 1948; 29: 107.

[26] Agrawal R, Kee AR, Ang L, *et al*. Tuberculosis or Sarcoidosis: Opposite ends of the same disease spectrum? Tuberculosis 2016; 98: 21-6.

[27] Available from: http://apps.who.int/iris/ bitstream/10665/79199/ 1/9789241505345_eng.pdf?ua=1. WHODarfwftruDIGWHOcM. (Accessed on: October 4, 2018)

[28] Hosoda Y, Sasagawa S, Yamaguchi T. Sarcoidosis and tuberculosis: Epidemiological similarities and dissimilarities. A review of a series of studies in a Japanese work population (1941-1996) and the general population (1959-1984). Sarcoidosis Vasc Diffuse Lung Dis 2004; 21(2): 85-93.

[29] Hosoda Y, Yamaguchi M, Hiraga Y. Global epidemiology of sarcoidosis: What story do prevalence and incidence tell us? Clin Chest Med 1997; 18(4): 681-94.

[30] Saboor S, McFadden J, Johnson NM. Detection of mycobacterial DNA in sarcoidosis and tuberculosis with polymerase chain reaction. The Lancet 1992; 339(8800): 1012-5.

[31] Gerdes J, Richter E, Greinert U, *et al*. Mycobacterial nucleic acids in sarcoid lesions. The Lancet 1992; 339(8808): 1536-7.

[32] Thoe S, Tay L, Sng E. Evaluation of Amplicor-and IS6110-PCR for direct detection of Mycobacterium tuberculosis complex in Singapore. Trop Med Int Health 1997; 2(11): 1095-101.

[33] van Soolingen D, De Haas P, Hermans P, Groenen P, Van Embden J. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of *Mycobacterium tuberculosis*. J Clin Microbiol 1993; 31(8): 1987-95.

[34] Farnia P, Mohammadi F, Masjedi MR, *et al*. Evaluation of tuberculosis transmission in Tehran: Using RFLP and spoloigotyping methods. J Infect 2004; 49(2): 94-101.