Prevalence of *Pneumocystis jirovecii* among immunocompromised patients in hospitals of Tehran city, Iran

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Abstract *Pneumocystis jirovecii* is an opportunistic organism that can cause extreme complications such as *Pneumocystis* pneumonia in immunocompromised individuals. There is no comprehensive study was conducted Iran to determine the prevalence of this infection in susceptible individuals. In the present study, 160 sera samples were collected from immunocompromised patients, including acquired immunodeficiency syndrome (AIDS) patients, diabetic patients, Hodgkin lymphoma patients and non-Hodgkin lymphoma patients. The specimens were collected from Imam Khomeini and army's 501 hospitals. The specimens were examined using indirect fluorescent antibody test. The results of the study showed that 39.30% specimens were found positive, with different rates in different groups, including 20, 22.50, 37.50, and 77.50% of diabetic patients, non-Hodgkin patients, Hodgkin lymphoma patients, and AIDS patients, respectively. This occurrence is relatively high and can be a potential life-threatening hazard to infected patients in studied groups, on the other hand the organism can be transmitted from infected people to other susceptible individuals.

Keywords *Pneumocystis jirovecii* · Immunocompromised individuals · IFA · Tehran

Introduction

When *Pneumocystis jirovecii* isolated for the first time, it was called *Pneumocystis carinii* and was classified as a protozoan parasite, but after that the name of this organism was changed to *P. jirovecii* and according to sequence homologies in 18S ribosomal RNA gene, it was considered as a member of Archiascomycetes class, Ascomycota phylum, and fungal kingdom (Uemura et al. 2008; Hof 2012). Unlike healthy individuals, the organism can cause important problems in individuals with impaired immune system. In the healthy people, *Pneumocystis* infection mainly can be controlled by T cell activity, on the other hand experimental and clinical data revealed that the humoral immunity play a role in the control of the infection, too (Vahid et al. 2007; Kelly and Shellito 2010). These days, due to increasing trend of the number of immunocompromised patients such as patients with AIDS and other immunosuppressed groups like patients suffering from malignancy, patients with autoimmune disorders, and transplant recipients, *P. jirovecii* should be considered as an important health problem (Cano et al. 2004; Alvarez-Martínez et al. 2006; Rodiño et al. 2011; Hof 2012). This organism can result in severe problems with poor prognosis like *P. jirovecii* pneumonia (PCP) in susceptible groups. Some drugs such as sulfadoxine-pyrimethamine can be used as prophylaxis in at-risk groups (Post et al. 1971). Also, co-trimoxazole can be used for the treatment of the infection, particularly in the transplant recipients (Struijk et al. 2011). The first choice for diagnosis of PCP is isolation of *P. jirovecii* from clinical samples, but in the lack of the culture system diagnosis will be based on microscopic methods with conventional staining methods such as Giemsa, Grocott-Gomori methenamine silver, toluidine blue O, Calcofluor White, and direct and indirect

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fluorescence immunocytochemical staining (Sing et al. 2000; Helweg-Larsen 2004; Azoulay et al. 2009). But the most applied and common three staining methods for diagnosis of PCP are Diff-Quik (D-Q is a rapid Giemsa-like staining) (Bigby et al. 1986; Ng et al. 1989), modified toluidine blue O (TBO) (Gosey et al. 1985), and Gomori-methenamine silver (GMS) (Grocott 1955). Recently, PCR based methods have increased the sensitivity of PCP diagnosis.

Monoclonal antibodies of the mouse, that is produced against cyst wall antigens of *P. jirovecii* and can be used for indirect fluorescent-antibody (IFA) test on induced sputa (IS) and BALs samples (Kovacs et al. 1986; Gill et al. 1987; Kovacs et al. 1988). It is proven that IFA method is more sensitive than D-Q, TBO, and GMS staining methods for detection of *P. jirovecii* using IS samples in an at-risk population or patients with AIDS (Kovacs et al. 1988; Ng et al. 1990). In comparison to the above-mentioned conventional staining methods IFA has several advantages for the detection of *P. jirovecii* such as being easy and rapid. For IFA, Preparation time takes 1–2 h. In the IFA, specificity of monoclonal antibody affects the detection of the organism, because the antibodies can be specific only for cysts or be specific for cysts, trophozoites, and sporozoites. The main problem with the test is its dependence to expertness of the technician and the familiarity of the technician with the different morphology of the organism. The main aim of the present study was the determination of the prevalence of *P. jirovecii* pneumonia in Iranian immunocompromised patients using IFA test.

Materials and methods

Specimen collection

In the present study, 160 sera specimens were obtained from the army's 501 and Imam Khomeini hospitals in 2011. Specimens were collected from immunocompromised patients, including acquired immunodeficiency syndrome (AIDS) patients, diabetic patients, Hodgkin lymphoma patients and non-Hodgkin lymphoma patients. 40 specimens were collected from each group. A part of the sera that were collected for routine tests were used in this study and no additional intervention was done on patients. In the present study, specimens were taken from patients 19–58 years old and both genders (88 males and 72 females). After that, samples were delivered to the Valiasr laboratory of the Imam Khomeini hospital within a day and were stored at -80°C .

Polyclonal antibody preparation

To prepare the polyclonal Ab against *P. jirovecii*, the infection was induced in immunosuppressed rats. In this study, 20 male rats (*Rattus norvegicus*) with an average weight of 280 g were used. The immune system of rats was suppressed using Dexamethasone, 1.43–4.28 mg/kg of Dexamethasone was injected intramuscularly twice a week for 14 weeks. On the other hand, free protein diet was used to induce chronic immune system suppression in rats. For the protection of rats against bacterial infections and prevention of losing them, Tetracycline HCl, Penicillin procainamide, and Streptomycin were administered. In order to induce the infection in rats, 5×10^6 – 10×10^6 *P. jirovecii* cysts were diluted in complete Freund adjuvant and injected subcutaneously, this step was repeated two times with the same number of cysts, but incomplete Freund adjuvant was used for second injection (Broaddus et al. 1985). After 14 weeks and observation of clinical signs (such as loss of hair and panting) in animals, necropsy carried out and impression smears were prepared from lung tissue. Prepared smears were coded and stored at -80°C .

IFA test

After fixation of previously prepared slides by ethanol, the slides were dried at room temperature for 15 min. After that, the slides were washed 3 times with phosphate-buffer saline (PBS) (pH 7.2). Then suspected sera with a dilution of 1:100 were added. The slides were incubated for 45 min at 37°C and then were washed with PBS for 3 times. Fluorescein-conjugated goat anti-human immunoglobulin G was added to each slide. The slides were incubated for 15 min in a moist chamber at 37°C , were washed for 3 times with PBS and dried. In the next step, one drop of evince blue with a dilution of 1:10 was added and washed with distilled water after 30 s. Finally, dry slides after mounting with glycerol fluid were examined under a microscope.

Data analysis

SPSS v. 15.0 software was used for analysis of obtained results. A descriptive method was used to analysis of data.

Results

In this study, 160 sera samples were included with following details: patients with different background disease, ages (19–58 years old with a median age of 32.53), and both genders (88 males and 72 females). Results revealed no significant difference between two genders (38.6% in

Table 1 Prevalence of *P. jirovecii* in patients with different background disease

Background disease	Samples no.	Positive no.	Prevalence %
Diabet	40	8	20
Hodgkin lymphoma	40	15	37.5
Non-hodgkin lymphoma	40	9	22.5
AIDS	40	31	77.5
Total	160	63	39.3

males and 40% in females) and also the infection was not significantly related to age. This study focused on the prevalence of *P. jirovecii* in patients with different background diseases. The total prevalence was calculated 39.3%. The highest prevalence was found in AIDS patients (77.5%), and the lowest prevalence was found in diabetic patients (20%). The prevalence of this infection in Hodgkin lymphoma patients and non-hodgkin lymphoma patients were calculated 37.5 and 22.5%, respectively (Table 1).

Discussion

The results of this study revealed that there is a high prevalence of *P. jirovecii* infection in studied groups, particularly in AIDS patients. This is a potential hazard to immunocompromised patients, because respiratory infections are among the important causes of death in these patients. On the contrary, the prevalence rate of *P. jirovecii* in healthy individuals is out of concern because healthy immune system is able to prevent and control this infection.

In Iran, the first epidemic of *P. jirovecii* reported by Post et al. (1964), which had occurred in two orphanages of Shiraz city between 1961 and 1963. In that epidemic, 40 children out of 168 studied children died and the autopsy proved the presence of *P. jirovecii* in six died patients (12.5%).

By decreasing the count of CD4⁺ lymphocytes to 200×10^6 , the risk of PJP increases up to five times (Masur et al. 1989). Sheikholeslami et al. (2013) reported that *P. jirovecii* infection resulted in up to 26.6% mortality rate in Iranian HIV positive patients.

There are few reports of PCP in diabetic patients around the world, for example, Marques et al. (2002) reported PCP in a diabetic 46-year-old man with no predisposing risk-factors, including primary immunodeficiency, immunosuppressive therapy, neoplasm, or HIV infection.

Kim et al. (2013) carried out a study to estimate the prevalence of *P. jirovecii* pneumonia in patients with non-hodgkin lymphoma, they reported the prevalence rate of 2%. Estimated prevalence in their study is different from

the present study, which can be due to different aims of studies as our study tried to detect organism but they surveyed the prevalence of PCP.

In Jarboui et al. (2010) study, 60 immunocompromised patient examined and 14 patient (23.3%) were found positive. In this study, the most of the *P. jirovecii* positive patient were HIV positive (11 out of 14), too.

As we expected, the prevalence of *P. jirovecii* in AIDS patients was higher than other groups. In comparison to other groups, *P. jirovecii* causes life-threatening pneumonia with high mortality rate in AIDS patients. So physicians should pay more attention to this infection in immunocompromised individuals, particularly AIDS patients and they should use suitable prophylactic methods and drugs to prevent the infection.

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