



The counterimmunoelectrophoretic detecting of serum response to *Rhizopus oryzae* and *Candida albicans* in diabetic and non-diabetic subjects

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Abstract

Background: Increased susceptibility to invasive fungal diseases is one of the most important issues in diabetic patients. The purpose of the present study was to assess immunologic status of diabetic and non-diabetic subjects regarding to the subclinical infections with *Rhizopus oryzae* and *Candida albicans*.

Methods: The classic counterimmunoelectrophoresis (CIEP) assay has been employed to detect serum response to *R. oryzae* and *C. albicans* in diabetic (n=100; mean fasting blood sugar 170±102) and non-diabetic (n=100; mean fasting blood sugar 90±12) subjects.

Results: The anti-*R. oryzae* and anti-*C. albicans* antibodies have been detected in diabetic subjects more than those of non-diabetic subjects (p<0.001). The relative risk of candidiasis and mucormycosis in diabetic subjects were 2.8 and 19.0 times more than non-diabetic subjects, respectively. Absolute risk increase was 0.27 (2700 per 10,000 people) for candidiasis compared to 0.18 (1800 per 10,000 people) for mucormycosis in diabetes mellitus (DM). The number needed to treat for the prevention of candidiasis and mucormycosis in DM was 3.7 (370 per 10,000 people) and 5.5 (550 per 10,000 people), respectively.

Conclusion: CIEP would be considered as the first tool to screen latent mucormycosis and candidiasis in DM, however its characteristics like sensitivity and specificity should be measured in comparison to a gold standard technique.

Keywords: Candidiasis, mucormycosis, diabetes mellitus, counterimmunoelectrophoresis

Background

Both clinical and experimental studies evidently showed that (mutual) relationship is between diabetes mellitus (DM) and opportunistic fungal diseases like zygomycosis (mucormycosis) and candidiasis [1]. In this context, zygomycosis is a group of severe angio-invasive infections caused by the zygomycetes like *Rhizopus oryzae* [2]. Although the most advantageous management of zygomycosis has not been defined, rapid diagnosis, identification and mitigation of risk factors, chemotherapy, surgical debridement where needed, and the use of adjunctive therapies all contribute to control this life-threatening disease [3]. The accelerated immunosenescence that occurred in DM and especially in poorly managed DM or in DM with ketoacidosis has been the most commonly recognized underlying cause associated with different clinical aspects of zygomycosis e.g., [2,4].

Candidiasis is another invasive fungal infection that is a frequent cause of morbidity and mortality in immunosuppressed patients like diabetic subjects and organ transplant recipients

[5]. Various clinical features of candidiasis caused by different species mainly *Candida glabrata* and *Candida albicans* that have been detected in diabetic subjects [6].

The main issue associated with an improved survival of diabetic or immunosuppressive patients that susceptible to invasive fungal diseases (IFDs) is a quick satisfactory antifungal therapy. In order to be able to arrange a successful chemotherapeutic program, a prompt diagnosis is mandatory. However, conventional mycological methods lack sensitivity and take several days from sampling to a positive outcome [7,8]. Therefore, other rapid methods are recommended to manage patients who are at risk of IFDs. However, these advanced methods usually employed for patients that showed some kinds of clinical signs [9]. It is noteworthy to launch a strategic plan for campaigning IFDs that may occur in immunocomplicated situation like DM. In this sense, rapid and reliable diagnostic tools like immunodiagnostic tests seem to be rational especially in preventive programme of public health.

According to a huge body of literature that states high

probability of occurrence of IFDs in DM [10], the present study was aimed to find the apparent immunologic status of diabetic and non-diabetic subjects in Iran regarding to the subclinical infections with *Rhizopus oryzae* and *Candida albicans* as two major pathogens involved in IFDs.

Methods

Study subjects

The Medical Ethical Committee of the Medical University of Kermanshah, Iran reviewed and approved this study. The study group comprised a total of 200 adults (100 diabetic patients and 100 healthy subjects) attending to the Kermanshah University of Medical Education Mycology Laboratory in Kermanshah province, west Iran (34°18'N, 47°3'E and 1420 m above sea level). The including criteria for diabetic patients were according to World Health Organization as a fasting plasma glucose concentration >126 mg/dl. The including criteria for non-diabetic subjects were lack of history of diabetic symptoms like polyuria, polydipsia and unexplained weight loss and a fasting plasma glucose concentration <126 mg/dl. The individuals included in the present study received no antibiotic treatment, immunosuppressive agents, corticosteroid therapy, X-ray exposure or radiotherapy. In addition individuals that suffered from leukemia and AIDS have been excluded from study.

Counterimmunoelectrophoresis (CIEP) assay

The sera were immediately harvested and stored at -70°C. CIEP was carried out on 8x10 cm² glass plates covered with type II agarose gel (*Sigma-Aldrich*, St Louis, MO) diluted in Veronal buffer solution, pH 8.2. Two parallel rows of wells (n=8) punched in the gel respectively received our laboratory-manufactured antigens of *Rhizopus oryzae* and *Candida albicans* (10µl for each; methodology not shown), normal saline solution (10µl; namely negative control) and immunized goat antiserum (10µl; namely positive control).

The slides were subjected to a 5 mAmp electric current for 120 min and then immersed in Veronal buffer solution, pH 8.2 for 24 h. After finishing electrophoresis, the slides were immersed in sodium nitrate for 10 min followed by immersion in saline solution (2%) for 24 h, then to make them dry, a piece of paper containing distilled water was laid on them and they

were incubated in 40°C degree for 48 h. After drying, the gel was stained with Coomassie brilliant blue (*Sigma-Aldrich*, St Louis, MO). The existence of precipitate bands was considered as positive reaction.

Statistical analysis

Statistical analysis of data was calculated by using SPSS ver. 16 software. P values of 0.05 or less were considered statistically significant. Chi-square was employed for categorical variables.

Results

Based on the interview and patients' self-report, an internalist categorized sample population into two groups; diabetic and non-diabetic ones (Table 1). The results of CIEP showed that diabetic patients were more affected to *Candida albicans* compared with non-diabetic individuals (p<0.001; Table 2).

The proportion of diabetic subjects with anti-*R. oryzae* antibodies was higher than those of non-diabetic ones (p<0.001; Table 2). The relative risks were greater than one for both studied fungi, so DM was associated with both *C. albicans* and *R. oryzae*. However, the relative risk of candidiasis in diabetic subjects was 2.8 times more than no-diabetic subjects (Table 2). The relative risk of mucormycosis in diabetic subjects was 19.08 times more than no-diabetic subjects (Table 2). Absolute risk increase that has been shown as risk difference (RD) in Table 2 is 0.27 (2700 per 10,000 people) for candidiasis compared to 0.18 (1800 per 10,000 people) for mucormycosis. The number needed to treat (NNT) for the prevention of candidiasis and mucormycosis is 3.7 (370 per 10,000 people) and 5.5 (550 per 10,000 people), respectively (Table 2).

Discussion

In spite of an array of advanced molecular methods like

Table 1. The characteristics of selected population participated in the study.

Group/Character	Diabetic (n=100)	Non-diabetic (n=100)
Mean age (year)	44±16.7	38±14.2
Mean FBS (mg/dl)	170±102	90±12

FBS: Fasting blood sugar

Table 2. Outcomes of fungal infections in the diabetic and non-diabetic subjects.

	<i>C. albicans</i> (+)	<i>C. albicans</i> (-)	Risk of <i>C. albicans</i>	<i>R. oryzae</i> (+)	<i>R. oryzae</i> (-)	Risk of <i>R. oryzae</i>
Diabetic (n=100)	42	58	0.42	19	81	0.19
Non-diabetic (n=100)	15	85	0.15	1	99	0.01
The relative risk		2.8			19.0	
Risk difference (RD)		0.27			0.18	
Number needed to treat (NNT=1/RD)		3.7			5.5	

polymerase chain reaction and traditional microbial culture methods, qualitative and quantitative assaying of antigens of most fungal species and antibodies against them in the patient serum is nowadays the standard methodology for diagnosis of IFDs [11-14]. In this continuum, qualitative immunodiagnostic methodologies are still more economic and fast for population-based screening tests for detecting IFDs [14]. Therefore we employed CIEP in the present study to detect serum response of diabetic and non-diabetic subjects to *R. oryzae* and *C. albicans*.

The co-occurrence of IFDs and DM is not new story, however an efficient and economic planning to detect DM patients that are susceptible to IFDs before advancing the course of diseases would be a necessity. In the present investigation, diabetic subjects showed a more response to *R. oryzae* than healthy counterparts. In this regard, previous studies also emphasized that this opportunistic microorganism leads to some kind of lethal diseases like rhinocerebral mucormycosis in DM and other immunosuppressive conditions [15,16].

High NNT (550 per 10,000 people) for *R. oryzae* affected diabetic patients in this study declares that special attention must be paid to these patients. The NNT (370 per 10,000 people) for *C. albicans* also showed that a good strategy must be planned to control and prevent candidiasis in diabetic patients. However lower relative risk for *C. albicans* compared with *R. oryzae* in diabetic patients represents the importance of mucormycosis vs. candidiasis in these patients. In this context, rhinocerebral mucormycosis is the most fatal fungal infection which is frequently reported in DM [17]. The most prevalent infectious cause of mucormycosis is also *R. oryzae* which belongs to the phylum of zygomycota and mucorales branches [17,18].

The relative risk of candidiasis in diabetic subjects was 2.8 times more than no-diabetic subjects. *Candida albicans* is one of the saprophytes yeast in mucosa such as oral cavity and in the diabetic patients can cause mucocutaneous candidiasis and skin infections but cannot lead to fatal disease like rhinocerebral mucormycosis [18]. The results showed the positive serum responses against both fungi were higher among diabetic patients than healthy subjects. In addition diabetic subjects had more positive response against *C. albicans* in comparison to *R. oryzae*. In this sense, DM has been identified as an important predictor of IFDs in intensive care unit [19].

Conclusions

In sum, successful therapy for IFDs like mucormycosis and candidiasis in diabetic patients depends on early diagnosis. Hence counterimmunoelectrophoresis assay can be considered in initial screening programme, however further investigations are essential to evaluate its clinical relevance.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	AM	IK
Research concept and design	✓	--
Collection and/or assembly of data	✓	--
Data analysis and interpretation	--	✓
Writing the article	✓	✓
Critical revision of the article	--	✓
Final approval of article	--	✓
Statistical analysis	--	✓

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