PREVALENCE OF PARACOCCIDIOIDOMYCOSIS INFECTION BY INTRADERMAL REACTION IN RURAL AREAS IN ALFENAS, MINAS GERAIS, BRAZIL

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SUMMARY

This study aimed to estimate the prevalence of paracoccidiodal infection by intradermal reaction (Delayed-Type Hypersensitivity, DTH) to Paracoccidioides brasiliensis in rural areas in Alfenas, Southern Minas Gerais (MG) State, Brazil, and to assess risk factors (gender, occupation, age, alcohol intake and smoking) associated with infection. We conducted a population-based cross-sectional study using intradermal tests with gp 43 paracoccidioidin in 542 participants, who were previously contacted by local health agents and so spontaneously attended the test. Participants underwent an interview by filling out a registration form with epidemiological data and were tested with an intradermal administration of 0.1 mL of paracoccidioidin in the left forearm. The test was read 48 hours after injection and was considered positive if induration was greater than or equal to 5 mm. Out of 542 participants, 46.67% were positive to the skin test. Prevalence increased in accordance with an increase of age. There was statistical significance only for males. Occupation, alcohol intake and smoking habits were not significantly associated with the risk of paracoccidiodomycosis infection. There is relevance of paracoccidioidomycosis infection in such rural areas, which suggests that further epidemiological and clinical studies on this mycosis should be done in the southern part of Minas Gerais State.

KEYWORDS: Paracoccidioidomycosis; Epidemiology; Health surveys; Intradermal tests.

INTRODUCTION

Paracoccidioidomycosis is a systemic mycosis, endemic and limited to Latin America. It is caused by the dimorphic species of fungus Paracoccidioides brasiliensis or Paracoccidioides lutzii which can exist as a mycelial stage and yeast1,10-16. The mycelial form is found in nature at temperatures between 18 °C and 25 °C and produces spores or yeast-like conidia which may cause infections. Spores inhaled by susceptible hosts are converted into yeast in the tissues. By inhalation spores target the lungs and later on reach any systemic structure through the lymphatic or the blood stream, especially affecting skin, mucous membranes, lymphatic tissue and adrenal glands1,13,13.

P. brasiliensis, or P. lutzii, lives in argillaceous or sandy soil with adequate humidity. This fact points out the possibility of saprophytic life in soil, rich in organic matter, humid and protected against sunlight17.

Paracoccidioidomycosis distribution is heterogeneous showing high and low endemicity in different areas, in accordance with the climate and the agricultural conditions in the region. In Brazil, which is responsible for more than 80% of paracoccidioidomycosis cases in the world, asymptomatic infection is revealed by a cutaneous (Delayed-Type Hypersensitivity) test using the antigen paracoccidioidin. This disease has higher prevalence in the Southeast, South and Central West regions20. It is believed that about 10% of the population may have been infected by the fungus, which represents a more impressive percentage than that related to other neglected diseases, e.g., schistosomiasis and leishmaniasis14,15. There are also reports of cases in non endemic areas, related to people who had lived in or visited Latin America before the beginning of signs and symptoms of the disease. Under this condition paracoccidioidomycosis is considered a traveler’s disease16,21.

The disease is mainly found among men who work in rural areas and it affects them between the ages of 30 to 50 years old when they are supposed to produce at their most productive age. For this reason, this disease has an important impact on the production chain and on public health1,19. Recently, it was included among the neglected diseases, that have a significant impact on public health and that are not sufficiently quantified due to the lack of available information, that paracoccidioidomycosis is not a disease of compulsory notification10,15.

With this in mind, the goal of this study is to quantify the prevalence of people prone to P. brasiliensis in rural areas, in Alfenas - Southern

List of Abbreviations and Acronyms: IDR: intradermal reaction; CI: Confidence Interval.
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MG, as well as to evaluate the influence of age, gender, alcohol intake, smoking and occupation associated with infection.

MATERIALS AND METHODS

Population studied: This study was carried out in the rural area, in Alfenas-MG, Brazil, from May to December 2009. The selected population to be studied consisted of 542 people, who lived in rural area. The selection was carried out on spontaneous demand. The participants were previously contacted by health agents. On the scheduled day, participants in the study (male and female, over 10 years old) were submitted to an interview, filling out a registration form, which presented epidemiologic data. Pregnant women were excluded. The written informed consent was obtained from each participant, taking into consideration the resolutions of the Brazilian National Health Council No. 196/1996 and 347/2005.

Intradermal tests: The antigen used in the present work for the intradermal test was the exoantigen of *P. brasiliensis* (strain B-339), rich of gp43 (minimum concentration of 70%) and was referred to as gp43 paracoccidioidin. The purified glycoprotein of 43 kD from *P. brasiliensis* (gp43) was tested as paracoccidioidin, in DTH tests, in both experimental animals and patients with paracoccidioidomycosis, and compared with the traditional polysaccharide Fava Netto antigen.

The use of gp43 as paracoccidioidin in humans showed that this molecule can be used to evaluate the DTH response in patients with PCM. They found that patients who were responsive to *P. brasiliensis* antigen, 92.3% reacted against gp43 and 53.8% reacted against Fava Netto antigen. Gp43 skin test responses were significantly higher than those obtained with Fava Netto antigen, demonstrating the efficacy of this preparation.

The intradermal reaction tests were done by injecting 0.1 mL of exoantigen in the left forearm and readings of possible intradermal reaction were taken 48 hours after the injection. Induration equal to 5 mm or over was interpreted as a positive result.

Clinical, radiological and serological evaluation: Blood samples were taken, in order to perform hemogram and double agar gel immune diffusion tests. All IDR positive individuals were submitted to chest x-rays and a medical examination (one examining doctor) to evaluate and exclude possible clinical disease in activity and/or sequelae.

Statistical tests: The data were submitted to statistical analysis by means of software R. Analysis of frequency distribution of the main variables was performed, in order to characterize the population of the present study. The existence of association among the qualitative variables was evaluated by the chi-square test and the level of significance was 5%. The logistic regression analysis was performed to confirm and inform the odds ratio, in relation to the factors that were significant, expressing the protectoral or potentializing effect on the studied disease.

RESULTS

A total of 557 intradermal reaction tests were performed but 15 individuals did not return for the results and were excluded from the research. Of the remaining 542 studied individuals, positive reaction to intradermal injection of paracoccidioidin was observed in 46.67% (Confidence Interval 95% 0.42 to 0.51) of participants, which corresponds to 253 individuals. Of the individuals tested, 270 (49.82%) belonged to the male gender and 272 (50.18%) to the female gender. The age varied from 11 to 86 years old and the average age was 43 years. In relation to occupation, most participants reported regular activity in rural areas (53.50%) revealing major exposure to *P. brasiliensis*. In relation to habits, 29.33% were addicted to smoking and 20.67% to alcohol (Table 1). None of the participants assessed presented clinical signs, radiological and serological findings indicative of disease activity. Table 2 shows the distribution of the intradermal reaction adjusted for age, gender, occupation, tobacco and alcohol use. Among subjects with positive IDR, 56.30% were men (Confidence Interval 95% 0.50 to 0.62) and 37.13% women (Confidence Interval 95% to 0.43) (*p* < 0.001). The prevalence rate of sensitization grew progressively, in accordance with increasing age. From 11 to 29 years old, the prevalence rate was 35.37%; from 30 to 49, it was 49.56% (*p* < 0.01). Over 50 years old, the rate was 52.66% (*p* < 0.01), being the highest one.

Table 1

| Variables       | Number of individuals | %   |
|-----------------|-----------------------|-----|
| *IDR*           |                       |     |
| Negative        | 289                   | 53.33|
| Positive        | 253                   | 46.67|
| Total           | 542                   | 100.0|

| Gender          |                       |     |
|-----------------|-----------------------|-----|
| Male            | 270                   | 49.82|
| Female          | 272                   | 50.18|
| Total           | 542                   | 100.0|

| Occupation      |                       |     |
|-----------------|-----------------------|-----|
| Farm work       | 290                   | 53.50|
| Others          | 252                   | 46.50|
| Total           | 542                   | 100.0|

| Smoking         |                       |     |
|-----------------|-----------------------|-----|
| Yes             | 159                   | 29.33|
| No              | 383                   | 70.67|
| Total           | 542                   | 100.0|

| Alcohol intake  |                       |     |
|-----------------|-----------------------|-----|
| Yes             | 112                   | 20.67|
| No              | 430                   | 79.33|
| Total           | 542                   | 100.0|

*IDR = Intradermal reaction.*

In relation to occupation (work in farming), 48.28% were positive to the IDR test. People addicted to tobacco were 52.2% positive; people addicted to alcohol were 55.36% positive. On the other hand, there was no statistical significant association between positive intradermal reaction (sensitization) and professional activity and a smoking habit (*p* > 0.05). However, addiction to alcohol revealed an influence to the level of positive

282
Table 2
Intradermal results distributed by gender, profession, age range, smoking and alcoholism variants

| Variants          | Intradermal reaction |
|-------------------|----------------------|
|                   | Positive | Negative | Total |
|                   | ***N (253) | %  | N (289) | %  | N (542) | %  |
| Age range         |          |         |        |        |         |
| 11-29             | 52       | 35.37   | 95     | 64.63  | 147     | 27.12 |
| 30-49             | 112      | 49.56   | 114    | 50.44  | 226     | 41.70 |
| > 50              | 89       | 52.66   | 80     | 47.34  | 169     | 31.18 |
| Gender            |          |         |        |        |         |
| Female            | 101      | 37.13   | 171    | 62.87  | 272     | 50.18 |
| Male              | 152      | 56.30   | 118    | 43.70  | 270     | 49.82 |
| Occupation        |          |         |        |        |         |
| Farm work*        | 140      | 48.28   | 150    | 51.72  | 290     | 53.51 |
| Nonfarm work      | 113      | 44.84   | 139    | 55.16  | 252     | 46.49 |
| Smoking           |          |         |        |        |         |
| Yes**             | 83       | 52.20   | 76     | 47.80  | 159     | 29.34 |
| No                | 170      | 44.39   | 213    | 55.61  | 407     | 70.66 |
| Alcohol intake    |          |         |        |        |         |
| Yes**             | 62       | 55.36   | 50     | 44.64  | 112     | 20.66 |
| No                | 191      | 44.42   | 239    | 55.58  | 430     | 79.33 |

*p-value* = 0.004674

**Table 3**
Parameters estimation of logistic regression, odds ratio and its confidence interval of 95% for infection prevalence by Paracoccidioides brasiliensis (intradermal positive reaction) only for significant variants

| Variables          | Estimation | Odds ratio | *CI95% | p-value |
|--------------------|------------|------------|--------|---------|
| Male gender        | 0.768424   | 2.16       | 1.53 to 3.05 | 1.33 x 10^-1 |
| 11 to 29 years     | -0.68334   | 0.50       | 0.32 to 0.81 | 0.00372 |
| 30 to 49 years     | -0.03918   | 0.96       | 0.63 to 1.46 | 0.85085 |

*CI = Confidence Interval.

Intradermal reaction (p < 0.05), which was not observed by the logistic regression analysis (Table 3).

**DISCUSSION**

Paracoccidioidomycosis is the commonest systemic mycosis in Latin America, predominantly affecting individuals who have frequent and close contact with soil. It mainly affects people who are in their most productive stage of life. Epidemiological surveys have been used to evaluate paracoccidioidomycosis-infection prevalence in Brazil and in other countries in Latin America. However, there is an impressive variability in relation to results that can be related to the characteristics of the studied population and to the nature or concentration of the used antigen. It should also take into consideration the chemical compounds and presence of fungus in soil.

Classically, paracoccidioidin, a polysaccharide extracted from different strains of *P. brasiliensis*, was the most used antigen for epidemiologic survey studies. More recently, it has been valued using purified antigens in intradermal tests with promising results using the exoantigen gp 43 as paracoccidioidin considered immunodominant and specific component. In Brazil, the paracoccidioidin Fava Netto antigen was used in epidemiological surveys, in order to assess the prevalence of PCM infection. Results are not homogeneous, with positivity rates ranging from 2% among children from different areas of Rio de Janeiro, to 82% in Cachoeira do Sul. Using the same antigen in Colombia, the prevalence of sensitization to *P. brasiliensis* can reach up to 77% of the population in certain rural areas, while in Venezuela, the prevalence rates of infection in recent years ranged from 10.2% to 19.7%. Argentina has lower prevalence rates of PCM compared to other Latin American countries, a variation being observed in prevalence between 1.6% to 10.2%. Some authors have currently used exoantigen purified gp43 in epidemiological surveys for the delimitation of endemic areas, also noting variable results (4-45%). In this study, we found a prevalence of 46.67% of positive tests in rural areas of Alfenas, MG, Brazil using gp43 paracoccidioidin (Table 1). Similar prevalence (49.5%) was also observed in other rural areas (Ibiá, MG) using Fava Netto paracoccidioidin and in the northwest of Paraná State (43.0%) using gp43 paracoccidioidin.

On the other hand, such antigen may induce cross-reaction in relation to other fungi antigens, e.g., *Histoplasma capsulatum, Coccidioides immitis* and *Sporothrix schenckii*. Simultaneous positive tests for histoplasmin and paracoccidioidin do not necessarily imply a dependence relation among them, taking into consideration the coexistence of fungi in the same area. Meanwhile, this result indicates that it is not possible to state that there is no cross-reaction.

Despite the fact that all participants lived in rural areas, only 53.50% reported to be farm workers (Table 1). And 48.28% had positive intradermal reaction to paracoccidioidin. This was not statistically significant as a risk variant for positive intradermal reaction (Table 2). This could be explained by the fact that they lived in an endemic area and had worked sporadically in farming such as coffee harvest.

Gender differences are one of the most important characteristics of this systemic fungal disease. The incidence and progression of PCM in endemic areas is much higher in adult men than women. In relation to gender and positivity to paracoccidioidin test, we found a significant...
The differences observed as to the prevalence between men compared to women are not due solely to less exposure or social conditions. Epidemiological data indicate that hormonal factors can have a strong role in the pathogenesis of the disease. Experimental studies in animal tests demonstrate the protective effect of feminine hormones, providing support for the role of 17 β-estradiol in the innate resistance of females to the PCM.

Moreover, the ability of the hormone to modulate the production of cytokines may be associated with improvement of immune response. Although women can be infected early, estrogen appears to affect the transition yeast and mycelial increase the secretion of IFN-γ and Th1 cells and lower levels of IL-10 as demonstrated in experimental models. Furthermore, confirmation of subclincial infection in healthy and asymptomatic, skin tests for delayed hypersensitivity, the paracoccidioidin reveals heterogeneity of response in relation to gender. One should take into account that the agent-host relationship factors such as higher or lower environmental exposure, nutritional status, comorbidities, alcoholism and smoking among others, probably interfere with the immune response favoring the progression of infection to disease.

Any individual exposed to this fungus is susceptible to infection. However, infection rates increase accordingly with age, as it was observed both in our present study and by SILVA-VERGARA & MARTINEZ (1998). This may be more likely related to a higher possibility of sensitization to the fungus, in relation to people living in an endemic area. Prevalence from 30 to 50 years old was 49.56%, the average age being 43 years old.

On the other hand, some life habits may be related to either acquisition of infection or development of the disease. Among other triggering factors, smoking and alcohol addiction have long been associated with the disease. A smoking habit is more likely to influence and/or facilitate the development of this infection due to some possible factors such as change of the mucociliary activity, diminishing of immunity and defects of immunological response of macrophages. In relation to alcohol addiction, it is thought that this drug is a risk cofactor associated with smoking. In the present study, 29.33% of the individuals reported to be addicted to tobacco and 20.67% to alcohol. Among the tobacco addicted, 52.20% were positive to a skin test and among alcohol addicted 55.36% were positive. Meanwhile, such variants were not statistically significant in accordance with the regression analysis (Table 3).

The intradermal test convinced us that it was an important way of identifying if someone was or was not sensitized by the antigen. This makes it easier for patient follow-up, in order to facilitate a future diagnosis and early treatment.

In addition to this, after the intradermal test, it is possible to detect paracoccidioidomycosis endemicity in rural areas. Our results showed a high prevalence of sensitization to \textit{P. brasiliensis} antigen in rural areas, in Alfenas - in Southern MG State. In our view, this data points out the necessity of public health policies to deal with mycosis in this region.

**RESUMO**

Prevalência da paracoccidioidomícosse por intradermorrrecação em áreas rurais de Alfenas, Minas Gerais, Brasil

Este estudo teve como objetivo estimar a prevalência de sensibilização da pele pelo \textit{Paracoccidioides brasiliensis} em áreas rurais em Alfenas, MG, Brasil, e avaliar os fatores de risco associados à infecção. Foi realizado um estudo transversal de base populacional utilizando testes intradérmicos com paracoccidioidina em 542 indivíduos selecionados por demanda espontânea. Os participantes foram submetidos a uma entrevista através do preenchimento de um formulário de inscrição com os dados epidemiológicos e os testes com a administração intradérmica de 0,1 mL de paracoccidioidina no antebraço esquerdo. O teste foi lido 48 h após a injeção e foi considerado positivo se enduremento era maior ou igual a 5 mm. De 542, 46,67% participantes foram positivos ao teste de pele. Prevalência aumentou de acordo com o aumento da idade. Houve significância estatística apenas para o sexo masculino. Profissão, alcoolismo e tabagismo não foram significativamente associados com o risco de infecção paracoccidioidomícosse. Há relevância da infecção paracoccidioidomícosse em áreas rurais, o que sugere mais estudos epidemiológicos e clínicos sobre esta micose no sul do estado de Minas Gerais.

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