EVALUATION OF NEGATIVE SERUM SAMPLES IN IMMUNODIFFUSION TEST FROM CONFIRMED PARACOCCIDIOIDOMYCOSIS PATIENTS.

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Serological tests are one of the most usefull diagnostic methods for paracoccidioidomycosis. However, approximately 10% of paracoccidioidomycosis patients with mycological diagnosis present negative double agar gel immunodiffusion test (DID). This study aimed at evaluating these cases. Serum samples from 32 patients with confirmed paracoccidioidomycosis but negative in DID before treatment were evaluated. As controls, positive sera from other 32 confirmed patients, paired according to clinical form and age, were analysed. These assays were carried out at the Research Laboratory of Tropical Diseases (RLTD) - FMB/UNESP and at Adolfo Lutz Institute (IAL) - SP. DID was performed using culture filtrate antigens from Pb-113, prepared at the Laboratory of Clinical Mycology – UNESP/Araraquara (DIDr), Pb-113 (DID_1) and Pb-B-339 (DID_2), prepared at IAL. Sera were also submitted to *immunoblotting* test with strains Pb-113 (IB_1) and PbB-339 (IB_2) for recognition of gp43 and gp70. Statistical analysis was carried out by McNemar's or binomial test and significance was set up at $p<0.05$. Analysis of these sera showed that DID evaluation in RLTD presented no difference in positivity when performed with the three antigens ($p>0.05$). DID evaluated in IAL presented no difference when used DID_1 and DID_2 ($p>0.05$), but these were higher than DIDr ($p=0.001$). Reproducitibility between laboratories was observed with DID_1 and DID_2 ($p>0.05$), but DIDr presented higher positivity in RLTD ($p=0.048$). *Immunoblotting* positivity presented no difference in recognizing IB_1-gp43, IB_2-gp70 and IB_2-gp43 ($p>0.05$), but a higher positivity than IB_2-gp70 recognition ($p<0.00001$). When DID was compared with i*mmunoblotting* the positivity was lower than IB_1-gp43, IB_2-gp43 and IB_2-gp43 recognition ($p<0.00001$), but higher than IB_2-gp70 recognition ($p<0.001$). These findings suggest that DID sensitivity is not increased when different antigens are used. Moreover, negative serum in DID should be evaluated by *immunoblotting* with gp43 recognition, using Pb-113 or Pb-B-339 antigen. However, *immunoblotting* specificity should be carefully evaluated.