Additionally, titers of serums from both groups were evaluated by indirect ELISA. Briefly, each serum was diluted in the range of 1:10 to 1:5120. Absorbance values under the cut-off point (0.081) were considered to be non-specific or negative reactions. The detection of *L. laeta* venom for Group 1 serum sample titration media was 160 (n = 10) (Fig. 1c). Similarly, the media of titer for Group 2 sera was 80 (n = 30). Group 1 sera had absorbance values over the cut-off point for *L. laeta* venom detection at low dilutions (Fig. 1c). However, at 1:160 dilution, samples 1 (dot A1), 2 (dot A2), 8 (dot H1), and 10 (dot B2) presented lower values of the cut-off point. The majority of Group 2 sera had absorbance values over the cut-off (Fig. 1d). Samples 11 (dot G4), 14 (dot F3), 18 (dot A4), 19 (dot H5), and 26 (dot H4) had lower values of the cut-off point at 1:40 dilution. This is consistent with the results of the dot blot. However, the trend of the samples from the non-loxoscelism group (Group 2) remained above the cut-off point up to the titer 80. Additionally, avidity index for both pooled serums showed a high affinity with antibodies present in sera of groups 1 and 2 for detection of *L. laeta* venom (Additional file 2A).

In contrast, the total IgG concentration (mg/dL) of Group 1 and Group 2 sera was similar and within the reference range of the assay (Table 2). Total serum IgG levels of Group 1 samples ranged from 825 to 1622.5 mg/dL, and the total serum IgG levels of Group 2 samples ranged from 993.6 to 1902.9 mg/dL. The sample with lowest level of total IgG of group 1 sera was the sample 8 (dot H1), with a concentration of 825 mg/dL, while the sample with lowest level of total IgG from group 2 was the sample 14 (dot F3), with a concentration of 993.6 mg/dL. The latter correlates with data showed by dot blot and indirect ELISA, in which both samples had the lowest detection levels of *L. laeta* venom observed for both groups. Moreover, no differences in total IgG level were observed related to gender among samples.

Sera from individuals with no history of loxoscelism recognize phospholipase D family proteins from *L. laeta*

In order to confirm the above results, the *L. laeta* venom component that was specifically recognized by sera from groups 1 and 2 was evaluated. Each serum was assessed by immunoblot of *L. laeta* venom separated by electrophoresis, showing that all sera from Group 1 and Group 2 recognized a protein component between 25 and 35 kDa (Additional file 3). Sera of Group 2 that could recognize different bands of *Loxosceles* venom, compared to those in the range of 25–35 kDa proteins, were excluded on suspicion of previous contact with the *Loxosceles* venom.

Additionally, considering that pooled sera from both groups recognized BSA in dot blot, and to discard reactions from antibodies other than anti-*L. laeta* venom in sera, we proceeded to purify IgG antibodies from the Group 1 and Group 2 serum pools and immunoselected against *L. laeta* venom. They were later evaluated by immunoblot for recognizing *L. laeta* venom separated by 1D and 2D electrophoresis (Fig. 2). As a detection control, mouse anti-*L. laeta* venom serum was used, noting that it recognizes a protein band pattern of *L. laeta* venom in the range of 25–35 kDa, while pre-immune mouse serum does not detect *L. laeta* venom (Fig. 2a), which was corroborated by densitometry analysis of bands (Additional file 1B).

Similar detection patterns of *L. laeta* venom were observed in Group 1 and Group 2 serum pools, as well as with purified IgG antibodies for both groups (Fig. 2b). By means of 2D venom electrophoresis, it was possible to see that the IgG antibodies of both study groups recognize a similar pattern of spots of *L. laeta* venom proteins, within the range of 25 and 35 kDa (Fig. 2c).

The protein components of *Loxosceles* venom between 25 and 35 kDa have been considered members of the phospholipase D family and are present in different *Loxosceles* species [30]. Therefore, we evaluated whether purified IgG antibodies from both groups could recognize the *L. laeta* phospholipase D1 protein (rLlPLD1), showing detection of the recombinant PLD with purified IgGs from both groups (Fig. 3a). In addition, *L. laeta* venom immunoprecipitation with pooled sera from groups 1 and 2 and subsequent immunoblot with a rabbit polyclonal anti-*L. laeta* venom serum (Fig. 3b, upper panel) or with monoclonal antibody anti-LlPLD1 (Fig. 3b, bottom panel) showed that PLD was the major protein immunoprecipitated from the venom. Moreover, since pooled sera from groups 1 and 2 could detect BSA in dot blot (Fig. 1a), we also carried out immunoprecipitation of *L. laeta* venom with an anti-BSA antibody as an unrelated antibody, which was not detected, corroborating the hypothesis that detection of *L. laeta* venom was a consequence of the presence of anti-PLDs antibodies in the serum samples of

Table 2 Human IgG levels in serum samples from patients with and without loxoscelism

Serum samples	IgG [a] (mg/dL)	Reference range (mg/dL)	p-value[#]
Loxoscelism group (n = 10)	1355 ± 117.1	710–1520	0.7445
Without loxoscelism group (n = 30)	1385 ± 36.14	710–1520	0.7445

[a]Values of media ± SEM. Human IgG concentration in serum were determined by radial immunodiffusion (RID). [#] t-test; $\alpha = 0.05$