Title: Clinico-epidemiological analysis of Post kala-azar dermal leishmaniasis (PKDL) cases in India over last two decades: a hospital based retrospective study

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Author’s response to reviews: see over
To

The Editor,
BMC Public Health

Subject: Submission of revised manuscript to BMC Public Health

Dear Sir,

We are thankful to the reviewers for providing useful comments which have helped us to improve the manuscript entitled “Clinico-epidemiological analysis of Post kala-azar dermal leishmaniasis (PKDL) cases in India over last two decades: a hospital based retrospective study”. We are providing the responses to the comments raised and the revised manuscript.

Sincerely,

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Reply to comments

Reviewer 1: Anna Bajer

Reviewer's report:
Minor revision
We thank the referee for the valuable comments. We have addressed all the queries raised by the reviewers’ and the manuscript has been revised accordingly.

Comment: Ethic statement: line 135: ‘… under the guidelines of the ethical committee’- Could you provide the number of license, if any?
Response: This study is retrospective one and the data refer to patients seen over the previous 20 years. All the patients were diagnosed and treated as per the National Health Policy applicable at the time and/or following the guidelines of the ethical committee of the Safdarjung Hospital, New Delhi, India. The clinico-epidemiological details of patients were collected maintaining the confidentiality of the subjects under study.

We have accordingly revised "Ethics Statement" in the revised manuscript.

Comment: Results: line 170- there is a statement of significant association between types of lesion and age. Could you provide here (not only in the discussion) the real result for it –i.e. what lesions were found in certain age classes? Similar problem with statistical output reported in lines 180-181: where is a real result for this?
Response: As advised, appropriate changes have been made in Result section of the revised manuscript. We added the following two points of concern. (1) There was a significant association between type of lesions and age ($\chi^2 = 40.775$ (6), $p = 0.001$), polymorphic/mixed lesions were predominant in adults aged between 19-44 years. (2) In addition, there was an evidence of association ($\chi^2 = 9.681$(2), $p=0.008$) between cases with history of VL and the place of origin, indicating that the majority of the PKDL cases with history of VL originated from high endemic zones.
Comment: Lines 185-192: associations between type of drug used for the primary VL treatment and the time lapse: I don’t think you can make any reliable analysis or conclusions on the basis of the group treated with miltefosine: there were only three persons treated with this drug at that time. Any generalization shall be not well justified, conclude with caution.

The same concern the part of discussion (lines 255-257), you cannot state that time laps (...) were independent of the type of drug used for VL treatment- not enough data, no stat significance.

Response: We agree with the reviewer and removed both the above mentioned statements from the revised manuscript.

Tables
Comment: Tables 2 and 3: please explain w.r.t.

Response: The abbreviation ‘w.r.t.’ means “with respect to” and the same has been added in the caption of Table 2 and 3 of revised manuscript.

Figures
Comment: Figure 1- please provides info, what are the numbers on map – number of cases from each area?

Response: Number in the bracket shown in the figure is the number of PKDL cases from district of origin.

Comment: Please, rephrase the title: you may delete “Graph illustrating’, exchange ‘over the year’ with ‘in reported period’; delete ‘duration’. In my pdf version fig 2 seems very small – can be bigger?

Response: As per your suggestion, the caption of figure 2 has been changed to “Incidence of PKDL in the reported period” and also the size of the figure 2 has been increased.

Comment: I don’t understand this title. Please rephrase and write precisely what is shown in these 3 pictures.

Response: The title of figure 3 has now been rephrased to “Diagnosis of PKDL based on minimally invasive sampling technique”.
Detailed corrections
Comments: Line 61: add ‘space’ behind ‘Sudan’
Response: Appropriately changed.

Comments: Line 84: correct to ‘develops’
Response: Appropriately changed.

Comments: Line 88: add dot in the end of the sentence
Response: Appropriately changed.

Comments: Lines 122-123: explain the use of ‘Mw vaccine’ – used as immunomodulator?
Response: It is known to be an immunomodulator for enhancing Th1 response of the host in diseases like tuberculosis and leprosy and a chance observation of its efficacy in PKDL in the mid-nineties, led us to try it along with SAG. However, our experience with Mw vaccine in containing PKDL was less impressive.

The same has been added in the revised manuscript in the “Discussion section”.

Comments: Line 304: you may use ‘recent’ or ‘next’ instead of ‘later’
Response: Appropriately changed.

Reviewer 2: Ewa J. Mierzejewska

Reviewer's report:
The manuscript „Clinico-epidemiological analysis of Post kala-azar dermal leishmaniasis (PKDL) cases in India over last two decades: a hospital based retrospective study” concerns epidemiological features of PKDL cases that can serve as durable reservoir of *L. donovani* in endemic regions. This long-term clinical based study provides data from high-endemic regions for visceral leishmaniasis and thus is a valuable source of information for evaluation of accuracy of diagnostic tools, used treatment, epidemiology of the VL and PKDL. I find this manuscript adequate for further publication. However, some details need corrections. They are mentioned below. I recommend minor essential revision.
**Minor revision**

We thank the referee for the valuable comments. We have addressed all the queries raised by the reviewer and the manuscript has been revised accordingly.

**Comments:** Lines 122-123 Mw vaccine” – please give broader description of this vaccine: give the full name and the name of the manufacturer if possible. In discussion, please write shortly about effect of this vaccine on leishmaniasis treatment (effective/non-effective???????????). Given that, in some sources expected immunomodulatory effect in VL therapy was showed to be insignificant, its worth to underline your evidence obtained during clinical studies.


**Response:** Mw vaccine (Immuvac, Cadila Pharmaceuticals, Ahmedabad, India) is derived from a non-pathogenic, saprophytic, Mycobacterium strain and is known to be an immunomodulator for enhancing Th1 response of the host in diseases like tuberculosis and leprosy. However, in experimental VL infection, it was demonstrated to be ineffective (Tandon 2013) and our experience with Mw vaccine in containing PKDL was also less impressive.

The same has been added in the “Result and Discussion section” of the revised manuscript. We also added two references [26] and [27].

**Comments:** Lines 130-131 “One case was exclusively treated with amphotericin B (i.v. 50 mg/day, total dosage of 4.5g), with strict monitoring of renal functions” – Please, explain why this particular patient was treated with amphotericin B?

**Response:** As stated in the article the patient had taken SAG without any response. So we considered him SAG resistant and gave only amphotericin B as miltefosine was unavailable with us at that time.

**Comments:** Lines 151-153 “Based on this classification, 63.5% (n=179) of PKDL cases originated from high endemic area as against 30.1% (n = 85) and 6.4% (n=18) from meso- and low endemic areas respectively (Fig. 1)” – these numbers don’t stick to your map. In summary
on the map 177 cases are from high-endemic regions, 84 cases are from meso-endemic regions and 21 from low-endemic regions. Please, put correct numbers on the map.

**Response:** We have revised the figure 1 and corrected the numbers on the map.

**Comments:** Lines 153-154 and 240 “We observed an upward trend in reporting of PKDL cases to Safdarjung Hospital, New Delhi, India since the year 2000 (Fig. 2).” – For the first 5 years counting from year 2000 the tendency is decreasing. This statement is misleading, given that on fig. 2 the upward trend is visible since 2005.

**Response:** As per your suggestions, corrections have now been made in both Result & Discussion sections.

**Comments:** Lines 165-169 “Polymorphic/mixed forms were predominantly present in both genders (53.3% males, n=120; 54.4% females, n=31) followed by papulonodular form in males (24.9%, n=56) and macular in females (36.8%, n=21). Papulonodular lesions were the least frequent in female (8.8%, n=5) cases whereas the macular lesions were the least frequent in males (19.5%, n=44). The unusual clinical lesions were exclusively observed in male cases (2.2%, n=5).” Can you explain in Discussion part possible reasons of these disproportions? Do you think the only reason is the lower number of cases among females?

**Response:** It is difficult to add in the Discussion section the possible reasons as data available is with small number of cases.

**Comments:** Line 170 – 171 “There was a significant association between type of lesions and age ($\chi^2 = 40.775(6), P = 0.001$)” – What kind of association it was? What means the number “(6)” and all other numbers in brackets in description of statistics?

**Response:** The kind of association is Chi square ($\chi^2$) test of association between two variables, here, the type of lesions and age group. The number in the bracket “(6)” reflects the degree of freedom.

**Comments:** Lines 176-179 “In the present study, 79.8% (n= 225) of PKDL patients reported history of VL (Table 1). Among PKDL patients with history of VL, 52.9% (n=119) had mixed/polymorphic lesions, 24.9% (n=56) had only macular lesions and rest 20.9% (n=47) had
either papular and/or nodular.” – Sorry, but 119+56+47=222, not 225. Please correct these numbers or explain the missing 3 cases.

**Response:** The missing 1.3% (n=3) cases had unusual clinical presentations like erythrodermic, fibroid or plaque and the same has now been added in the revised manuscript in the “Result Section”.

**Comments:** Line 182 and 188 “median time of manifestation of PKDL after VL treatment was 36 months (range = 1 - 384 months).” – 384 months means 32 years - is it a correct number? Please explain this number.

**Response:** Yes, the duration of 384 months (32 years) is correctly mentioned and we found it in one PKDL case. Other studies too had similar observations, one stating PKDL might develop 20-40 years following cure from VL (Mukhopadhyay 2013) while another report (Mukhopadhyay 2012) from India had reported a case of PKDL with history of VL of 45 years.

**Comments:** Lines 191-192 and 256-257 “Besides, our data indicated that there was no association between type of drug used for VL treatment and the time lapse between VL treatments and PKDL incidence (χ²=1.994 192 (2), p = 0.369).” – the number of cases treated alternatively to monotherapy with SAG is very small (12 with amphotericin B, 3 with miltefosine), thus statistical analysis in this case seems to be pointless. If you decide to carry on with this analysis, please explain in ‘Discussion’ part the weakness of it rather than using statement: “type of used drug had no effect on the time laps between VL and onset of PKLD.”

**Response:** We agree with the reviewer and removed the statements for above mentioned analysis in the revised manuscript.

**Comments:** Lines 206-210 “We earlier demonstrated that rK39 strip test produced identical results with slit aspirate or serum as diagnostic sample for PKDL [20]. Furthermore, we reported skin slit aspirate as a good diagnostic specimen which offers sensitivity and specificity comparable to that of tissue biopsy with qPCR [20]. Therefore, we propose confirmatory diagnosis of PKDL using minimally invasive skin slit aspirate samples as described in figure 3c.” – The whole part should be replaced to the ‘Discussion’ section.
Response: As suggested we have moved above mentioned part from result section to the Discussion section of revised manuscript.

Comments: Lines 216-233 the whole paragraph ‘PKDL treatment’ – once patients treated with SAG who completed treatment were described only using percentages (%), next patients treated with miltefosine and/or amphotericin B who completed treatment are described using full numbers and percentages (%). Please, unify this in the text and rather use full numbers as well as percentages.
Response: As suggested we have now made the appropriate changes in the revised manuscript.

Comments: Table 1 is needless. The whole description is in the text.
Response: As advised, we have removed the Table 1 from the revised manuscript.

Comments: Table 2. What means an abbreviation in caption: w.r.t?
Response: The abbreviation ‘w.r.t.’ means “with respect to” and the same has been included in the caption of Table 2 and 3 of revised manuscript.

Comments: Figure 1 – The map is difficult to read. The contours should be thinner to leave space for names of districts. Specific names should be written with smaller, but more distinct letters. Other way it is hard to read some of those names. The meaning of numbers showed on the map should be explained in caption, i.e. number of cases in each district is showed in brackets.
Response: As suggested, the quality of figure 1 has been improved and number is explained.

Comments: Figure 3a, 3b is needless. Those two numbers are explained in the text.
Response: As per suggestion, we have removed figure 3a, 3b from the revised manuscript.