Reviewer’s report

Title: TNF-α−308 G/A and IFN-γ+874 A/T gene polymorphisms in Saudi patients with cutaneous leishmaniasis

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Reviewer: Bhavi Modi

Reviewer's report:

The manuscript by Ahmed AA et al describes the results of a case-control study testing association between individual polymorphisms in the TNF-α and IFN-γ genes and the susceptibility to infection by different Leishmania species among Cutaneous Leishmaniasis patients in central region of Saudi Arabia. While the study findings are of great interest, several important key issues require clarification and consideration. The manuscript in its current state lacks significant details in outlining the background and known literature surrounding the host susceptibility to Leishmania infections, etiology and health impact of CLs, role of immunologic factors and more importantly genetic factors (genotype differences) in disease pathology. Adding this will further help to define the rationale and study objectives in a better fashion to fully appreciate the significance of the study findings. In addition, several grammatical errors & issues with sentence framing and structure need to be fixed as these are impeding proper understanding of the manuscript and make digesting the information provided difficult.

Specific comments and questions that need addressing:

Background: Specific questions that need addressing in this section are: What are the different factors that influence different outcomes of Leishmania infections? What is Cutaneous Leishmaniasis and what is the disease etiology? Are specific Leishmania species more commonly associated with CL in certain geographical areas? Are variances in outcomes mostly related to environmental factors such as temperature, host nutrition status, specific species, co-infection with other pathogens or do genetic factors influencing host immune response and therefore creating a 'genetic susceptibility' model play a major role as well? While the authors may have loosely tried address some of these questions (the prose makes it difficult to follow), a more in-depth review of existing literature addressing these questions should be included to demonstrate the progression to current study objectives.

The two specific variants under study should be identified by their 'rs' identifiers and with standard HGVS nomenclature so readers can easily evaluate specific criteria associated with the variant that they might be interested in. Other information about their population prevalence/allelic frequency (from databases like gnomad), predicted or known functional impact and relevance should be reported. This will not allow for comparison (of allelic frequencies etc) of controls used in the study with the general population but will also help understand study objectives and findings in a more meaningful context.

Methods: What were the exclusion criteria (if any) established for selection of the control subjects in the study? For e.g. were they screened for presence/absence of infections (Leishmania
and/or other pathogens?) before inclusion in the study?. In addition, how were the cases/CL patients identified? What were the diagnostic criteria used? Were any immunologic criteria used to inform selection in the study? Reporting the specific inclusion and exclusion criteria for all study subjects is a requirement of a well-designed case-control study and the authors fail to provide this information.

Results: Tables 3 and 4 outline the key results of the study and are very interesting findings. Another study question that could add value to this section is testing the association of both minor alleles combined (i.e. presence of both TNF-α A allele and IFN-γ T allele in an individual) and infection susceptibility in cases compared to controls. Does having both minor alleles increase/decrease susceptibility to Leishmania infection in general or to individual L. species? This might provide additional insights into genetic mechanisms of host immune response as these factors work together in a pathway and not in isolation.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Unable to assess

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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Please indicate the quality of language in the manuscript:

Not suitable for publication unless extensively edited

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