TITLE: Impact of inflammasome on hypopigmentation in patients with Post Kala-azar Dermal Leishmaniasis (PKDL)

INTRODUCTION:
Post kala-azar Dermal Leishmaniasis PKDL is a neglected tropical disease resulting as a complication of apparently cured cases of kala-azar, the causative species being the protozoan parasite, *Leishmania donovani*. PKDL caused by *Leishmania donovani* is confined to South Asia (India, Bangladesh and Nepal) and East Africa (mainly Sudan). PKDL can present either as papulonodular (polymorphic) or hypopigmented lesions (macular, Chatterjee et al., 2020). Disfigurement, scarring, and stigmatisation have devastating and long-lasting effects on the quality of life of those affected. Additionally, in the ongoing visceral leishmaniasis (VL) elimination program 2021-2030 in South Asia, PKDL a dermal sequel of VL, is an obstacle to success of this program as these PKDL patients harbour parasites in skin lesions, which being accessible to sand flies can sustain VL transmission.

Skin hypopigmentation reflects loss or defective melanocyte function, but the underlying mechanisms remain unclear. Recent studies have revealed that a variety of inflammatory mediators participate in the regulation of melanogenesis in melanocytes and include IL-18, IL-33, granulocyte-macrophage colony stimulating factor, IFN-γ, IL-1, IL-4, IL-6, IL-17 and tumor necrosis factor (Fu et al., 2020). The inflammasome is an important protein complex that activates caspase 1, which proteolytically activates the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18. It has a critical role in eliciting innate immune responses, IL-1β has been suggested to contribute to various skin diseases, including psoriasis, vitiligo, systemic lupus erythematosus (SLE), and atopic dermatitis (AD) (Tang et al.,2020). Therefore, it may be envisaged that activation of the inflammasome might have a role in hypopigmentation in case of PKDL and will be the focus of this study.

OBJECTIVES:
(i)To establish the status of the inflammasome across the clinical spectrum of PKDL using Immunohistochemistry/ cytokine profiles in PKDL vs. controls
(ii) Correlate the histopathological and functional profile of T-cells and keratinocytes in relation to inflammasome activation and pigmentary loss.

METHODOLOGY:

1. **Characterization of inflammasome related genes and T-cell populations in lesional skin from patients with PKDL**

Microscopically/PCR-confirmed cases of PKDL across the clinical spectrum (Polymorphic and macular, n = 10 in each group), with matched healthy controls (n = 10) will be included. Archival tissues will be repurposed, necessary permissions are in place. The status of inflammasome related genes as well as tissue localization (Caspase 1, Il-1β and NLRP3) along with melanocyte activity and pathogen load will be studied by using high-resolution immunohistochemistry (IHC). So the study will involve study of the following featires:

(i) Measurement of parasite load by kinetoplast DNA based quantitative PCR (Moulik et al., 2018)
(ii) Study of the inflammasome by IHC
(iii) Status of lymphocyte populations CD4, CD8 and CD20 will be examined by IHC (Mukherjee et al., 2019)

(iv) Study of melanogenesis by examining the status of melanocyte-specific markers namely tyrosinase (TYR), microphthalmia-associated transcription factor (MITF) and tyrosinase-related protein-1 (TYRP1) (Sengupta et al., 2023b).

2.Analysis of circulating cytokines in PKDL

Local dermal inflammatory factors can impact on skin pigmentation, possibly via apoptosis of melanocytes accordingly, the lesional status of pro-inflammatory cytokines, IFN-γ, IL-6, IL-2, TNF-α and IL-1β will be measured (Mukherjee et al., 2019).

OUTCOMES:

Leishmania infections are associated with activation of the NLRP3 inflammasome, possibly with a view to limit parasite growth. In patients of cutaneous leishmaniasis, the role of inflammasome responsible for the hypopigmentation associated with macular PKDL remains a pertinent yet unanswered question (Sengupta et al., 2023a). In addition, NLRP1 and IL-1β levels in the skin may also represent better markers than detection of lymphocyte infiltration in PKDL patients. Specifically, this project will lead to (a) in-depth knowledge about the regulation of hypopigmentation across the different forms of PKDL and whether this relates to control of microorganisms along with (b) study of molecules and/or cellular responses locally and/or systemically that may predict disease development. Importantly, the knowledge gained from understanding PKDL pathogenesis will facilitate improved diagnosis and development of novel treatment concepts for pathogen-driven hypopigmentation.

TIMELINE:

- Months 1-2: Characterization of inflammasome related genes and T-cell populations in lesional skin of patients with PKDL
- Months 2-3: Deciphering the exact step which is impaired in melanogenesis pathway leading to hypopigmentation in PKDL
- Months 4-5: measurement of parasite load by qPCR and analysis of cytokine profile in PKDL.
- Month 6: Compilation and analysis of data obtained.

References:

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