Differential expression of Vitamin D related genes in Macular vs. Polymorphic Post Kala-azar Dermal Leishmaniasis

Background: Indian Post kala-azar dermal leishmaniasis (PKDL) is the cutaneous aftermath of visceral leishmaniasis (VL) that manifests as macular or polymorphic PKDL in the ratio of 1:10. However, ensuing the ongoing active surveillance for PKDL as part of the Leishmaniasis elimination programme, a substantial increase in the proportion of macular PKDL has been established. The lesional distribution of polymorphic cases is predominantly in sun-exposed areas whereas it is disseminated in the macular variant, suggesting a differential role for Vitamin D. Accordingly, this study aimed to delineate the status of Vitamin D_3 expressing genes in peripheral blood and dermal lesions of patients with polymorphic vs. macular PKDL.

Methods: Patients clinically diagnosed with PKDL were recruited from active field surveys conducted in VL endemic districts of West Bengal. Blood and dermal biopsies were collected at disease presentation. Upon ITS-1 PCR positivity, parasite load was quantified, and mRNA expression of Vitamin D receptor (*VDR*), 25-Hydroxyvitamin D_3 1-alpha-hydroxylase (*CYP27B1*), *LL-37* (cathelicidin) and *β-actin* was measured by qPCR in PBMC of PKDL cases (n=20, macular: polymorphic, 1:1) and VL (n = 6) along with reverse transcriptase-PCR from dermal biopsies (n=10, macular: polymorphic 1:1). Plasma levels of 25(OH) Vitamin D_3 was measured by sandwich ELISA at disease presentation (n=23, macular: polymorphic 11:12).

Results: As compared to healthy controls, plasma 1α,25-dihydroxyvitamin D3($1,25D_3$) levels were significantly raised in the polymorphic variant and positively correlated with parasite load at disease presentation. In circulating monocytes, irrespective of the variant, the mRNA expression of VDR (responsible for nuclear signaling of 1,25D3), CYP27B1 (converts vitamin D to its active form, 1,25D3) and *LL-37* was significantly increased, and they positively correlated with parasite load. Additionally, Plasma $1,25D_3$ positively co related with mRNA expression of *CYP27B1* and *LL37*. In dermal lesions, normal skin demonstrated expression of *VDR* but *CYP27B1* and *LL37* was not detectable. The mRNA expression of *CYP27B1* was significantly raised in both variants, but an increase of *LL37* was restricted to the polymorphic cases. The mRNA expression of Vitamin D related genes in circulating monocytes of VL patients was comparable with healthy individuals.

Conclusion: In PKDL, the enhanced polarization of monocytes-macrophages towards alternate activation accounted for the enhanced expression of Vitamin D related genes *VDR*, *CYP27B1* and *LL37* in both variants in their peripheral blood. Importantly, in dermal lesions which are the site of the disease, an increase in the mRNA expression of *VDR* and *CYP27B1* was demonstrated. However, the failure to detect mRNA expression of *LL37*, in the macular variant suggests its putative impact on the altered melanogenesis, a classical feature of macular cases. Taken together, Vitamin D appears to play a contributory role in the pathogenesis and disease progression of PKDL.