A Pilot Study to Measure Serum IL-6 Levels in Erythema Nodosum Leprosum Cases and Correlate These Levels with Severity of Reaction

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Erythema Nodosum Leprosum (ENL), a type 2 leprosy reaction, is an immune-mediated inflammatory complication that leads to significant morbidity in leprosy patients, particularly those with lepromatous and borderline lepromatous leprosy. Recently, interleukin-6 (IL-6) has gained attention as an inflammatory biomarker for leprosy reactions. This study investigates serum Interleukin-6 (IL-6) levels in patients with Erythema Nodosum Leprosum (ENL). Using a case-control design, the study aimed to quantify IL-6 levels and correlate them with ENL severity, measured by the 16 points ENLIST ENL severity scale. Blood samples were collected from 35 ENL patients and 35 healthy controls, with IL-6 levels quantified through chemiluminescent immunoassay. Statistical analyses, including Spearman's correlation and ROC analysis, were employed. Results demonstrated significantly elevated IL-6 in ENL cases compared to controls, with a positive correlation between IL-6 levels and ENL severity. Findings suggest IL-6 as a potential biomarker for ENL, contributing to earlier detection and enhanced management strategies. The study focused on single biomarker. Other inflammatory markers like TNF-α, IL-1β, or IL-10 were not studied, limiting the exploration of holistic view of immune mechanisms in ENL. Future research should consider larger, multi-center studies with longitudinal designs and disease controls without evidence of reaction to validate IL-6 as a therapeutic target for ENL.

Keywords: Erythema Nodosum Leprosum, Severity, IL-6, Biomarker

Introduction

Leprosy, caused by *Mycobacterium leprae*, is a chronic infection primarily targeting the skin and peripheral nerves, often resulting in sensory loss and motor dysfunction (Bhat & Prakash 2012). Erythema Nodosum Leprosum (ENL), a type 2 leprosy reaction, is an immune-mediated inflammatory complication that leads to significant morbidity in leprosy patients, affecting about 50% of lepromatous leprosy patients and 10% of borderline lepromatous leprosy patients (Pocaterra et al 2006). It can be encountered either before, during the course of anti-leprosy treatment, or after the completion of anti-

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leprosy treatment (IAL Workshop 2003). ENL is characterized by the sudden appearance of crops of evanescent (lasting for few days) pink (rose) colored tender papules, nodules or plaques and constitutional symptoms (fever, malaise, arthralgia, and myalgia), often accompanied by neuritis, uveitis, orchitis, or other systemic organ involvement (Walker et al 2015). Despite effective multi-drug therapy (MDT), many patients experience ENL episodes, which may result in permanent nerve damage and disability.

ENL is often associated with a hyperactive immune response, sometimes referred to as a "cytokine storm." IL-6, along with other cytokines like TNF-α and IL-1β, is significantly elevated during ENL episodes (Dias et al 2016). IL-6 contributes to the systemic inflammatory response, enhancing the acute phase reaction and driving the clinical manifestations of ENL. IL-6 promotes the differentiation of B cells, increases T cell proliferation, and upregulates the expression of adhesion molecules on endothelial cells, which facilitates the migration of leukocytes to inflammation sites (Grebenciucova & van Haerents 2023).

Recent studies have identified cytokines like IL-6 as potential biomarker for inflammatory conditions. IL-6 is a pro-inflammatory cytokine involved in immune response modulation, and its elevated levels have been observed in various autoimmune and infectious diseases (Uciechowski & Dempke 2020). However, limited research exists on the correlation between IL-6 levels and ENL severity. This study aims to fill this gap by examining IL-6 as a biomarker to better understand ENL's immunopathology and potentially guide clinical management.

Materials and Methods

This observational, hospital-based case-control study was conducted at the Department of Dermatology, Venereology, and Leprosy at Sawai

Man Singh Medical College, Jaipur, Rajasthan. The study was conducted after obtaining Ethical clearance from the institutional ethical committee over a duration of 1 year, from May 2023 to April 2024. The study included 35 patients diagnosed with ENL and 35 healthy controls, matched for age and gender. All the patients with confirmed ENL who were willing to participate were taken as cases and healthy individuals who were willing to participate in the study were taken a control. All patients who have known disorders and diseases that interfere with levels of IL-6 (autoimmune diseases, chronic systemic and dermatological inflammatory diseases, viral and bacterial infections), patients receiving treatment that interfere with levels of IL-6, and patients of ENL who took treatment for ENL in last 1 month were excluded from the study. After obtaining informed consent, demographic data and clinical findings were recorded.

The ENL severity of each patient was assessed using the 16 points ENLIST ENL Severity Scale (EESS) described in Table 1(Walker et al 2016). Categorization of ENL severity was based on the sum of score of all 16 items. Male patients with total score up to 16 and females with score up to 15 (since orchitis was not applicable) were categorized as mild ENL. Male patients with a score of more than 16 and females with a score of more than 15 were categorized as moderate - severe ENL. Blood samples were collected under aseptic conditions for both cases and control group, and serum IL-6 levels were quantified using chemiluminescent immunoassay (Talwar et al 2022).

Descriptive statistics (means, standard deviations) were calculated for continuous variables, while frequencies and percentages were used for categorical data. Student's t-test compared IL-6 levels between ENL cases and controls. Spearman's correlation analyzed the relationship

between IL-6 levels and ENL severity. A p-value of <0.05 was considered statistically significant.

Results

The study sample consisted of 35 ENL patients and 35 healthy controls. There were 27 males (77.14%) and 8 females (22.86%) in each group. The average age of participants was approximately 41 years, with no statistically significant age difference between groups (p= 0.7). 34 cases and all control group patients were from Rajasthan while 1 case was from Uttar Pradesh. 26 cases and 25 controls were from rural area, while 9 cases and 10 controls were from urban area. Mean BMI of cases was 20.84 (standard deviation = 2.42) while mean BMI of controls was 21.42 (standard deviation = 1.91). The difference in BMI of both groups was not statistically significant (p = 0.22).

Clinical assessment of all 35 cases of ENL was done using 16 points EESS scale given by Walker et al (2016) (Table 2). 60% of cases reported a Visual Analog Scale (VAS) pain score of 1.

Regarding wellbeing, 57.14% had a VAS wellbeing score of 1. In the fever score, 57.14% had a score of 1 (i.e. no fever during examination but history of fever was present in the last 7 days). Almost half of the cases (45.71%) had 11-20 ENL skin lesions (score - 2). Three-fourth of cases had painful lesions (score - 2). 60% had extension of lesions to 5-7 regions (score - 3). There was no peripheral edema in 62.86% cases. 37.14% of cases did not experience bone pain, 34.29% had a bone pain score of 1 (present but not limiting activity). Majority of the cases (85.71%) did not experience inflammation of joints. There was no eye inflammation in 60% cases, while 22.86% had a score of 1 (redness or pain). Orchitis was absent in 68.57% of cases. Almost half of the cases (54.29%) had a lymphadenopathy score of 1(enlarged). In the Nerve tenderness score, 31.43% had a score of 0 (no tenderness), 34.29% patients had a score of 1 (absent if attention distracted). Majority of cases (82.86%) did not experience new sensory or motor nerve function
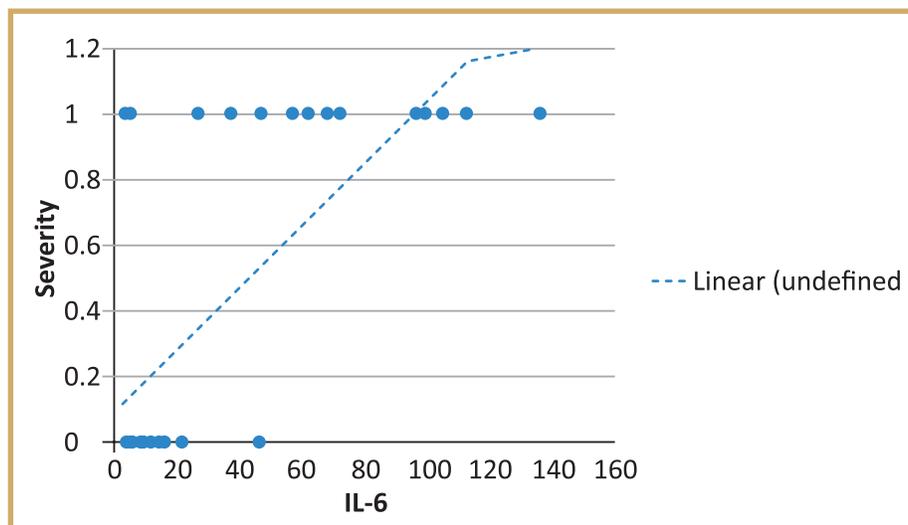

Fig. 1: Spearman's rho Analysis for the correlation of IL-6 and EESS score of ENL cases included in the study.

Table 1: ENLIST ENL 16 points scale (Walker et al 2016).

Item	Scores			
	0	**1**	**2**	**3**
1. VAS – Pain (mm)	0	1–39	40–69	70–100
2. VAS Wellbeing (mm)	0	1–39	40–69	70–100
3. Fever	None (37.5 or less)	No fever now, but history of fever in last 7 days	37.6–38.5	38.6 or higher
4. Number of ENL skin lesions	None	1–10	11–20	21 or more
5. Inflammation of ENL skin lesions	Non-tender	Redness	Painful	Complex
6. Extent of ENL skin lesions	0	1–2 regions	3–4 regions	5–7 regions
7. Peripheral oedema due to ENL	None	1 site of Hands or Feet or Face	2 sites	All three sites (Hands and Feet and Face)
8. Bone pain	None	Present, not limiting activity	Sleep or activity disturbed	Incapacitating
9. Inflammation of Joints and/or digits due to ENL	None	Present, not limiting activity	Sleep or activity disturbed	Incapacitating
10. Eye inflammation due to ENL	None	Redness or pain	Redness and pain	Blurring and/or reduced vision
11. Orchitis due to ENL	None	Tenderness	Pain on walking or movement	Pain at rest
12. Lymphadenopathy due to ENL	None	Enlarged	Pain or tenderness	Pain or tenderness in 2 or more groups
13. Nerve tenderness due to ENL	None	Absent if attention distracted	Present even if attention distracted	Patient withdraws limb on examination
14. Number of nerves with NEW sensory NFI due to ENL	None	1	2	3 or more
15. Number of nerves with NEW motor NFI due to ENL	None	1	2	3 or more
16. Urinalysis for protein	None	Trace	1 or 2+	3+

Table 2: Clinical assessment score of ENL cases based on 16 items EESS scale.

16 items scale		Number of patients (% of patients)			
S.No.	Item	Score 0	Score 1	Score 2	Score 3
1.	VAS - Pain	4 (11.43%)	**21 (60%)**	7 (20%)	3 (8.57%)
2.	VAS - Wellbeing	5 (14.29%)	**20 (57.14%)**	7 (20%)	3 (8.57%)
3.	Fever	4 (11.43%)	**18 (51.43%)**	10 (28.57%)	3 (8.57%)
4.	Number of ENL skin lesions	0	9 (25.71%)	**16 (45.7%)**	10 (28.57%)
5.	Inflammation of ENL skin lesions	0	8 (22.86%)	**26 (74.29%)**	1 (2.86%)
6.	Extent of ENL skin lesions	0	3 (8.57%)	11 (31.43%)	**21 (60%)**
7.	Peripheral edema due to ENL	**22 (62.86%)**	7 (20%)	5 (14.29%)	1 (2.86%)
8.	Bone pain	**13 (37.14%)**	12 (34.29%)	8 (22.86%)	2 (5.71%)
9.	Inflammation of joints and/or digits due to ENL	**30 (85.71%)**	1 (2.86%)	4 (12.43%)	0
10.	Eye inflammation due to ENL	**21 (60%)**	8 (22.86%)	6 (17.14%)	0
11.	Orchitis* due to ENL	**24 (68.57%)**	2 (5.71%)	1 (2.86%)	0
12.	Lymphadenopathy due to ENL	7 (20%)	**19 (54.29%)**	6 (17.14%)	3 (8.57%)
13.	Nerve tenderness due to ENL	11 (31.43%)	**12 (34.29%)**	5 (14.29%)	7 (20%)
14.	Number of nerves with new sensory NFI** due to ENL	**29 (82.8%)**	0	5 (14.3%)	1 (2.86%)
15.	Number of nerves with new motor NFI** due to ENL	**29 (82.8%)**	0	5 (14.3%)	1 (2.86%)
16.	Urinalysis for protein	**23 (65.7%)**	8 (22.8%)	4 (11.4%)	0

*Orchitis score was NOT APPLICABLE on female cases (**8** out of total 35 cases evaluated).

**NFI - Nerve Function Impairment

Table 3: Comparison of serum IL-6 levels in ENL cases and control group.

	Serum IL-6					P-Value
	Mean±SD	Median	Minimum	Maximum	Range	
Case Group	33.02±37.77	11.7	3.6	136	132.4	<0.01
Control Group	3.16±1.59	2.6	1.4	9.5	8.1	

impairment (NFI). Almost two - third of cases (65.71%) did not have protein in urine.

The distribution of ENL severity among females and males showed distinct differences. Among females, 50% experienced mild ENL, and 50% reported moderate - severe ENL. In contrast, among males, 70.37% cases had mild ENL while 29.63% had moderate - severe ENL.

The mean score in mild ENL group was 10.17 with a standard deviation of 3.11. For moderate - severe ENL, the mean score was 27.83 with a standard deviation of 8.04.

The mean serum IL-6 level was significantly higher in ENL patients (33.02 ± 37.77 pg/mL) compared to the control group (3.16 ± 1.59 pg/mL), with a p-value of <0.01, indicating a notable difference between cases and controls. The median serum IL-6 level was also significantly higher in ENL cases (11.7) compared to the control group (2.6), with a p-value of <0.01 (Table 3).

A positive correlation was found between IL-6 levels and ENL severity. Patients with mild ENL had a mean serum IL-6 level of 10.3 ± 8.97 pg/mL, those with moderate - severe ENL had a mean IL-6 level of 76.57 ± 33.1 pg/mL. The difference was statistically significant (p < 0.01), suggesting a strong correlation between IL-6 levels and ENL severity.

Spearman's rho analysis shows a strong positive correlation between serum IL-6 levels and EESS,

with a correlation coefficient (r-value) of 0.61 and a p-value of <0.01. This indicates a statistically significant relationship between higher IL-6 levels and increased severity of ENL (Fig. 1).

ROC analysis demonstrated an area under the curve (AUC) of 0.86 for IL-6 as a predictor of ENL severity, with an optimal IL-6 cut-off value of 24.15 pg/mL. This cut-off provided a sensitivity of 85.7% and specificity of 95.2%, supporting IL-6's potential utility as a biomarker for ENL severity assessment.

Discussion

Erythema Nodosum Leprosum (ENL) is an immune-driven inflammatory complication affecting around 50% of patients with lepromatous leprosy and 10% of those with borderline lepromatous leprosy, potentially occurring before, during, or after completing MDT-MB therapy. It is clinically characterized by intensely painful, erythematous nodules or plaques. Recent case studies highlight diagnostic delays, with necrotic ENL sometimes presenting before any leprosy reaction, during early treatment, or in unusual clinical presentations.

Interleukin-6 (IL-6), a proinflammatory cytokine, is pivotal in ENL reactions by promoting neutrophil activation and antibody production. Neutrophil infiltration marks the early stages of ENL, but progressively decreases over nine days as the reaction moves from an acute to a

regressive phase (Polycarpou et al 2017). Given that IL-6 is produced in response to infection or tissue damage and induces the production of acute-phase proteins in the liver, the observed reduction in serum IL-6 levels after 1 month of treatment may be linked to the resolution of the inflammatory process (Vilani-Moreno et al 2021). Therefore, measuring IL-6 levels in ENL cases could help correlate its levels with the severity of the reaction.

In the present study, severity of ENL was classified into two categories after clinical assessment of cases using EESS 16 points scale: mild ENL and moderate - severe ENL. Out of 35 cases assessed, 23 patients (19 males, 4 females) had mild ENL, 12 patients (8 males, 4 females) had moderate - severe ENL. For patients with mild ENL, average severity score was 10.17 (SD- 3.11), while those with moderate - severe ENL had a mean score of 27.83 (SD- 8.04).

In our study, in the case group, the median serum IL-6 level was 11.7 pg/mL, with values ranging from a minimum of 3.6 to a maximum of 136 pg/mL, resulting in a range of 132.4. In contrast, the control group had a median serum IL-6 level of 2.6 pg/mL, with levels ranging from 1.4 to 9.5 pg/mL, giving a range of 8.1. This difference was statistically significant, with a p-value of <0.01.

Serum IL-6 levels varied significantly across different severities of ENL in the case group. For mild ENL, the mean serum IL-6 level was 10.3 pg/mL with a standard deviation of 8.97. In patients with moderate - severe ENL, the mean serum IL-6 level was 76.57 pg/mL, with a standard deviation of 33.1. The differences across these severity levels were statistically significant, with a p- value of <0.01.

Spearman's rho analysis (Fig. 1) revealed a strong positive correlation between serum IL-6 levels and the Erythema Nodosum Leprosum Severity Score (EESS), with a correlation coefficient (r-value) of 0.61 and a p-value of <0.01. This result indicated a statistically significant relationship, demonstrating that higher IL-6 levels were associated with increased severity of ENL.

The ROC (Receiver Operating Characteristic) analysis for serum IL-6 showed an area under the curve (AUC) of 0.86, indicating strong diagnostic performance for distinguishing disease severity. The p-value of <0.01 confirmed the statistical significance of these findings. The optimal IL-6 cutoff value was determined to be 24.15 pg/mL, with a sensitivity of 85.7%, specificity of 95.2%, and an overall accuracy of 83%. These results suggest that IL-6 is a reliable biomarker for differentiating between disease states in ENL (Erythema Nodosum Leprosum) and potentially for other inflammatory conditions as well.

Vilani-Moreno et al (2021) assessed serum biomarkers in ENL patients, finding significantly elevated IL-6 and TNF-alpha in patients compared to controls. IL-6 levels were highest in patients with severe ENL and decreased after treatment.

Nahrisyah et al (2024) found a strong, significant positive correlation between serum IL-6 and EESS suggesting serum IL-6 may help in early detection of ENL severity. Conversely, Dubey et al (2020) found no statistical difference in IL-6 levels between ENL patients and controls, with ENL patients exhibiting IL-6 levels of 5.63 ± 11.29 pg/mL, highlighting variability in study findings regarding IL-6 levels in ENL patients.

Our study demonstrates a significant elevation in IL-6 levels among ENL patients compared to healthy controls, with IL-6 levels correlating positively with ENL severity. These findings align with previous studies indicating IL-6 as a pro-inflammatory marker involved in immune responses, further supporting its role in inflammatory complications such as ENL

reactions associated with leprosy (Vilani-Moreno et al 2021, Nahrisyah et al 2024).

The study's results propose IL-6 as a viable biomarker for monitoring ENL severity, which could aid in identifying patients at risk of severe reactions. IL-6 level measurements could facilitate early intervention, potentially minimizing the risk of complications such as permanent nerve damage. Furthermore, the promising specificity and sensitivity of IL-6 (as evidenced by ROC analysis) underscore its potential as a clinical tool.

The study's single-center design and limited sample size may restrict the generalizability of findings. By capturing data at only one point in time, the study design restricts insight into the temporal progression of IL-6 levels in response to ENL episodes and transition from BL/ LL without reactions to ENL episodes. Our study focused primarily on only single biomarker, IL-6, limits the study's exploration of other inflammatory markers like TNF-α, IL-1β, or IL-10 that could provide a more holistic view of immune mechanisms in ENL.

Future research should incorporate larger, multi-center cohorts and longitudinal designs to validate IL-6's role over the course of ENL. Additionally, examining other cytokines alongside IL-6 could provide a more comprehensive view of ENL's immunopathology, potentially uncovering additional biomarkers or therapeutic targets.

Acknowledgements

References

1. Bhat RM, Prakash C (2012). Leprosy: An overview of pathophysiology. *Interdiscip Perspect Infect Dis*. **2012**: 181089.

2. Dias AA, Silva CO, Santos JPS et al (2016). DNA sensing via TLR-9 constitutes a major innate immunity pathway activated during erythema nodosum leprosum. *J Immunol*. **197(5):** 1905–1913.

3. Dubey A, Gaur R, Arela N et al (2020). Soluble mediators of immune significance in sera of leprosy patients. *Lepr Rev*. **91(4)**: 403–412.

4. Grebenciucova E, Van Haerents S (2023) Interleukin 6: at the interface of human health and disease. *Front Immunol.* **14**: 1255533.

5. IAL workshop on reactions in leprosy, Jamnagar, Gujarat, 11-12 January 2003. *Indian J Lepr.* **75**: 295-303.

6. Nahrisyah, Lubis R D, Siregar R et al (2024). Correlation between serum interleukin-6 levels and the severity of Enlist ENL Severity Scale (EESS) in erythema nodosum leprosum (ENL) patients. *Bali Med J*. **13(1**): 373-376.

7. Pocaterra L, Jain S, Reddy R et al (2006). Clinical course of erythema nodosum leprosum: An 11-year cohort study in Hyderabad, India. *Am J Trop Med Hyg*. **74(5**): 868–879.

8. Polycarpou A, Walker SL, Lockwood DN (2017). A systematic review of immunological studies of erythema nodosum leprosum. *Front Immunol*. **8**: 233.

9. Talwar D, Kumar S, Acharya S et al (2022). Interleukin 6 and its correlation with COVID-19 in terms of outcomes in an intensive care unit of a rural hospital: A cross-sectional study. *Indian J Crit Care Med*. **26(1):** 39–42.

10. Uciechowski P, Dempke WC (2020). Interleukin-6: a master player in the cytokine network. *Oncology*. **98(3**): 131–137.

11. Vilani-Moreno FR, Brito-de-Souza VN, Silva SUR et al (2021). Increased serum levels of inter-leukin-6 in erythema nodosum leprosum suggest its use as a biomarker. *Indian J Dermatol Venereol leprol*. **87(2)**: 190-198.

12. Walker SL, Balagon M, Darlong J et al (2015). ENLIST 1: an international multi-centre cross-sectional study of the clinical features of erythema nodosum leprosum. *PLoS Negl Trop Dis*. **9(9)**: e0004065.

13. Walker SL, Knight K, Pai VV et al (2016). The development of a severity scale for erythema nodosum leprosum – the ENLIST ENL severity scale. *Lepr Rev.* **87**: 332–346.

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