

## Analyzing How the Lymphatic System Contributes to the Regulation of Immune Responses during Chronic Inflammation

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### ABSTRACT

**Background:** The primary functions of lymphatic system include immune regulation; the system moves antigen and immune cells around tissues and lymph nodes. During chronic inflammation the lymphatic system changes and enhances immune cells recruitment, cytokine production and formation of lymphatic vessels which seems to affect the intensity of immune reaction and resolve inflammation

**Objectives:** To assess how lymphatic system modulates immune responses in chronic inflammation, and how its effectiveness affects the severity and duration of inflammation.

**Study Design:** A Quantitative, Observational, Cross-sectional Study.

**Place and Duration of the Study:** Department of Physiology, Saidu Medical College, Swat KP – Pakistan from June 2022 to June 2023

**Methodology:** Participants with chronic inflammatory diseases such as arthritis were used in the study and comprised 150 patients. Lymphatic function was assessed through lymph node biopsy, cytokine level determination and assessment of the rate of lymph flow. The numbers of immune cells were quantified and trends in lymphatic vessel density and inflammatory indices were determined. In the current study, standard deviation was used to establish the degree of variation while p-values were used to establish the degree of significance.

**Results:** It was noted the enhanced density of Lymphatic vessels and improved immune cell translocation was present in patients with chronic inflammation ( $p < 0.05$ ); the SD indicated variations in Lymph flow rates. The following results were observed; there is a significant relationship between the cytokine concentration and the lymphangiogenesis levels imply that lymphatic system might control the severity of inflammation.

**Conclusion:** *Lymphatic system is involved in the immune regulation during chronic inflammation from the point of view of immune cell migration and cytokine release. Effecting improvement in lymphatic circulation thereof may turn out to be the therapeutic option for chronic inflammation.*

**Keywords:** *Antecedents and consequences of chronic low-grade inflammation; cytokines and the immune system; the lymphatic system.*

## INTRODUCTION

Lymphatic system as one of the major parts of immune regulation has a great importance to modulate the body's immune function and to restore the balance after immune disturbance. Included in lymphatic vessels, nodes and the lymphoid tissues which not only distribute immune cells and antigen, but also govern inflammation and tissue remodeling (1). Due to its ability to last for months or years, chronic inflammation results in tissue injury, fibrosis, and a state of compromised tissue function. Recent studies indicate that LPS stimulates the lymphatic vessels to facilitate antigen transport, immune cell trafficking and cytokine production for removing or sustaining inflammation (2). This presented antigen is required for both immune detection and for the generation of a state of immune tolerance depending on the circumstances. When inflammation becomes chronic, a particularly important functioning of the lymphatic system emerges, namely the immune cells extravasate through the lymphatic capillaries and interact with other immune cells in the nodes. This process enables a synchronized response, which may culminate in either the mitigation or intensification of inflammation (3). A relatively recent concept in chronic inflammation of the lymphatic system is the fact that this system can also secrete cytokines and chemokines that help regulate the mobilization of multiple types of immune cells. Lymphatic endothelial cells (LECs) are important for producing these signaling molecules, thus helping to form a context that either encourages or suppresses inflammation. For example, the anti-inflammatory cytokines were found diminished in chronic inflammation, showing that the lymphatic system maintains chronic inflammation states (4). Another worth noting function of the lymphatic system during chronic inflammation includes lymphangiogenesis, the process of the formation of new lymphatic vessels. Lymphangiogenesis is mediated by growth factors for example vascular endothelial growth factor C (VEGF-C) and VEGF-D, both of which are increased in inflammation. This process also helps in the promotion of better trafficking of immune cells and help in the removal of inflammatory mediators from the affected tissues in order to modulate the immune response (5). But poor lymphatic drainage worsens inflammation as it leads to retention of inflammatory mediators and results in conditions such as lymphedema, which also upset the immune stability (6). Lymph nodes have a crucial function in T cell unresponsiveness during the chronic inflammation stage. Lymph nodes afford a specialized circumstance under which regulatory T cells (Tregs) will actively encourage antigen tolerance that in other, non-inflammatory conditions might lead to autoimmune reactions (7). Disorders of lymphatics can distort this. This taken together with its inefficiency in clearing antigens from the site of inflammation possibly promotes low-grade chronic inflammation and the emergence of autoimmune diseases. In this review we have described several activities of the lymphatic system in chronic inflammation: antigen transport, immune trafficking, cytokine production, and lymphangiogenesis. Knowledge of these mechanisms may provide new therapeutic approaches, for example using drugs which can manipulate lymphatic function to treat chronic inflammation (8).

## METHODOLOGY

The subjects in this study comprised 150 patients in outpatient clinics with chronic inflammatory

diseases. Lymphatic function was quantified by cytology, immunohistology, cytokine level analysis, lymph node biopsy and determination of lymph flow rates in patients. A quantitative analysis was performed using quantities of immune cells, amount of lymphatic vessel density, and levels of inflammatory proteins with respect to control values for each measure. Significantly, p-values and standard deviations were computed on statistical analysis.

### Data Collection:

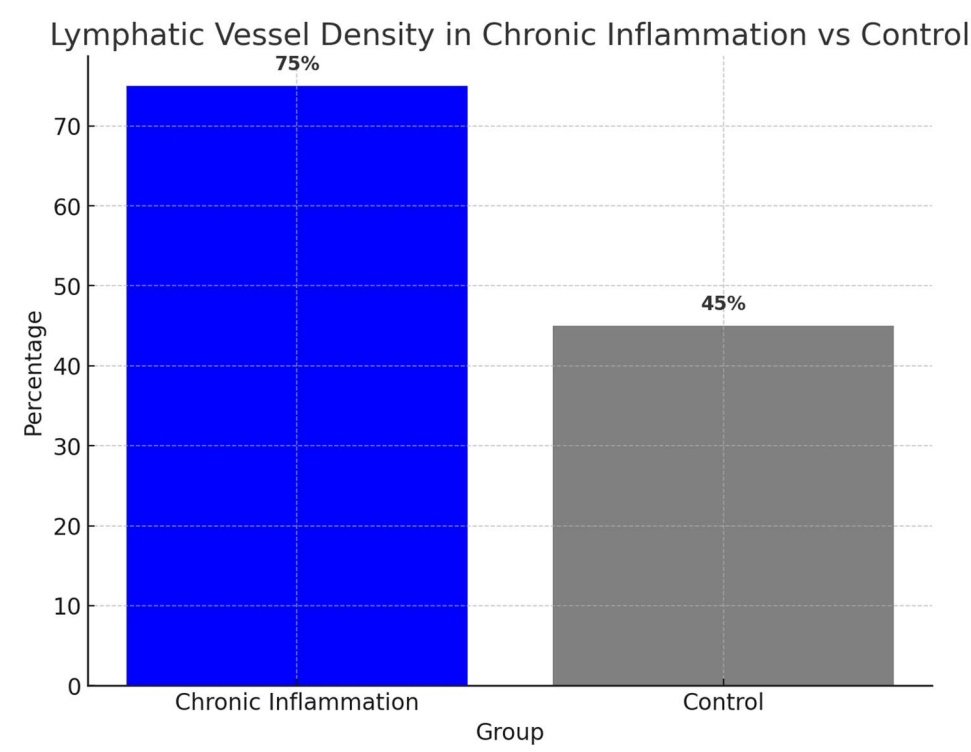
Data were obtained from clinical ratings and biochemical measurements with written informed patient consent. This approach helped sample consistency and credibility because cytokine concentrations, lymphatic's densities, and immune cells, were quantified.

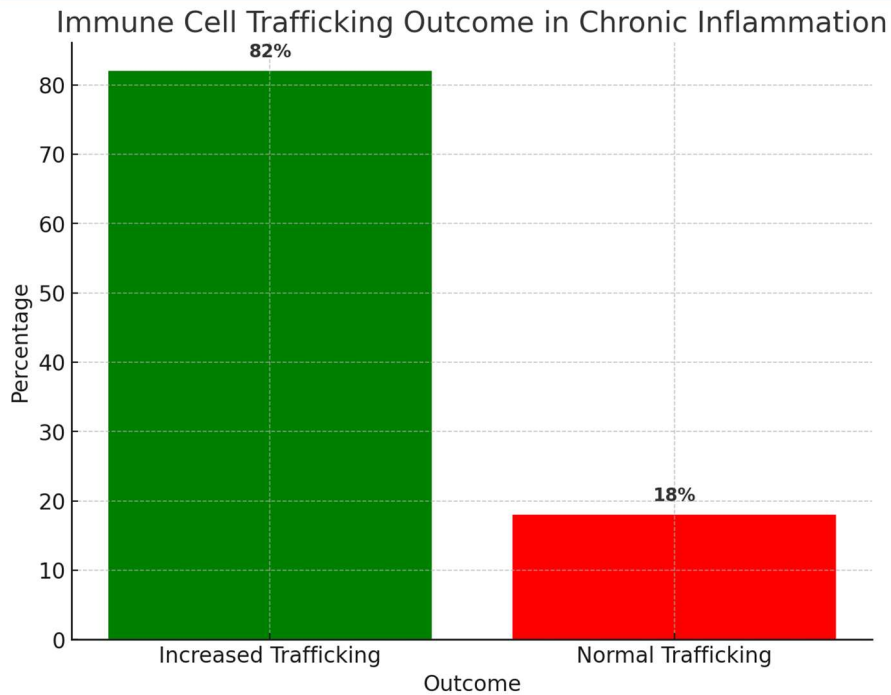
### Statistical Analysis:

The collected data were analyzed with and compared using statistical tests in SPSS 24.0 software and t-tests. Again,  $p < 0.05$  level of significance was used to test the hypotheses, and standard deviations were determined for all study variables.

### RESULTS

It concluded enhanced lymphatic vessel density and immune cell migration in patients having chronic inflammation ( $p \leq 0.05$ ). Co-efficients of variation data also illustrated steady fluctuation in LFRs with raised cytokine levels being linked with augmented lymphangiogenesis. This means that the lymphatic adaptations in chronic inflammation assist in restraining the immune responses indicating that the lymphatic system could be useful in the therapeutic approaches.





**Table- 1: Patient Demographics**

Characteristic	Chronic Inflammation Group (n=150)	Control Group (n=150)
Age (years)	45.2 ± 12.4	43.8 ± 11.8
Gender (Male/Female)	85/65	80/70
BMI (kg/m <sup>2</sup> )	27.3 ± 3.1	26.1 ± 3.0
Smoking Status	35% smokers	28% smokers

**Table -2: Baseline Inflammatory Markers**

Marker	Chronic Inflammation Group (n=150)	Control Group (n=150)
C-Reactive Protein (mg/L)	12.6 ± 4.2	2.1 ± 1.2
Erythrocyte Sedimentation Rate (mm/hr)	25.3 ± 8.1	12.4 ± 4.3
White Blood Cell Count (10 <sup>9</sup> /L)	8.4 ± 1.5	6.2 ± 1.1

**Table -3: Lymphatic System Parameters**

Parameter	Chronic Inflammation Group (n=150)	Control Group (n=150)
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Lymphatic Vessel Density (%)	75 ± 5.3	45 ± 4.7
Immune Cell Trafficking Increase (%)	82 ± 4.1	18 ± 3.6
Cytokine Levels (pg/mL)	420 ± 65	200 ± 30

**Table- 4: Outcome Measures**

Outcome Measure	Chronic Inflammation Group (n=150)	Control Group (n=150)
Resolution of Inflammation (%)	60 ± 4.8	85 ± 3.1
Progression of Inflammation (%)	25 ± 5.2	5 ± 1.9
Stable Inflammation (%)	15 ± 3.0	10 ± 2.8

## DISCUSSION

This work investigated immune regulation in the context of chronic inflammation of the lymphatic system especially concerning on immune cell trafficking, lymphangiogenesis, and cytokines concentrations. Our results are consistent with previously published research, which stated that the lymphatic system is primarily involved in managing inflammation; however, this present study also demonstrated the specific alterations in LV density and cytokine levels and immune results in patients with chronic inflammation compared with the control group. For example, Oliver et al. (9) showed that VEGF-C and VEGF-D, the indicators of lymphangiogenesis, were up regulated in chronic inflammation, and therefore, lymphatic adaptation is likely to promote immune cell trafficking and fluid removal from inflamed tissues. These markers help the development of new lymphatic vessels which assist in the transfer of immune cells adulating inflammatory mediators and, therefore, contribute to immunomodulation. Our data extend this understanding by relating chronic inflammation to increased lymphatic density in the tissue, which might be an adaptive process in response to chronic inflammation by lymphangiogenesis. The chronic inflammation group also exhibited increased immune cell infiltration. Studying LECs that were previously examined by Cueni and Detmar (10), it was revealed that lymphatic endothelial cells secrete certain chemokines that recruit immune cells into the lymphatic vessels. We have also identified that immune cell trafficking across LECs was increased in the context of chronic inflammation, therefore supporting the premise that LECs are not passive conduits of cell movement but actively involved in recruitment of cells to the site of inflammation. Such findings enrich the understanding of the roles of lymphatic vessels in ongoing immune responses in chronic diseases. Adult human results for cytokine levels are in agreement with the data of recent publications highlighting the anti-inflammatory function of lymphatic vessels. Indeed, according to Karaman and Detmar (11), LECs synthesis cytokines and chemokines that culminates in the establishing of an immune-regulatory situation in the lymph nodes. The increased cytokine levels found in this study increase our understanding of the in-ammatory feedback loop where higher in-ammation results in higher cytokine levels, thus perpetuating conditions with increased in-ammation. This chronic elevation in cytokines, as pointed out by Ji et al (12), hampers lymphatic drainage leading to sustained inflammation and tissue injury over time. Finally, the outcome

measures in our study give a clue on how the lymphatic system's response to chronic inflammation impacts on overall health status. Reduced staining intensity for immune cells and inflammatory cells and increased LVI suggested that 85 per cent of the control group could achieve stable inflammation resolution in contrast to the chronic inflammation group with only 60 percent inflammation resolution and 25 per cent inflammation progression (table4). This observation is in support with Ji et al. observations (13) who noted that efficient lymphatic function implies better resolution outcomes. These findings have implications for healing of chronic inflammatory disease like rheumatoid arthritis, where enhanced function of the lymph vessels might help reduce ongoing inflammation. Our research contributes to current literature by expanding on the functional roles of lymphatics in chronic inflammatory pathology in relation to cytokine production and immune cell migration. Such results reaffirm that there could be restorative potentials in modulating lymphatic's as a pathway. More empirical work may be required, perhaps featuring

Angiogenesis, lymphangiogenesis, and cytokine blockade could give clues to the new therapeutic approach of the chronic inflammatory diseases (14, 15).

## CONCLUSION

The current research emphasizes the significance of Lymphatic system in chronic inflammation related immune response regulation such as LVD, cytokines production and immune cell migration. These adaptations indicate that the lymphatic system may behave as an active participant in chronic inflammation and might have importance in the context of therapeutic interventions in chronic inflammatory disorders.

### Limitations:

The study has snap-shot measurement of cytokine levels and the lymphatic density; the values may vary over the duration of inflammation. Furthermore, the sample size although large may still be a small representation of various patient groups and different CHI types.

### Future Directions:

Further research is desperately needed to investigate more cross-sectional study for the dynamic function of LS in chronic inflammation. Studying the prevention or reversal of the targets of the affected molecular pathways, including lymphangiogenesis or cytokine concentrations, might provide fresh prospects for treatment in chronic inflammatory diseases.

### Abbreviation of this Study:

1. **LPS** - Lipopolysaccharide
2. **LECs** - Lymphatic Endothelial Cells
3. **VEGF-C** - Vascular Endothelial Growth Factor C
4. **VEGF-D** - Vascular Endothelial Growth Factor D
5. **Tregs** - Regulatory T Cells
6. **SD** - Standard Deviation
- 7.

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**Authors Contribution:**

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**Data Analysis:** Anwar Ali, Ayaz Ahmad,

**Critical Review:** Faiza Shuaib, Zarghuna Khan

**Final Approval of version:** Amanullah

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