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# Pentraxin-3 and its Association with C1q-CIC, hsCRP and Pro-Inflammatory Cytokines (TNF-A and IL-1β) among SLE Patients from India

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## Abstract

**Introduction:** Systemic Lupus Erythematosus (SLE) is a prototype autoimmune disease with alternating periods of flares and remission. Disease pathogenesis involves vast inflammatory responses, characterized by impaired cell signaling and T-cell dysfunction with interplay of cytokines and a wide network of protein cascades. Aim of this study is to understand the correlation between PTX-3 and pro-inflammatory cytokines (TNF-α and IL-1β) and their role in disease pathogenesis of SLE.

**Materials and Methods:** Sixty-three SLE patients classified as per ACR 1997 criteria were included, of which 36 had renal involvement (LN). Autoantibodies were detected by IFA and ANA BLOT techniques. Serum Complement levels and hsCRP (by nephelometer), Pentraxin-3 and C1q-CIC (by ELISA), TNF-α and IL-1β levels (by Multiplex immunoassay) were assessed.

**Results:** ANA was present in 90.5% patients, anti-dsDNA antibodies were present in 87.3% patients. Reduced C3 (<90 mg/dl) and C4 (<15 mg/dl) levels were found in 58.7% patients. hsCRP was elevated (>5 mg/L) in 49.2% patients. C1q-CIC levels were high (>50 µg/ml) in 50.8% patients. Serum PTX-3 levels and TNF-α levels were significantly higher in SLE patients than healthy controls ($p<0.0001$ and $p<0.0001$ respectively). It was observed that PTX-3 and IL-1β levels were significantly higher in LN patients as compared with nonLN patients ($p=0.0107$; $p=0.0022$ respectively). PTX-3 positively correlates with C1q-CIC and negatively correlates with hsCRP and IL-1β levels among SLE patients.

**Conclusion:** This study suggests that serum PTX-3 though do not induce an immediate inflammatory response, its positive correlation with C1q-CIC levels, suggested its possible role in classical complement pathway activation. Further studies in this regard are needed to understand the role of PTX-3 in pathogenesis of SLE.

**Keywords:** Pentraxin-3; SLE; Lupus Nephritis (LN); C1q-CIC; Cytokines (TNF-α and IL-1β)

## Introduction

Systemic Lupus Erythematosus (SLE) is a prototype systemic autoimmune disease classical for differential diagnosis owing to the varied clinical manifestations. Clinical manifestations like arthralgia (arthritis), malar rash, synovitis, serositis, photosensitivity, anemia, thrombosis are the common features in this disease [1]. Involvement of renal tissues (lupus nephritis) is also a major presentation which is significant in disease diagnosis and treatment of SLE. Lupus Nephritis (LN) is classified into six classes (Class I to Class VI) according to increasing renal pathogenesis [2]. The symptoms seen among SLE patients may be unexplained and may evolve and disappear unpredictably. Immune responses in SLE patients are directed to synthesize autoantibodies against self-antigens. Vast inflammatory responses set in are mainly characterized by impaired signaling and T-cell dysfunction. Interplay of various cytokines (interleukin 1β [IL-1β], tumor necrosis factor α [TNF-α] etc.) is prominent, with a wide network of protein cascades (pentraxin-3, C1q etc.) being induced. With advancement in lupus research, role of inflammation and related molecules in the disease pathogenesis have gained prominence [3]. The active disease stage (flares) may present with multiple manifestations simultaneously, which are mainly governed by significant increase in the circulatory levels of pro-inflammatory cytokines and associated proteins which are involved in the disease pathogenesis.

Pentraxin-3 (PTX-3) is released by phagocytes, dendritic cells, fibroblasts and endothelial cells in response to primary inflammatory signals like toll-like receptor (TLR) engagement, TNF-α and IL-1β etc. PTX-3 binds with high affinity to the complement component C1q and extracellular matrix component TNF-α induced protein 6. PTX-3 is known to facilitate pathogen recognition through macrophages and dendritic cells. PTX-3 is also known to inhibit recognition of apoptotic bodies by dendritic cells, thus helps in preventing the onset of autoimmune disorders. The role of PTX-3 in clearance of apoptotic cells through complement pathways has been studied in SLE. PTX-3 causes deposition of complement factor C3 on surfaces of apoptotic bodies, which are further presented by PTX-3 C1q complex to the complement pathways for immune complex clearance [4,5].

Complement factor I (C1) is a complex protein which binds to the Fc region of immunoglobulins that are bound to antigens i.e. immune complex (IC). These circulatory immune complexes (CIC) are trapped by the fenestrated vascular bed of glomeruli, with assistance from C1q. C1q activates C3 to C3b which facilitates IC deposition by binding to surfaces. The C1q mediated IC deposition induces a complex cascade leading to an inflammatory response at the site of IC deposition [6]. PTX-3, which is stimulated by TNF-α and IL-1β, has higher affinity for binding with C1q and inducing the classical complement pathway. In apoptosis C1q binding is enhanced by PTX-3, indicating the role of PTX-3 in complement mediated clearance of apoptotic cells.

The role of PTX-3 in SLE is still unclear as its actual involvement in disease pathogenesis is still not fully understood. Studies on PTX-3 levels in SLE patients across the globe also show variations [7].

The aim of this study is to understand the correlation between PTX-3 and hsCRP, C1q-CIC and pro-inflammatory cytokines TNF-α and IL-1β and their role in disease pathogenesis of SLE.

## Materials and Methods

### Ethics Approval

All aspects of this study were approved by the ethics committee of National Institute of Immunohaematology (NIIH-ICMR).

### Characteristics of patient population

Sixty-three (63) clinically diagnosed SLE patients were selected for the study. Clinical presentations at the time of evaluation were recorded and clinical severity score (SLEDAI) was calculated as per ACR/EULAR 1997 criteria. Patients with renal involvement (LN) were classified as per ISN/RPS 2003 classification criteria.

Out of sixty-three (63) SLE patients studied there were 57 females (90.5%) and 6 males (9.5%) with female: male ratio of 9.5:1. Thirty-one patients (49.2%) were treatment naïve (<1 year) cases. Severe disease activity (SLEDAI Score>18) was calculated in 17 patients (26.9%), while moderate activity (SLEDAI Score 8-18) was seen in 39 patients (61.9%) and 7 patients had mild disease activity (SLEDAI Score<8). Lupus Nephritis (LN) was observed in 36 patients (57%) on basis of renal biopsy findings. On basis of the ISN/RPS classification, 15 patients (23.8%) were Class II LN, whereas 20 patients (31.8%) were diagnosed with Class IV LN and one patient (1.6%) was diagnosed with Class III LN. Class 1 & 5 Malar (facial & skin) Rash and oral ulcers were reported in 26 patients (41.3%) each, while 22 patients (35%) had photosensitivity. 32 patients (50.8%) had Arthritis & arthralgia, while 17 patients (27%) had serositis. It was observed that 8 patients (12.7%) had neuropsychiatric complications.

Anti-nuclear antibodies (ANA) and anti-dsDNA antibodies was detected by Indirect Immunofluorescence assay (IFA) [Biorad, USA]. ANA specificities to identify specific autoantibodies was performed using LINE BLOT assay (EUROIMMUN, Germany). Serum complement levels (C3 and C4) and high sensitivity C-reactive protein (hsCRP) levels were detected using nephelometer [BN ProSpec Siemens, Germany]. Serum levels of IL-1β and TNF-α were analysed using Multiplex Immunoassay (Merck Millipore, LUMINEX platform). Serum levels of Pentraxin-3 (PTX-3) were tested using Qayee Biotech (Shanghai, China) kit and C1q-CIC using Calbiotech (USA) kit ELISA and optical density readings were recorded at 450 nm with TECAN Infinite 200 multimode reader (Switzerland). GraphPad Prism V6.01 was used for all statistical analysis.

### Autoantibody profile in SLE patients

Anti-nuclear antibodies (ANA) were present in 57 patients (90.5%), where as anti-dsDNA antibodies were present in 55 patients (87.3%). ANA BLOT revealed specificities for anti-Smith antibodies in 34 patients (54%), anti-nucleosome antibodies in 17 patients (27%), anti-Histone antibodies in 24 patients (38.1%) anti-ribosomal P antibodies in 15 patients (23.9%), anti-SS-A/Ro 52 and/or 60 kD antibodies in 33 patients (52.4%), anti-SS-B/La antibodies in 4 patients (6.4%), anti-U1RNP antibodies in 15 patients (23.8%).

### Complement levels

Reduced levels of C3 levels (<90 mg/dl) were found in 37 patients (58.7%) while 37 patients (58.7%) reported low C4 levels (<14 mg/dl). Thirty-five patients (55.6%) had reduced levels for both the complement components (C3 & C4). Thirty-one patients (49.2%) had elevated hsCRP levels (>5mg/L).

## Results

### Evaluating serum levels of PTX-3, C1q-CIC, hsCRP and cytokines

The mean PTX-3 levels in SLE patient sera (Mean ± SD) (68.8 ± 20.2 ng/ml) were significantly higher than healthy controls (38.8 ± 27.1 ng/ml) (p=0.0001***). The mean C1q-CIC levels in SLE patients were 57.4 ± 22.9 µg/ml. Thirty-two patients (50.8%) had higher levels (>50 µg/ml) of C1q-CIC as compared to healthy individuals (<50 µg/ml). TNF-α levels in SLE patient sera (29.3 ± 20.3 ng/ml) were higher than healthy controls (12.6 ± 2.9 ng/ml) (p<0.0001****). Serum IL-1β levels were marginally higher in SLE patients (10.3 ± 5.5 ng/ml) than healthy controls (8.6 ± 4.3 ng/ml) (Figure 1).

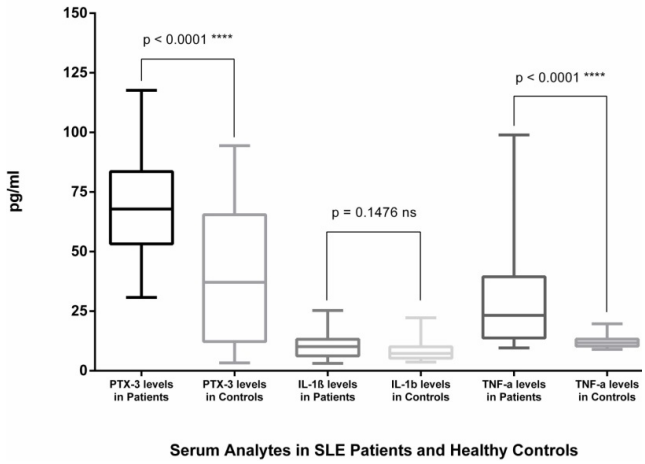


**Figure 1:** Serum levels of PTX-3, IL-1β and TNF-α in SLE patients (n=63) and healthy controls (n=37).

PTX-3 levels in SLE patients with LN (68.9 ± 19.9 ng/ml) were significantly higher as compared to SLE patients without LN (50.1 ± 33.6 ng/ml) (p=0.0 107*). IL-1β levels in LN patients (12.1 ± 5.8 ng/ml)

were also found to be significantly higher than non-LN patients (7.9 ± 3.861 ng/ml) (p=0.0022**). There was no statistically significant difference (p>0.05) for TNF-α levels among LN patients (30.9 ± 20.5 ng/ml) as compared with non-LN patients (26.9 ± 20.2 ng/ml). Higher C1q-CIC levels were found in 19 out of 36 LN patients (52.8%) and 13 out of 27 non-LN patients (48.2%) (p>0.05) (Figure 2).

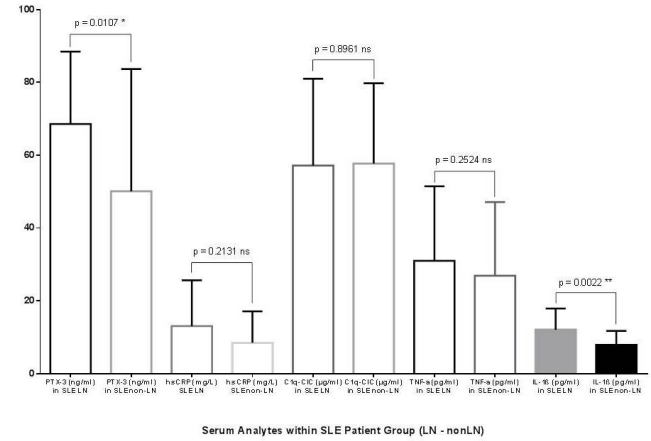


**Figure 2:** Serum levels of PTX-3, C1q-CIC, IL-1β and TNF-α in LN (n=36) and SLE without nephritis (n=27).

## Association between Pentraxin-3 and C1qCIC, hsCRP and cytokines

Pentraxin-3 shows no significant association with C1q-CIC among SLE LN and patients without renal involvement; however, a significant positive correlation is seen among treatment naïve cases of SLE (r=0.3831; p=0.0366*) (Figure 3).

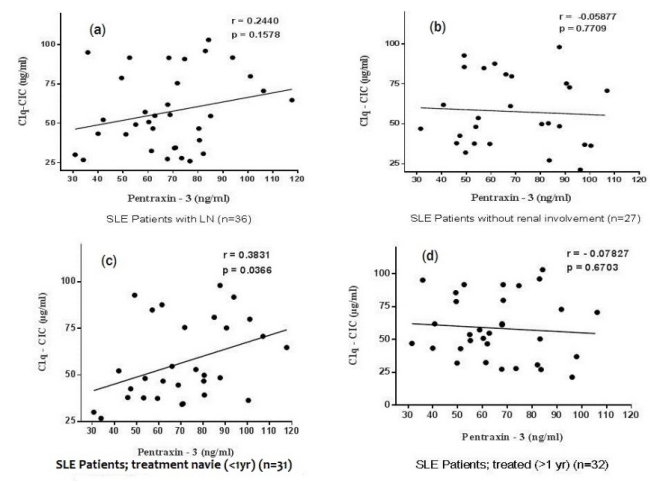


**Figure 3:** Correlation between Pentraxin-3 and C1q-CIC in SLE patients (a) with Lupus Nephritis (n=36) and (b) SLE without Renal Involvement (n=27); (c) treatment naïve patients (n=31) and (d) treated patients (n=32).

Pentraxin-3 also is negatively correlated with hsCRP (r=-0.3828; p=0.0340*) in treatment naïve patients (Figure 4). Pentraxin-3 does not show a significant correlation with TNFα (Figure 5), while a negative correlation with IL-1β is seen (r=-0.3828; p=0.0335*) (Figure 6). PTX-3 does not bear any significant association with C1q-CIC, hsCRP, TNF-α and IL-1β in patients with more than 1 year of treatment.

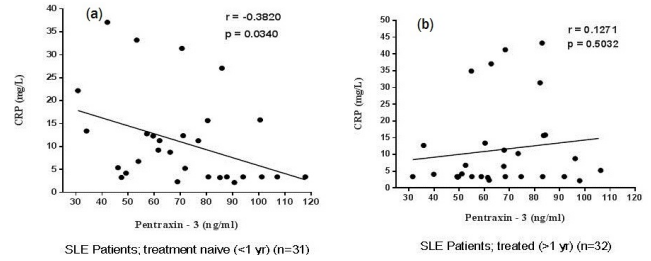


**Figure 4:** Correlation between Pentraxin-3 and hsCRP in (a) treatment naive SLE patients (n=31) and (b) treated SLE patients (>1 year) (n=32).

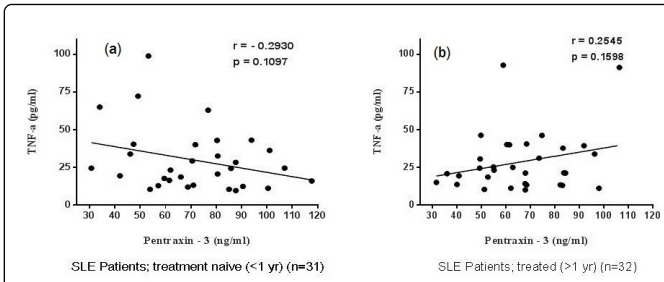


**Figure 5:** Correlation between Pentraxin-3 and TNF-α in (a) treatment naive SLE patients (n=31) and (b) treated SLE patients (>1 year) (n=32).

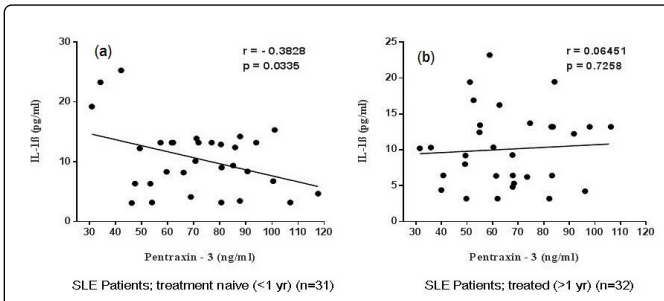


**Figure 6:** Correlation between Pentraxin-3 and IL-1β in (a) treatment naive SLE patients (n=31) and (b) treated SLE patients (>1 year) (n=32).

## Discussion

In an autoimmune disease like SLE, multiple protein cascades are involved in the pathogenesis. Activation of classical complement cascades is a key event in the innate immune system that contributes to the placid clearance of antigenic determinants. PTX-3 and C1q and the subsequent immune-complex formation, play important role complement activation. C1q which is the initiator protein for the Classical pathway carries out major clearance of immune complexes

(ICs). Activation and interplay of classical complement pathway is also important in the pathogenesis of SLE [8].

Recently Bassi *et al.* (2015) had reported significantly lower levels of PTX-3 among SLE patients from Italy as compared to healthy controls (p=0.009), whereas renal involvement did not show any significant variation in PTX-3 levels [9]. However, in another study published by Assandri *et al.* (2015), significantly elevated levels of PTX-3 (plasma) were reported in Italian SLE patients as compared to healthy individuals (p<0.001**) [10]. El-Rawi H *et al.* (2011) had also reported significantly lower levels of PTX-3 among SLE patients as compared to healthy controls among Egyptian population (p<0.0001). Fazzini *et al.* (2001) reported significantly higher concentrations in untreated SLE patients from Italy, as compared to patients in remission [11]. Hollan *et al.* (2010) also reported lower concentrations of PTX-3 in SLE patients from Norway as compared to healthy individuals [12]. Kim *et al.* (2009) had reported significantly raised PTX-3 levels among Korean SLE patients when compared to healthy controls (p<0.001) [13]. Shimada *et al.* (2014) reported significantly elevated levels of PTX-3 in SLE patients (p<0.001**) [14]. In our study, we have observed significantly elevated levels of PTX-3 among SLE patients as compared to healthy controls (p<0.0001). PTX-3 levels in SLE LN patients were also significantly raised as compared to patients without renal involvement (p=0.0107*). This suggests a progressive role of PTX-3 in disease activity and severity in SLE patients.

Ishihara *et al.* (2011) found significantly higher concentrations of PTX-3 in active SLE patients than inactive patients (p<0.001) and healthy controls (p<0.001) in Japanese population. They also documented higher PTX-3 levels among active SLE patients having normal hsCRP [15]. Shimada *et al.* (2014) reported significant association between PTX-3 and elevated CRP (p<0.001**) [14]. We observed significant inverse correlation between PTX-3 and hsCRP in treatment naïve SLE patients (r=-0.3820, p=0.034*).

Inflammatory cytokines TNF-α and IL-1β are known to induce the long chain PTX-3 [16]. Pang *et al.* (2016) reported However, the possible role of PTX-3 and its interplay with pro-inflammatory cytokines in SLE pathogenesis remains unclear. Our group had recently reported raised serum levels of the pro-inflammatory cytokines IL-6, TNF-α and IL-1β in active SLE patients as compared to inactive patients suggesting the role of these cytokines as inflammatory mediators in active stage of disease [17]. Present study however did not find any association between TNF-α and IL-1β and PTX-3, which does not indicate a direct link between cytokines and PTX-3 in disease pathogenesis.

## Conclusion

There are very few reports available on PTX-3 and C1q serum levels and their association with clinical manifestations in SLE. Present study showed a positive correlation between PTX-3 and C1q, supporting the role of PTX-3 in complement activation. However, the negative correlation between PTX-3 and pro-inflammatory cytokines (TNF-α and IL-1β) questions the direct role of PTX-3 in disease activity. Our study also showed a negative correlation between PTX-3 and CRP, both of which are components of the pentraxin super family. The exact mechanism of alternation of these two proteins should be studied further for elucidating the pathogenic events in SLE.

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