Original Article

A Comparative Study on Serum Levels of "Thymic Stromal Lymphopoietin" Between Patients with Psoriasis Vulgaris and Healthy Individuals: A Case-Control Study

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Abstract

Background: Thymic stromal lymphopoietin (TSLP) is a cytokine initially implicated to be associated with allergic disorders inducing Th2 response. Emerging studies have shown that TSLP is also involved in autoimmune diseases. In psoriasis, TSLP acts in synergy with T cell-derived CD40L to promote the release of IL-23 from dendritic cells. IL-23 is responsible for the inappropriate immune reaction and keratinocyte proliferation in psoriasis. Targeting TSLP could be a novel therapeutic approach in the treatment of psoriasis. **Objective:** To compare the serum levels of TSLP between patients with psoriasis and healthy individuals. **Materials and Methods:** A prospective hospital-based case-control study was carried out on 38 patients with psoriasis. The severity of psoriasis was graded into mild, moderate, and severe according to PASI. A total of 30 healthy individuals with matched age and sex were taken as controls. 5 ml of venous blood was collected, centrifuged, and the collected serum was stored at -80°C until quantitative assessment by sandwich enzyme-linked immunosorbent assay (ELISA) technique. **Results:** TSLP has been found to be significantly elevated in the sera of cases (0.1380178 pg/ml) than in controls (0.1125974 pg/ml). There was also a significant proportionate increase in the mean TSLP with the mean PASI score. **Limitations:** The sample size was small and we could not follow-up the cases to study the changes in TSLP levels with remission of the lesions. **Conclusion:** We found that serum TSLP was elevated in psoriasis patients and correlated with disease severity, indicating a possible pathogenetic role.

Keywords: *IL-23, psoriasis, serum, TSLP*

Introduction

Psoriasis is a common, chronic, relapsing, inflammatory, immune-mediated disorder of the skin, affecting 2%–3% of the world's population.[1] From being known as a mere epidermal disorder,[2] to an immune disorder,[3] to a Th1-mediated disorder to predominantly Th17-mediated disorder,[4] the understanding of psoriasis has taken many leaps till date based on many trial and error therapies and serendipitous discoveries. One such discovery is the critical role of thymic stromal lymphopoietin (TSLP) in psoriasis, which is the basis of our study.

TSLP was initially known as a Th2 cytokine involved in allergic disorders but its significant presence in chronic inflammatory and autoimmune disorders and in malignancies is gaining interest recently. Interactions with various components of the local immune milieu determine different TSLP functions in different diseases.[5] There is currently a paucity of information on TSLP in psoriasis, but it is found to be a novel player within the complex cytokine network. The present study is done to compare the serum levels of TSLP between patients with psoriasis and healthy controls.

Materials and Methods

A prospective case-control study was conducted in accordance with the ethical guidelines in the Declaration of Helsinki and was approved by the Institutional Ethics Committee of a tertiary care center in Andhra Pradesh, India. A written informed consent was obtained from each participant. A total of 38 subjects as cases and 30 subjects as controls were recruited from the Department of Dermatology, using a convenient sampling technique.

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Inclusion criteria included psoriasis patients irrespective of age and sex, untreated or who had stopped taking any systemic/topical treatment for at least two weeks before taking the blood sample. Individuals without psoriasis, atopic dermatitis, other chronic inflammatory disorders, infections, and malignancies were taken as controls, matching age and sex as closely as possible.

Exclusion criteria included those with conditions like atopic dermatitis, allergic rhinitis, chronic obstructive pulmonary disease, celiac disease, rheumatoid arthritis, inflammatory bowel disease, and infections and also smokers where TSLP is known to be elevated. The diagnosis of psoriasis was made clinically, and a thorough medical and dermatological examination was performed for everyone. Grading was done according to Psoriasis Area Severity Index (PASI) into mild (PASI < 10), moderate (PASI 10–20), and severe (PASI > 20).

Under strict aseptic conditions, 5 ml of venous blood was collected from both cases and controls into a vacutainer with a clot activator and allowed to clot for 30 min. The tube was then centrifuged for 15 min at 1,000 rpm. 1 ml serum in two aliquots (0.5 ml in each) was stored at -80°C until quantitative assessment by enzyme-linked immunosorbent assay (ELISA).

TSLP was determined quantitatively using Human TSLP Duo Set Sandwich ELISA Kit (with catalog number DY1398-05 and DY008 manufactured by R and D systems, USA). We performed the experiment as per the instructions given by the manufacturer and we established the standard curve, as it represents the relationship between the known concentrations in a given sample. We derived a standard curve for various concentrations which matched our test sample by measuring optical density values using Multiskan Sky High with Touch Screen and µDrop Duo Plate reader, Thermo Fisher Scientific. From this standard curve we determined the unknown concentrations in both cases and control samples. The cases were treated according to the severity of the disease and were followed up to look for the achievement of PASI-75, and a second sample was collected to assay the changes in levels of TSLP before and after treatment.

Data were analyzed using the statistical software MEDCALC version 24.0 (online version). TSLP concentration levels and association with psoriasis incidence when compared with control serum samples were considered as primary outcome variables. The frequency of PASI score and disease severity were considered as primary descriptive variable. Statistical analysis was performed using descriptive and inferential statistics with Chi-square test/Fisher's exact test for categorical data. A descriptive analysis of all the variables for the data which were collected during the study period was done using mean and standard deviation for quantitative variables and frequency and percentages for categorical variables. The sociodemographic factors like age and sex were considered as other explanatory variables. The mean TSLP values were compared between the controls and cases using a *t*-test. *P* value less than 0.05 was considered significant at 95% confidence level.

Results

Demographic data

The mean age among the cases was 40.34 ± 9.89. Maximum number of patients were in the age group of 31–40 years. This age group constitutes about 26.3% of patients. The mean age among controls was 33.33 ± 21.92. The maximum number of controls were in the age group of 21–30 years constituting 43.33%**.** In this study, the percentage of males and females among cases was 71% and 29% and the percentage of males and females among controls was 50% each. According to the PASI scores, majority of the cases had mild psoriasis (47%), followed by severe (29%) and moderate (24%) psoriasis. In the present study, we performed sandwich ELISA to estimate the TSLP concentrations in cases and control samples. As there is no standard range or method to estimate the concentrations of TSLP to correlate, we performed the experiment as per the protocol **(**Human TSLP Duo Set sandwich ELISA Kit (with catalog number DY1398-05 and DY008) instructed by the manufacturer. As per the instructions provided, we established the standard curve as it represents the relationship between the known concentrations of the given sample. This procedure helped us to determine the unknown concentrations (TSLP concentration in samples) from one that is more easily measured (TSLP concentration in standards). We derived a standard curve for various concentrations, which matched our test sample optical density values and we have established the standard curve for cytokine concentration which is illustrated in Figure 1, and their range in Table 1.

Of 30 controls and 38 cases, we measured the concentrations of TSLP by ELISA, and the concentrations were measured as per the standards (pg/ml) in Table 1. In this present study, we found that an average of 0.11259 ± 0.008 pg/ml concentration of TSLP was measured in controls, whereas in cases it was found to be 0.138 ± 0.017 pg/ml. We compared the standard deviations of cases and controls

Table 1: Concentrations and their corresponding optical density (OD) measured

Standard concentrations (pg/ml)	OD Measured (450 nm–540 nm)
0.25	0.462
0.125	0.279
0.0625	0.194
0.0312	0.122
0.0156	0.0995

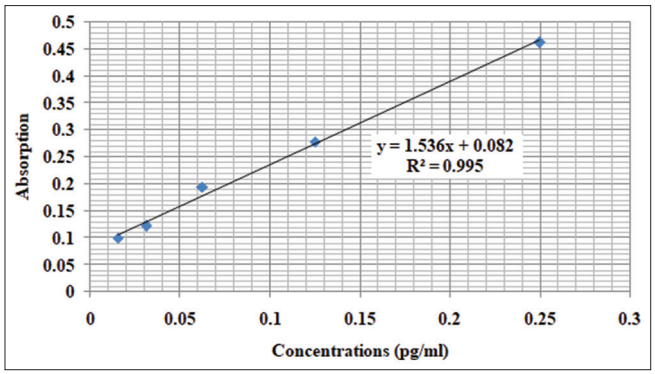

Figure 1: Derivation of TSLP standard curve using standard reagents provided by the manufacturer

by F-test and found that the difference was statistically significant with a *P* value of < 0.001 as depicted in Table 2.

The mean difference of measured TSLP concentrations between cases and controls was 0.0254 pg/ml. *P* value was estimated using *t*-test, which turned out to be <0.0001 implying the significance of the observation**.**

There is also a significant association between the mean PASI score and mean TSLP concentrations when the number of cases falling above and below the mean values are correlated, as shown in Table 3.

Two cases of severe psoriasis were followed and their levels of TSLP (based on optical density) before initiating treatment and after achieving PASI-75 are depicted in Table 4 and Figures 2a and b. There was a significant downfall in the levels of TSLP after treatment when compared to before treatment.

Discussion

In a genetically predisposed individual, upon exposure to various precipitating triggers of psoriasis, there is release of various cytokines like TSLP, IL-1, and TNFα from keratinocytes. TSLP binds to TSLPR and IL-7R present on dendritic cells leading to activation and maturation of dendritic cells. Matured dendritic cells release IL-23 which is the main cytokine responsible for the differentiation of naïve T cells into Th17 cells. Th17 cells further release various cytokines that contribute to keratinocyte hyper-proliferation seen classically in psoriasis.

TSLP is a pleiotropic cytokine, released mainly from cells of the barrier epithelia like skin, gastrointestinal mucosa, bronchial epithelium in response to various stimuli like allergens, fungi, viruses, bacteria, and cigarettes. The TSLP receptor is expressed on a wide variety of immune and non-immune cells like dendritic cells, monocytes, eosinophils neutrophils, mast cells, macrophages, B cells and T cells, NKT cells, platelets, and sensory neurons.[5]

TSLP was initially considered an important cytokine of Th2 immunity, but the growing evidence disclosed the role of TSLP in autoimmune disorders like rheumatoid arthritis,[6]

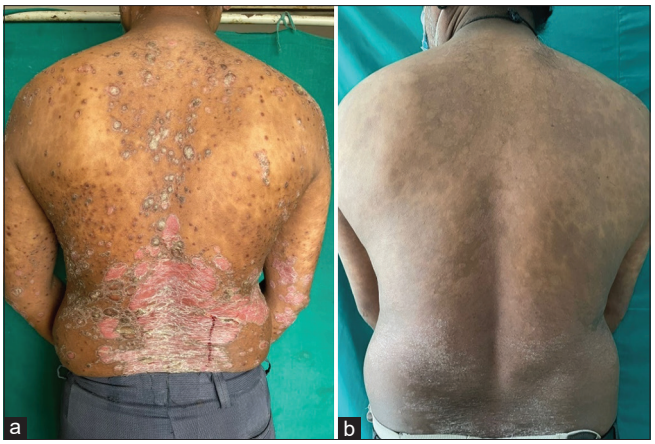

Figure 2: (a) A case of unstable psoriasis whose serum TSLP before treatment was 0.0151 pg/ml. (b) The same case after achieving PASI-75. Serum TSLP at this time was 0.0104 pg/ml

Table 2: Comparison of mean TSLP concentrations among cases and controls

Variable	Cases (*n*=38)	Controls (*n*=30)	Significance (*P*)*
TSLP (pg/ml) Mean±SD	0.1380178±0.01714	0.1125974±0.008	<0.001**

*Fisher's test, ** highly significant. *n*=number; SD=standard deviation

psoriasis,[7] chronic obstructive pulmonary disease, and celiac disease. These findings confirm the complexity of TSLP and its interactions with a multitude of immune cells, resulting in different immuno-modulatory effects depending on the milieu. TSLP can be cleaved by many endogenous proteases in pathological conditions, taking the complexity to the next level.

Volpe *et al*.[7] in 2014 were the first to detect a potential role of TSLP in the IL-23/IL-17 pro-inflammatory pathway, as evidenced by the significant upregulation of the cytokine in the lesional skin of psoriasis when compared to the non-lesional skin. They observed in their *in vitro* studies that keratinocyte-derived TSLP induces dendritic cell maturation and subsequent CD40 ligand induced IL-23 production. The recently activated, CD40L+, T cells bind to the CD40 on dermal DCs in the inflamed skin. They have observed that co-stimulation of dendritic cells by binding of TSLP to TSLPR and CD40L to CD40 leads to a synergistic and strong maturation signal for the release of IL-23, IL-6 from DCs in psoriasis.

Later in 2019, El Ghareeb and other authors estimated the levels of TSLP in the serum of 53 patients of psoriasis and compared with the serum levels of TSLP in healthy individuals. Results showed a statistically significant increase in TSLP in the sera of cases when compared to controls[8] similar to our study.

Suwarsa *et al*. in 2019 analyzed the expression of TSLP in the lesional skin, in addition to the levels in serum. There was

Table 3: Correlation between mean PASI score and mean TSLP concentrations

Variable	Number of cases ≤ Mean±SD	Number of cases ≥ Mean±SD	95% CI	Significance*,**
Mean±SD of PASI 14±26.33	27	11	-22.47–5.23	0.002
Mean±SD of TSLP 0.1380±0.0171 pg/ml	31	07		

*t-test, ** highly significant

Table 4: TSLP before and after treatment in two cases

Case No	TSLP OD before treatment	TSLP OD after treatment
1.	0.0098 pg/ml	0.0074 pg/ml
2.	0.0151 pg/ml	0.0104 pg/ml

Table 5: Comparison of mean TSLP value among other studies

STUDY	Serum TSLP in cases Mean (pg/ml)	Serum TSLP in controls Mean (pg/ml)	*P*
El-Ghareeb *et al*.	1042.7±812.93	314.21±220.78	<0.001
Suwarsa *et al*.	332.18±170.531	121.11±53.501	0.000
The present study	0.1380178±0.01714	0.1125974±0.008	<0.001

a statistically significant difference ($P < 0.05$) in the serum levels of TSLP between cases and controls, but the difference between TSLP expression in lesional and non-lesional skin of psoriasis was not statistically significant ($P > 0.05$).[9]

Here, in the present study, serum levels of the cytokine TSLP in psoriasis patients versus healthy controls were estimated using sandwich ELISA. According to our results, TSLP has been found to be elevated significantly ($P < 0.001$) in the sera of cases (0.1380178 pg/ml) than in controls (0.1125974 pg/ml).

El-Ghareeb *et al*.[8] in their study found a statistically significant increase in serum TSLP levels with an increased PASI score. Pearson's and Spearman's correlation value (r) between TSLP and PASI score was 0.86 which was very highly significant ($P < 0.001$).

However, in our study, there was no proportionate increase in TSLP levels with an increase in severity or area of involvement of psoriasis. Our findings were consistent with the study done by Suwarsa *et al.*, where they did not observe any proportionate increase in TSLP with increasing severity of the disease. Nevertheless, when the mean PASI score is co-related with the mean TSLP concentrations there is a significant association between the two, as seen in Table 3**.** This could be attributed to the subjective variations in the assessment of PASI and needs further study. From the above results in our study, TSLP was found to be significantly elevated in psoriasis than in healthy controls, indicating a possible role in the pathogenesis of psoriasis.

It was also observed that there is a substantial decline in the levels of TSLP in cases after resolution of the disease as depicted in Table 4. As many cases were lost to follow-up owing to the COVID pandemic, we could not study this objective in greater detail. We recommend further studies in this aspect to assess and confirm the declination in TSLP concentration with a variety of treatment options. A comparison of mean TSLP among other studies which were similar to ours and our findings are elucidated in Table 5**.**

Specific targeting of the long isoform of TSLP could be a potential therapeutic option in psoriasis and other autoimmune diseases. A thorough review of literature revealed the established pathogenic role of TSLP in allergic Th2 predominant disorders. Treatment with human monoclonal antibody targeting TSLP, i.e, *tezepelumab* in patients with severe uncontrolled asthma exacerbations, improved the quality of life.[10] These observations widen the role of TSLP from allergic disorders to various other autoimmune disorders, especially psoriasis as detected in our study.

Limitations

The elevation of TSLP levels in our study was on par with the other two studies.[8,9] But the absolute concentrations of TSLP in our study were quite low when compared to the same two studies as seen in Table 5. This could be attributed to the differences in standardization, reagent sensitivity, sample handling, and biological reasons like age, sex, and genetic polymorphisms. The ELISA kits used in the other two studies are different from the kits used in our study. Owing to the COVID-19 pandemic, the sample size was small. and we could not follow-up on the cases to study the changes in TSLP levels with remission of the lesions. We highly recommend further research on this cytokine with a large sample size.

Conclusions

TSLP is a cytokine originally thought to play a key role in Th2-mediated allergic disorders. Recent studies have revealed the role of TSLP in various other autoimmune disorders. Psoriasis vulgaris is one of the autoimmune disorders where TSLP was found to have paramount importance in initiating and driving the inflammation.

Serum levels of TSLP were assessed using sandwich ELISA technique and compared with healthy controls. There is a

significant elevation in the serum levels of TSLP between cases and controls. As TSLP is a new cytokine which has complex interconnections in the immune web, much research into this should be encouraged to get elaborate details of this pleiotropic cytokine. Monoclonal antibodies directed against either TSLP or TSLPR could pave a therapeutic way for most of the autoimmune disorders in which TSLP is known to play a significant pathogenic role. We highly recommend further research into this cytokine keeping in mind the role of TSLP in many autoimmune diseases.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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