Relevance of Inflammatory Cytokine mRNA Expression of Tumour Necrosis Factor- Alpha (TNF α), Interleukin 17A (IL 17A) and Interleukin 6 (IL 6) in Indian Patients with Psoriasis

Abstract

Background: Psoriasis, a chronic, immune-mediated skin disorder, has systemic manifestations as well as an ample negative impact on the quality of life (QOL) of the patient. An abnormal proliferation of keratinocyte and dermal infiltration by immune cells is a characteristic feature. It involves components of both innate and adaptive immunity, and the interaction of T cells with macrophages. Keratinocytes and dendritic cells are mediated by the secreted cytokines. This study was taken up to look into changes at the molecular level that occur during the expression of three cytokines namely tumour necrosis factor–alpha (TNFa), interleukin 17A (IL-17A) and interleukin 6 (IL-6) in Indian patients with psoriasis. Methods: A case-control study was conducted with samples from 15 psoriasis vulgaris patients and 10 healthy control subjects. Clinical parameters were recorded. Blood samples were analysed for peripheral blood messenger ribonucleic acid (mRNA) expression of TNFa, IL-17A and IL-6 using real-time polymerase chain reaction (RT-PCR). Results: The mRNA expression of TNFa, IL-17A and IL-6 in psoriasis patients were increased as compared to that in normal subjects. Conclusions: The elevated levels of Interleukins indicates a systemic inflammatory process that is akin to the cutaneous inflammation. This study indicates that the targeted therapies against these cytokines are likely to be beneficial in Indian psoriasis patients.

Keywords: Inflammatory cytokines, psoriasis, targeted therapy

Introduction

Psoriasis vulgaris is a non-communicable, chronic, disfiguring and disabling disease with sizable negative impact on the patient’s quality of life (QOL).[1] It is an immune-mediated skin disorder with an underlying polygenic inheritance with environmental effects. Silvery-white scales covering well-delineated inflammatory plaques preferentially over the elbows, knees, scalp, and lumbar region are the hallmark of the disease [Figure 1].[2] Its worldwide prevalence is about 2%. These patients have increased prevalence of comorbid conditions like arthritis, cardiovascular diseases and, metabolic disorders.[3] A sustained inflammation with unrestricted proliferation and dysfunctional differentiation of the keratinocyte are characteristics of psoriasis.[4] The difference in phenotype and the treatment response in psoriasis could be the result of inflammatory cascade, particularly the cytokine profile.[4] Being a T cell–mediated disease, proliferation and differentiation of pathogenic T cell responses resulting in TNFα and IL-17 production has been responsible for the onset and maintenance of inflammation in psoriatic skin.[5] IL-6, a pleiotropic cytokine, elevated in serum and psoriatic skin lesions may act synergistically with TNFα.[6] The role of anti–IL 6 therapies have been explored in psoriatic arthritis and pustular psoriasis.[7] Targeted biological therapies with monoclonal antibodies against some of the cytokines are approved and used in the management of psoriasis. Baring itolizumab, other biologicals are not primarily invented and studied in India.[8] Cytokine profile of psoriasis patients can vary significantly since genetic diversity determines the secretion of cytokines. Genetic polymorphisms may also influence the response to therapy.[9] It is hence desirable to understand the cytokine profile of a population with psoriasis...
in a geographic region to predict the cytokine profile in patients with psoriasis and to predict the response to targeted therapies.

Methodology

This case-control study was approved by the ethical committee, and informed consent was taken from all study subjects. Clinically diagnosed patients of psoriasis vulgaris [Figure 1] without any associated comorbidities like arthritis, diabetes, and hypertension, along with age and sex matched healthy subjects were included in the study. Recipients of phototherapy or systemic therapy for psoriasis, current or in the past 3 months, were excluded from the study. Blood samples were collected in non-heparinized collection tube and preserved at -80°C until analysis. Further analysis has been described in Figure 2.

Analysis of interleukins mRNA expressions

The cytokine mRNA expression in psoriasis patients was determined using RT-PCR and compared with healthy controls. Blood samples of patients and controls were subjected to RBC lysis buffer and centrifuged to obtain cell pellets. The pellets were subjected to TRIzol RNA Isolation (TRI) reagent (Sigma-Aldrich) to isolate the total pure RNA and were converted into cDNA using...
Table 1: Primers for PCR utilized for the cytokines tested for mRNA response in psoriasis

| Gene      | Species | Forward sequence           | Reverse sequence           |
|-----------|---------|-----------------------------|-----------------------------|
| TNF-αA    | Human   | CTCTTCTGCCTGCTGCACCTTTG     | ATGGGGCTACAGGGCTTGTCACTC    |
| IL-17A    | Human   | CATTGGGTGTCACTGCTAC         | TCGGTTGTAATCTGAGG           |
| IL-6      | Human   | CCAGCTATGAACCTCTCTTC        | GCTTTCTCCATCATCTCTC         |

TNF: Tumour Necrosis Factor, IL: Interleukin

Table 2: Details of patients in the study on mRNA expression of cytokines in psoriasis

| Age (years) | Sex | Duration (years) | PASI | PPK | Nail Disease | Type |
|-------------|-----|------------------|------|-----|--------------|------|
| 24          | M   | 2.5              | 5.1  | -   | -            | PV   |
| 58          | M   | 1.5              | 9.0  | +   | -            | PV   |
| 42          | M   | 14               | 18.6 | -   | -            | PV   |
| 38          | M   | 4                | 14.0 | -   | +            | PV   |
| 44          | M   | 18               | 13.6 | +   | -            | PV   |
| 39          | M   | 4                | 7.2  | -   | +            | PV   |
| 37          | M   | 20               | 22.2 | -   | +            | PV   |
| 33          | M   | 0.5              | 23.5 | -   | +            | PV   |
| 26          | F   | 5                | 15.8 | -   | +            | PV   |
| 36          | F   | 5                | 9.1  | -   | -            | PV   |
| 52          | F   | 2                | 14.5 | -   | -            | PV   |
| 40          | F   | 5                | 12.7 | +   | +            | PV   |
| 40          | F   | 7                | 9.0  | -   | +            | PV   |
| 50          | F   | 1                | 4.6  | -   | -            | PV   |
| 32          | F   | 1                | 5.4  | -   | -            | PV   |

PASI: Psoriasis Area Severity Index, PPK: Palmoplantar Keratoderma, PV: Psoriasis vulgaris

PrimeScript™RT reagent Kit (Takara, Japan) according to the guidelines of the manufacturer. The mRNA levels were detected with iQ universal SYBR green supermix (Takara, Japan) using qRT-PCR system (BioRad, California, USA). The levels of interleukin mRNA were quantified with an aliquot of reverse transcribed total RNA and specific primers as mentioned in Table 1 by RT-PCR. All samples were run in triplicate. To quantify the relative gene expression, the comparative threshold cycle method was employed.

Results

In total, 15 patients and 10 healthy subjects were included in the study during a period of September 2018 to January 2019. Patient details are given in Table 2. Group matching was done for the selected subjects. Mean age of the cases was 37.8 ± 0.15 and that of control was 36.73 ± 9.55, with no statistically significant difference (P = 0.769). Duration of disease ranged from 6 months to 20 years. PASI ranged from 4.6 to 23.5 with mean of 12.29.

Analysis of peripheral blood indicated elevated mRNA expressions of TNFα, IL-17A and IL-6 in psoriasis patients as compared to healthy controls [Figure 3]. The fold change elevation for TNFα, IL-17A and IL-6 were 3.2, 1.5 and 2.4 respectively [Figure 3].

Discussion

Psoriasis is a genetically determined disorder activated by immune components and environmental influences. Continuous stable interactions between cells of innate and adaptive immunity and the keratinocytes possibly boost and sustain the chronic inflammation. The activated dendritic cells play a pivotal role in initiating the disease process by secreting TNFα, IL-6, IL-23, and IL-12 in large amounts. They convert to antigen-presenting cells and then interconnect with naïve T cells. These cytokines increase the proliferation of keratinocytes and neutrophil recruitment at the inflammatory site, thereby setting in an inflammatory cascade. While TNFα is involved in the inflammatory milieu and keratinocyte proliferation, IL-23 and IL-12 modulate the differentiation and proliferation of T helper 17 (Th17) and Th1 cell subsets respectively. Th17 cells secrete pro-inflammatory IL-17. Variations in the gene expression of patients with psoriasis are known and well studied. Diverse phenotype of psoriasis patients represent polygenicity and discrepancy in cytokine profile, thereby showing varied presentation and response to different forms of therapy including biologicals.

Cytokines in psoriasis are typically measured by methods like ELISA or radioimmunoassay which measure only the immunologically reactive materials and not the biologically inactive ones. Reference values of the cytokine levels vary considerably in various studies. PCR detection of cytokine mRNA is highly sensitive with the advantage of detecting gene expression rather than the ultimate production of cytokine. But this technique is not inherently quantitative. We employed a control group to enable comparison of the mRNA activity and thus fold change elevation measurement was feasible. In this study, we chose two fairly studied cytokines, namely IL-17 and TNF-α, whose drug targets have already been explored in psoriasis, and a lesser-known IL-6 whose role in the pathogenesis is not clearly understood. This was aimed at understanding the pathogenesis of inflammatory events occurring in psoriasis among our subset of patients. Currently biologicals are sparingly used in India, but they are likely to be used more frequently in the future. This study gives the data on the cytokine profile of the Indian patients which enables clinicians to be more convinced while prescribing the targeted therapies.

TNFα plays a significant role in the pathogenesis of psoriasis. Macrophages, monocytes, lymphocytes, and keratinocytes are the sources of TNFα. It contributes...
Psoriasis is a disease that needs a multidisciplinary approach. Therapy should not only focus on the management of the cutaneous inflammatory lesions, but also address the systemic inflammation and comorbidities. This study suggests a possibility of biological therapies against these cytokines being effective in Indian patients as a comprehensive management strategy. Small sample size and evaluation of only 3 cytokines are the limitations of this study. The findings need to be validated with a larger sample of Indian patients representing different regions.

Complex interaction between the genetic denominator of an individual coupled with the cytokine secretions and their interaction with the stakeholder cells determines the ultimate occurrence and phenotype of psoriasis as described in Table 3. Our study concludes that the mRNA response of the tested cytokines was higher in psoriasis as compared to controls, indicating their role in the pathogenesis of psoriasis in our patients too. It is also important to understand the diversity in the genetics and immune response in patients with polygenic disorders like psoriasis so that the newly developed therapies have global significance.

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Conflicts of interest
There are no conflicts of interest.

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