Circulating levels of chemokines in patients with psoriasis vulgaris and their association with disease severity: A case–control study from North India

Neha Joshi, Tarun Narang[1], Sunil Dogra[1], Seema Chhabra

Departments of Immunopathology and [1]Dermatology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Abstract

Background: Psoriasis is a chronic inflammatory skin disease characterized by hyperproliferation and incomplete differentiation of epidermis, and accumulation of neutrophils and proinflammatory T cells in epidermis and dermis. Chemokines are believed to be the main players mediating the chemotaxis of leucocytes to the lesional site. Previous studies have established the role of various chemokine ligands and receptors at the lesional site in psoriasis.

Aims: In this study, we have compared the serum levels of various chemokines, namely, inducible protein-10 (IP-10) (CXCL10), MCP-1 (CCL-2), monokine induced by gamma interferon (MIG) (CXCL-9), RANTES (CCL5), interleukin (IL)-8, and eotaxin in patients with chronic plaque psoriasis with that of healthy controls. We also studied whether the chemokine levels varied within different patient groups based on various clinical and demographic parameters, and if any of these chemokines correlated with disease activity.

Methods: We studied 40 patients with chronic plaque psoriasis from a single center. Their clinical and demographic details were recorded in predesigned prforma. Patients with unstable forms of psoriasis like guttate, erythrodermic, or pustular psoriasis were excluded. The serum chemokine levels were measured by flow cytometry–based bead array set system. The serum levels of the patients were compared with that of 25 healthy controls. A subgroup analysis was also done to study the correlation of chemokine levels with age, sex, duration, and severity of disease.

Results: We observed a significant decrease in serum level of all these chemokines in patients, when compared with that of healthy controls. We also found that MIG levels showed a positive correlation with disease severity based on Psoriasis Area and Severity Index.

Limitations: The major limitation of the study is lack of data on the lesional chemokine levels compared to serum chemokines.

Conclusion: The inflammatory process in psoriasis is orchestrated through chemokines. MIG is a potential serum biomarker for assessing disease severity.

Key words: Chemokine, cytometric bead array, monokine induced by gamma interferon, psoriasis

Correspondence:
Dr. Seema Chhabra,
Department of Immunopathology, Fourth Floor, Research Block A, Postgraduate Institute of Medical Education and Research, Sector-12, Chandigarh, India.
E-mail: drseemachhabra@gmail.com

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Introduction

Psoriasis is a chronic inflammatory skin disease with a worldwide prevalence of 2%,[1] and 0.44%–2.8%[2] in India. It is associated with significant morbidity, with no effective therapy available for long-lasting remissions. Chemokines contribute to local and systemic inflammation in patients with psoriasis. Previous studies have demonstrated the importance of chemokine ligands and receptors in the recruitment of T cells into psoriatic lesional skin and synovial fluid.[3-7] The recruitment of leukocyte also depends on the gradient of local versus systemic chemokines, rather than only on increased local chemokine levels.[8] Thus, studying the change in levels of chemokines in circulation of patients and their association with various clinical parameters such as disease severity, age at onset, and manifestation of comorbidities may help in understanding the underlying immune mechanisms that orchestrate the disease progression and relapse. In this study, serum levels of inducible protein-10 (IP-10) (CXCL10), MCP-1 (CCL-2), monokine induced by gamma interferon (MIG) (CXCL-9), RANTES (CCL5), and eotaxin along with interleukin (IL)-8 in patients were compared with that of healthy controls, as well as between the clinical subgroups. The study establishes the potential of MIG as a biomarker to assess disease severity.

Methods

Study population and controls

This was a prospective study conducted at dermatology outpatient department at the Postgraduate Institute of Medical Education and Research, Chandigarh, involving 40 newly registered patients with chronic plaque psoriasis and 25 age- and sex-matched healthy controls. Other inclusion criteria were: no systemic treatment for at least 4 weeks or topical treatment for at least 2 weeks before enrolment, no evidence of infections, or immuno suppression. Patients with unstable psoriasis, guttate psoriasis, erythrodermic psoriasis, pustular psoriasis, psoriatic arthritis and patients with renal, hepatic, or cardiovascular/cerebrovascular diseases and diabetes were excluded. The study was approved by Institutional Ethics Committee of PGIMER (PGI/IEC-06/2015-204).

Estimation of serum chemokine levels

After obtaining informed consent from all participants, venous blood samples were collected. Serum levels of various chemokines were estimated by BD flow cytometric bead array set system (552990 Hu Chemokine CBA Kit; BD Biosciences, USA), as per the manufacturer's instructions. Samples were acquired on BD-LSR Fortessa Cytometer, and the results were analyzed using FCAP Array software.

Statistical analysis

All results of comparison between different groups were represented as mean ± standard deviation. The mean chemokine levels between different groups were compared using one-way analysis of variance, Mann–Whitney, or unpaired *t*-test, as applicable. Correlations between Psoriasis Area and Severity Index (PASI) score and various chemokine levels were calculated using Pearson's correlation coefficient. Statistical analyses were performed using GraphPad Prism® software (v4; GraphPad Inc., La Jolla, CA, USA).

Results

Demographics

The basic demographics of the patients and controls are summarized in Table 1. The patients were further categorized into different subgroups based on various parameters of clinical importance as summarized in Table 2.

Serum chemokine levels between patients and healthy controls

We estimated and compared the serum levels of various chemokines such as IP-10, MCP-1, MIG, RANTES, IL-8, and eotaxin, in all patients and controls, as shown in Figure 1. The mean serum IP-10 levels in control and subjects

Table 1: Basic demographic profile of the patients (*n*=40) and healthy controls (*n*=25)

Variable	Patients	Controls
Total number (*n*)	40	25
Age (years), mean±SD (*P*=0.1369)	39.9±13.9	35.8±7.8
Age range (years)	19-70	20-46
Male:female	3:1	3:1
Baseline PASI score, mean±SD	6.75±3.14	NA

SD: standard deviation, PASI: Psoriasis Area and Severity Index, NA: not applicable

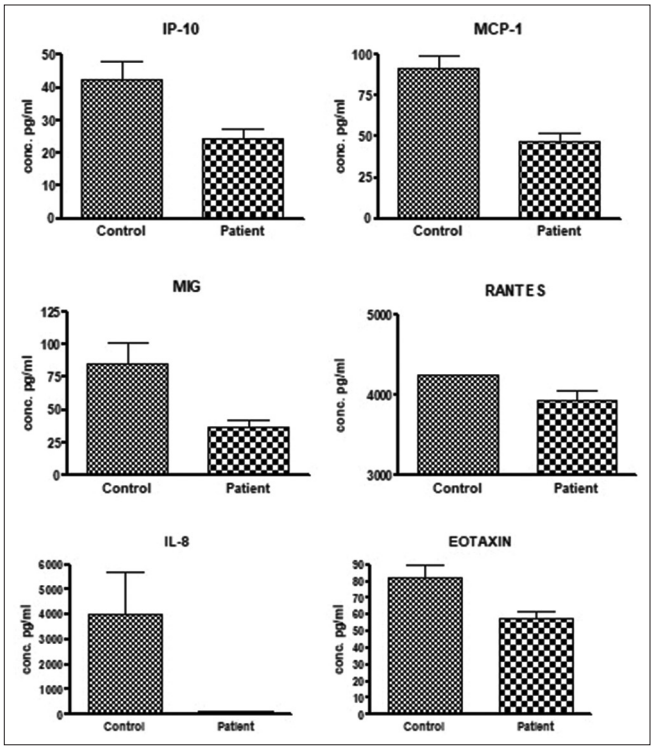

Figure 1: Comparison of serum chemokine levels between controls and patients IP-10- Inducible protein-10; MCP-1- Monocyte chemoattractant protein -1; MIG- Monokine induced by gamma interferon; RANTES- Regulated on activation, normal T cell expressed and secreted; IL-8-Interleukin-8

Table 2: Serum chemokine levels between different demographic and clinical subgroups within the patients

Classification criteria	Subgroups	IP-10	MCP-1	MIG	RANTES	IL-8	Eotaxin	MIF
Gender	Male (*n*=30)	24.32	43.59	59.33	3890	72.51	59.53	11.73
	Female (*n*=10)	24.89	55.35	34.33	4100	184.3	51.91	12.24
	P	0.9284	0.3264	0.9626	0.9858	0.8513	0.4407	0.8457
Age on onset (years)	≤40 (*n*=29)	26.94	49.72	57.31	3903	128.7	54	12.29
	>40 (*n*=11)	18.15	38.69	41.96	4018	25.93	67.17	10.98
	P	0.1523	0.2887	0.3478	0.7307	0.1262	0.1655	0.5844
BSA	≤10 (*n*=20)	21.75	48.63	31	3902	121.5	53.74	12.03
	>10 (*n*=20)	27.04	44.69	75.17	3978	79.44	61.51	11.82
	P	0.3425	0.7081	0.4989	0.8326	0.7150	0.2915	0.9246
PASI	≤10 (*n*=30)	24.28	44.05	30.89	3952	80.98	58.14	10.92
	>10 (*n*=10)	24.99	54.03	119.7	3902	158.9	56.08	14.95
	P	0.9128	0.4058	0.0269*	0.9861	0.2543	0.8361	0.0973
Duration of disease (years)	<1 (*n*=4)	35.24	31.84	35.57	4255	67.78	52.01	12.39
	1-5 (*n*=16)	19.22	38.75	26.96	3823	60.81	49.56	12.68
	>5 (*n*=20)	26.24	55.46	73.5	3974	150.9	65.2	11.25
	P	0.2057	0.2011	0.5185	0.5316	0.4403	0.1981	0.8101
Comorbidities	With comorbidities (*n*=30)	22.5	43.42	49.83	3828	104.6	56.32	10.99
	Without comorbidities (*n*=10)	30.15	55.87	62.84	4255	88.1	61.55	14.73
	P	0.2289	0.2983	0.0632	0.1319	0.0588	0.5971	0.1255

*Significant. *n*: Number of subjects, BSA: body surface area, PASI: Psoriasis Area and Severity Index, IP-10: inducible protein-10, MIG: monokine induced by gamma interferon, IL: interleukin, MIF: Macrophage migration inhibitory factor, MCP: Monocyte chemoattractant protein, RANTES: Regulated on activation, normal T cell expressed and secreted

with psoriasis were 42.2 ± 29.7 and 24.46 ± 17.1 pg/mL, respectively ($P = 0.0026$). The mean serum MCP-1 levels in controls and patients were 91.3 ± 41.5 and 46.6 ± 32.2 pg/mL, respectively ($P < 0.0001$). The mean serum MIG levels in control and patients were 85.2 ± 75.9 and 36.4 ± 36.6 pg/mL, respectively ($P = 0.0026$). The mean serum RANTES levels in control and patients were 4225 ± 0.001 and 3940 ± 695.4 pg/mL, respectively ($P < 0.0001$). The mean serum IL-8 levels in control and patients were 4033 ± 8277 and 100.5 ± 256.4 pg/mL, respectively ($P < 0.001$). The mean serum eotaxin levels in control and patients were 82.2 ± 38.1 and 57.6 ± 26.6 pg/mL, respectively ($P = 0.0025$). Thus, it was found that the circulating levels of all chemokines were lower in patients with psoriasis when compared with that of controls.

Serum chemokine levels between different clinical subgroups within the patients

We categorized the patients into different subgroups and compared the levels of individual chemokines within these subgroups [Table 2]. The levels of MIG were significantly higher in patients having severe disease as per PASI score, when compared with patients with mild to moderate disease.

Correlation between serum chemokines with disease severity based on PASI (Isnt it repetition of the above statement?)

Only MIG showed a significant correlation with disease severity. This result is depicted in Figure 2.

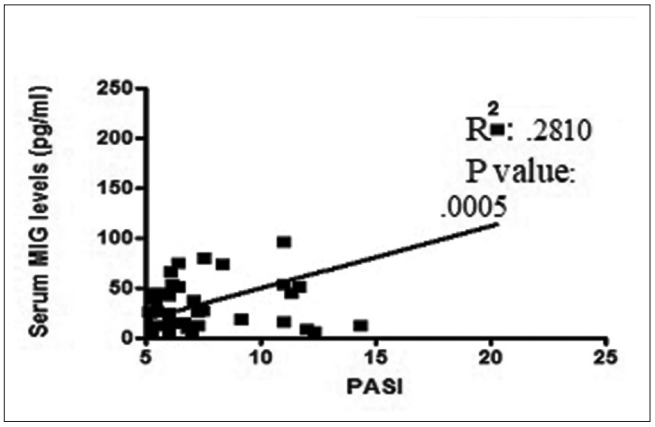

Figure 2: Correlation between disease severity (Psoriasis Area and Severity Index) and serum monokine induced by gamma interferon levels in patients with psoriasis vulgaris (Pearson r = .5301)

Discussion

Chemokines are low-molecular weight peptides having a role in chemotaxis of leukocytes to the site of inflammation. In this study, we found a decrease in the serum levels of all the chemokines. IP-10 (CXCL10) is known to have a role in both effector T-cell generation and trafficking.[9] Earlier, studies have reported IP-10 in psoriatic plaques,[10] and the kerationocytes cultured from patients with psoriasis vulgaris showed much higher level of constituent and induced IP-10.[11] Concurrent to reports of increased level of production at site of inflammation, our study shows a decrease in serum level which may help in recruitment of proinflammatory T cells to lesional sites.

MCP-1 (CCL2) has been shown to contribute to the monocyte/macrophage trafficking in inflammatory skin disorders.[12] Previously, keratinocytes cultured from patients with

psoriasis vulgaris showed much higher level of constituent and induced MCP-1.[11] Increased expression of MCP-1 gene has also been reported in the skin from patients with psoriasis when compared with healthy control.[13] However, contrary to our result, most of the previous studies on MCP-1 levels have observed an increase in serum MCP-1 level. One group has even reported a positive correlation of serum MCP-1 level with PASI.[14] One explanation for this difference is that during the acute phase of disease, all immune cells are in an overdrive mode, secreting chemokines in quantities enough to not only increase their levels locally but also also to increase their serum levels. However, during a prolonged ongoing chronic inflammatory response, immune cells may eventually reach a state of exhaustion, thus bringing about a decrease in their chemokine production, decreasing their serum levels.[15]

Migration of leucocytes from dermis to epidermis suggests the involvement of chemotactic agents such as chemokines.[16] Studies have shown, increased RANTES production in the lesion, but not in the perilesional skin, by immunohistochemistry[17], while the expression of its receptor CCR5 was found elevated only in the T cells of epidermis.[18] We observed a decrease in serum levels of this chemokine. The importance of RANTES in pathogenesis of psoriasis is further highlighted by the fact that narrow-band ultraviolet treatment used in psoriasis was found to reduce RANTES production in leisonal skin.[19] MIG or CXCL9 is highly expressed in the papillae of psoriatic lesion which is produced by activated macrophages and dermal microvasculature endothelial cell.[20] The mRNA expression of CXCL9 has been primarily located in dermal infiltrates in psoriasis.[21] Enhanced plasma levels of CXCL9 have also been reported in patients with psoriasis.[22] However, in our study we observed a decrease in serum MIG levels in patients with chronic plaque psoriasis. Moreover, it was the only chemokine whose level correlated with disease severity based on PASI. Thus, serum levels of this chemokine may serve as an important biomarker in studying disease severity.

IL-8 mRNA expression has been reported in the skin of patients with psoriasis but not healthy controls.[23] The presence of IL-8 has been reported within the psoriatic scales and epidermis; however, no circulating IL-8 levels were observed.[24] Another study reported an increase in serum IL-8 levels in psoriasis, without any correlation with disease activity.[25] However, an increase in IL-8 levels in the serum of patients with psoriasis when compared with healthy control, which correlated with the degree of erythema, has also been reported.[26] We observed a decrease in the serum levels of this chemokine. Beljaards *et al*. reported that in psoriasis, IL-8 receptor A was observed in neutrophilic granulocyte, while IL-8 receptor B was expressed in keratinocytes of suprabasal epidermis. In the same study, the authors have also reported that following therapy, IL-8 type B receptor expression reduced.[27]

Eotaxin, an eosinophilic chemotactic protein, has been implicated in pathogenesis of various skin related inflammatory disorders.[28,29] We found a decrease in serum eotaxin levels in patients with psoriasis.

This study reaffirms that change in chemokine production locally and in serum can be useful in monitoring disease activity, as well as in identifying new therapeutic targets for chronic inflammatory disorders such as psoriasis. Moreover, the stage of disease, that is, acute versus chronic, may have a considerable bearing in the chemokine levels.

The major limitation of the current work is the lack of corresponding chemokine data from lesional site. Another obvious query is why the serum concentration in patients should be lesser than control? A possible explanation is that since it is a chronic inflammatory condition, a sustained high amount of chemokine in circulation may cause more damage to host. However, the homeostasis attained in such a situation must still favor the migration of immune cells from circulation to the site of inflammation, and this may be a possible mechanism of how a gradient is achieved in such a situation.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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