CURRENT TRENDS IN **PHARMACEUTICAL RESEARCH 2012,1(1):1-12**

# ASSESSMENT OF MOLECULAR, PHYSICAL AND BIOCHEMICAL RISK FACTORS ASSOCIATED WITH DEVELOPMENT OF PSORIASIS - A PILOT STUDY

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## ABSTRACT

Psoriasis is a chronic, non-contagious skin disease with a predicted auto-immune etiology and limited success with the available medical interventions. Therefore, a clear understanding of the underlying molecular or immunological manifestations and other external and internal risk factors is indispensible for future therapeutic approaches and success. Psoriasis is a global problem, and there is a huge load of patients nationally and in Northeast India. Therefore, the present pilot study was planned to assess the risk factors associated with the development of psoriasis. Blood samples were collected from clinically diagnosed psoriasis patients (n=7) and age and sex matched healthy controls (n=3) with detail history of smoking, alcoholism, medication, blood proofing. Differential mRNA expression for IL-12, IL-10 and TNF-α was studied by Real-time PCR method. IL-2 and IL-4 expression was studied by ELISA. Statistical analysis was performed by SPSSv13 software. Differential mRNA expression showed many fold increase in IL-12 and TNF-α (Th1 markers) as well as IL-10 (Th2); whereas protein based expression of IL-2 and IL-4 showed statistically significant up regulation and down regulation, respectively, compared to controls. Smoking and alcohol drinking habits were found to play an associative role with immune deregulations in the development of psoriasis along with use of drugs such as anti-diabetic, antibiotics and higher serum cholesterol levels. Our present data proves that altered immuno-

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modulation status is critical for psoriasis development, and the risk factors for the disease may be multi-factorial with smoking, alcoholism playing an associative role in psoriasis development.

**Key words:** Psoriasis, IL-12, IL-10, TNF-α, Risk factors.

## INTRODUCTION

Psoriasis is a chronic, autoimmune disease that appears on the skin when the immune system sends out faulty signals that speed up the growth cycle of skin cells. Psoriasis is not contagious (Christopher and Henselar 1987). It commonly causes red, scaly patches to appear on the skin, although some patients have no dermatological symptoms. The scaly patches commonly caused by psoriasis are known as psoriatic plaques, are areas of inflammation and excessive skin production. According to world Psoriasis consortium about 125 millions of people all over the world suffer from this disease (Gelfand *et al* 2005). The cause of psoriasis is not fully understood, but it is believed to have a genetic component and local psoriatic changes can be triggered by an injury to the skin known as Koebner phenomenon (Krueger and Bowcock 2005). Various other factors have also been suggested as aggravating to psoriasis which includes infections (e.g. streptococcal infection and HIV), reaction against few drugs like Chloroquine, ACE inhibitors, Beta-blockers, Progesterone, Lithium and Indocin, Antibiotics (e.g. Penicillin and Tetracycline), withdrawal of systemic corticosteroid, excessive alcohol consumption and smoking, abnormal plasma lipid metabolism and diabetes (Tagami 1997; Fry and Baker 2007; Pietrzak and Lecewicz-Tourn 2002; Shapiro *et al* 2007; Lima and Lima 2011).

Psoriasis is clinically considered as a T cell-mediated autoimmune disease, and the Th1 response has been established as the major immune agent in its patho-mechanisms (Krueger and Bowcock 2005; Guttman-Yassky *et al* 2011; Guilloteau *et al* 2010). Available literature suggests that deregulation of host immune status plays a pivotal role in the development of psoriasis; based on which few drugs like Ustekinumab is currently being used for treatment, but with limited results (Ghoreschi *et al* 2003; Vander Zee 2011; Croxtall 2011; Clemmensen *et al* 2011; Collamer *et al* 2008).

There is also a sharp increase in the number of cases of psoriasis globally, nationally and regionally. Taking into account the lacunae in the existing knowledge of the underlying molecular etiology of psoriasis, the present study was undertaken to study IL-2, IL-4, IL-10, IL-12 and TNF-α expression profile in Psoriatic patients blood samples and to compare with healthy control blood samples. We hypothesized that altered immunomodulation of Th1 and Th2 response is instrumental in the development of psoriasis.

## MATERIALS AND METHODS

### Patient enrolment

Blood samples were collected under the supervision of a registered medical practioner and informed consent from patients suffering Psoriasis vulgaris (Plaque Psoriasis, n=7) and age and sex matched community controls (n=3). The study was approved by the Institutional Ethical Committee of Gauhati University, Guwahati. Blood samples were collected in EDTA and non-EDTA vials. Out of the seven patients, six patients were male. The identification of the psoriasis patients was based on physical and clinical examination which included presence of clubbed red pegs on biopsy, Auspitz's sign and PASI (Psoriasis Area Severity Index); which is characteristics of psoriasis. The details history of alcohol consumption and smoking habit and medication history (intake of drugs such as diabetic, anti-hypertensive and antibiotics) was also documented thorough interaction based on a questionnaire with the patients.

### Immunomodulation studies

For studying the role of alterations studies on immune status in the development, both differential mRNA and protein expression based approach was used. Briefly, total RNA extraction was done from 200 µl of patients as well as controls whole blood by using the trizol method. The total RNA thus isolated was screened for quality by running the RNA on 1.2% agarose gel. The total RNA was converted to cDNA using random hexamers and MuLV reverse transciptase. The relative quantification of the alteration of critical immunomodulatory genes IL-12 and TNF-α (both markers of Th1 type of response) and IL-10 (marker of Th2 type of response) was performed using the Real-Time PCR method using the SYBr green fluorescent dye and β-actin as an internal control (Table 1). The fold change in the expression of the studied

genes compared to those in controls was done by the $2^{-\Delta\Delta Ct}$ method, which is also known as the comparative threshold method. The mRNA levels of the target genes are normalized to the transcripts of the target genes from healthy control blood samples were determined. The quality and reproducibility of the results were done by melt-curve analysis and repetition of randomly selected samples.

The IL-2 and IL-4 protein concentration in serum was evaluated using ELISA (endogen human IL-2 and IL-4 ELISA kit) using 50 µl of patients and controls serum and following the manufacturer's protocol. Statistical Analysis done by SPSSv13 software, and an adjusted to tail value (corrected) less than 0.05 at 95% confidence level was considered statistically significant.

## RESULTS AND DISCUSSION

Relative quantification using real time RT-PCR was done for IL-10, IL-12 and TNF-α keeping β-actin as an internal control. The Ct values of IL-10, IL-12 and TNF-α were obtained by comparing with β-actin for Psoriatic samples and healthy

**Table 1: Primer sequences for respective genes used for mRNA based expression analysis**

| Genes evaluated | Primer sequences | Tm |
|---|---|---|
| IL-12 F | 5' CGCTCCCCAAGAAGACAG 3' | 58°C |
| IL-12 R | 5' GCCAGAGGGCTGATTAGAGA 3' | |
| TNF-α F | 5' AAGGAGGCGAGGTTCTAAGC 3' | 58°C |
| TNF-α R | 5' GCAGGTGAACGTCAGAAT 3' | |
| IL-10F | 5'TCTTGCAAAACCAAACCACA 3' | 58°C |
| IL-10R | 5' ACTCTGCTGAAGGCATCTCG 3' | |
| β-act F | 5' AGATAGTGGATCAGCAAGCAG 3' | 57°C |
| β -act R | 5' GCGAAGTTAGGTTTTGTCA 3' | |

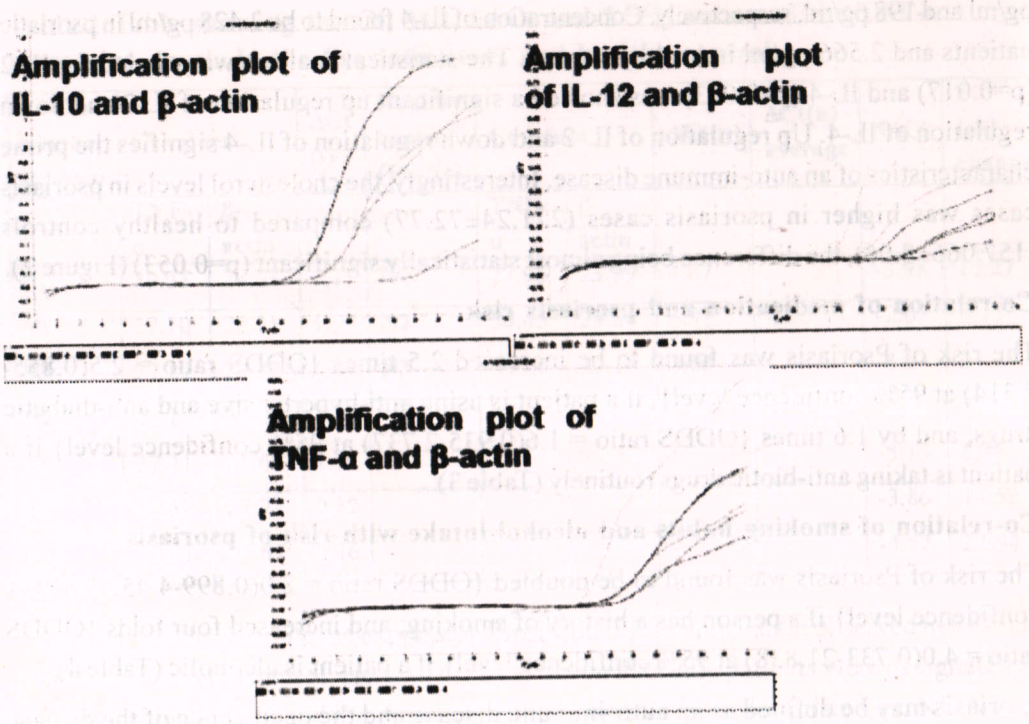


**Figure 1: Amplification plot for IL-10, IL-12 and TNF-α expression analysis performed by Real-Time PCR analysis using β-actin as the internal control**

normal. ΔCt, ΔΔCt and $2^{-\Delta\Delta Ct}$ were calculated from the data to find out the fold change in the m-RNA expression levels of psoriatic patient to that of healthy control (Figure 1). The data showed that there is a almost a 7 fold increase in the mRNA expression of IL-10 and IL-12, and almost a 14 fold increase in the expression profile of TNF-α (Table 2). The present data is suggestive of an altered hyper-activated immune status is playing a critical role in the development of psoriasis, and TNF-α expression analysis is a suitable marker for accessing the predisposition to psoriasis.

The concentration of IL-2 and IL-4 was measured by using ELISA KIT. The average concentration of IL-2 in psoriatic patients and healthy controls were found to be 297.14

pg/ml and 198 pg/ml, respectively. Concentration of IL-4 found to be 2.428 pg/ml in psoriatic patients and 2.566 pg/ml in healthy control. The statistical analysis was carried for IL-2 ($p=0.017$) and IL-4 ($p=0.033$) was showed a significant up regulation of IL-2 and down regulation of IL-4. Up regulation of IL-2 and down regulation of IL-4 signifies the prime characteristics of an auto-immune disease. Interestingly, the cholesterol levels in psoriasis cases was higher in psoriasis cases ($271.24\pm72.77$) compared to healthy controls ($157.066\pm8.06$), the difference being almost statistically significant ($p=0.053$) (Figure 2).

**Co-relation of medication and psoriasis risk**

The risk of Psoriasis was found to be increased 2.5 times {ODDS ratio = 2.5(0.855-7.314) at 95% confidence level}, if a patient is using anti-hypertensive and anti-diabetic drugs, and by 1.6 times {ODDS ratio = 1.6(0.935-2.737) at 95% confidence level} if a patient is taking anti-biotic drugs routinely (Table 3).

**Co-relation of smoking habits and alcohol intake with risk of psoriasis**

The risk of Psoriasis was found to be doubled {ODDS ratio = 2.0(0.899-4.452) at 95% confidence level} if a person has a history of smoking; and increased four folds {ODDS ratio = 4.0(0.733-21.838) at 95% confidence level} if a patient is alcoholic (Table 4).

Psoriasis may be defined as an auto-immune disease and the occurrence of the disease around the world varies from country to country. Till date no medical interventions has been found to be efficient enough to cure it completely. The re-emission tendency of the disease is a major drawback for its treatment. Psoriasis generally occurs due to altered immune status. Cytokines plays a vital role in protection of the body from

**Table 2:Table showing the Ct, ΔCt, ΔΔCt and $2^{-\Delta\Delta Ct}$ values of the TNF-α transcript levels in the Psoriatic cases and healthy control**

| Case number (Id. No) | Ct values | | ΔCt (t) | Healthy control (Id. No) | Ct values | | ΔCt(n) | ΔCt(n) average | ΔΔCt | $2^{-\Delta\Delta Ct}$ fold change |
|---|---|---|---|---|---|---|---|---|---|---|
| | TNF-α | β-actin | | | TNF-α | β-actin | | | | |
| P1 | 29.03 | 28.33 | 0.7 | N1 | 27.96 | 23.89 | 4.07 | | -3.98 | 15.27 |
| P2 | 27.10 | 25.11 | 1.99 | N2 | 36.45 | 29.54 | 6.91 | 4.68 | -2.67 | 6.45 |
| P3 | 27.12 | 27.12 | 0.09 | N3 | 30.26 | 27.18 | 3.08 | | -4.6 | 24.25 |
| P4 | 28.15 | 26.29 | 1.89 | | | | | | -2.82 | 7.06 |
| P5 | 27.21 | 25.66 | 1.55 | | | | | | -3.13 | 8.75 |
| P6 | 28.54 | 27.72 | 0.82 | | | | | | -3.86 | 14.52 |
| P7 | 27.81 | 27.65 | 0.16 | | | | | | -4.52 | 22.94 |

*The above table illustrate the expression Profile of TNF-α and β-actin observed in Psoriatic patient and healthy control. Here all the total Seven Psoriatic patients were designated as: P1, P2, P3, P4, P5, P6 and P7. And the healthy controls were designated as: N1, N2 and N3.

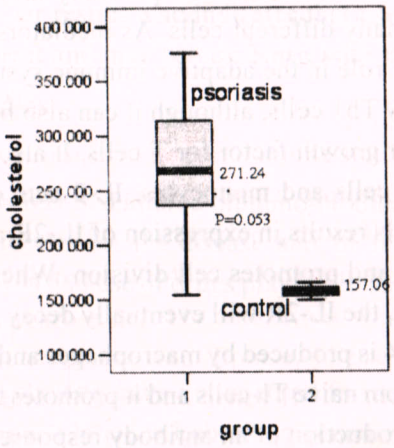


**Figure 2: Box-plot analysis showing higher serum cholesterol levels in psoriasis patients (group 1) compared to controls (group 2)**

**Table 3: The drug intake profile of individual patients and healthy controls**

| Case number (Id. No) | Anti-hypertensive | Anti-diabetic | Any other | Healthy control (Id. No) | Anti-hypertensive | Anti-Diabetic | Any other |
|---|---|---|---|---|---|---|---|
| P1 | yes | yes | sedatives | N1 | none | none | none |
| P2 | yes | yes | antibiotics | N2 | none | none | none |
| P3 | yes | yes | anti-thyroid | N3 | none | none | none |
| P4 | yes | no | none | | | | |
| P5 | no | no | antibiotics | | | | |
| P6 | yes | yes | none | | | | |
| P7 | no | yes | none | | | | |

*The above table illustrate the various Drug intake Profile observed in Psoriatic patient and healthy control. Here all the total Seven Psoriatic patients were designated as: P1, P2, P3, P4, P5, P6 and P7. And the healthy controls were designated as: N1, N2 and N3.

various pathogens and external stimuli, and importantly they can be produced by many different cells and act on many different cells. As mediators of adaptive immunity the Cytokines that play a major role in the adaptive immune system include: IL-2 and IL-4. Interleukin 2 is produced by Th1 cells, although it can also be produced by Tc cells to a lesser extent. It is the major growth factor for T cells. It also promotes the growth of B cells and can activate NK cells and monocytes. IL-2 acts on T cells in an autocrine fashion. Activation of T cells results in expression of IL-2R and the production of IL-2. The IL-2 binds to the IL-R and promotes cell division. When the T cells are no longer being stimulated by antigen, the IL-2R will eventually decay and the proliferative phase ends. Similarly Interleukin-4 is produced by macrophages and Th2 cells. It stimulates the development of Th2 cells from naive Th cells and it promotes the growth of differentiated Th2 cells resulting in the production of an antibody response. It also stimulates Ig class switching to the IgE isotype.

In our experiment, we have found that Psoriasis is accompanied by higher expression of IL-2 expression, which is corresponds to Th1 cells. And in case of IL-4, which is corresponds to Th2 cells were found to be decreased when compared with healthy control. This follows an up regulation of IL-2 and a down regulation IL-4, which is a prime characteristic of an auto-immune disease. As fibroblast produces IL-8, and the IL-8 thus produced IL-12 but the production of IL-10 is also significant, as found in our experiment. For treatment of Psoriasis, common p40 subunit of IL-12 and IL-23 were chosen and found effective as the expression level of IL-12 is found higher. In our experiment, we had also found that increased of IL-12 expression profile in Psoriatic patients as compared to healthy control. TNF-α which is responsible for activation of inflammation, fever and acute phase reaction; and a critical representative of Th1 type of immunomodulation, was found out to be a crucial marker for psoriasis development.

Smoking and alcohol drinking habits were found to play an associative role with immune deregulations in the development of psoriasis along with use of drugs such as anti-diabetic, antibiotics. One of the limitation of our work is that the limited number of psoriasis cases evaluated, which resulted in limited pattern for the occurrence of psoriasis in this region, but the consistency of results indicates the critical role of these factors in the development of psoriasis which needs to be confirmed by larger cohort based studies along with analysis of other panel of markers. Our results also indicates that use of anti-TNF-α drugs holds promise in psoriasis treatment and may be combined with currently used anti-psoriatic drugs for clinical interventions.

## CONCLUSION

From the current study, it is clear that altered immuno-modulation is critical for psoriasis development and the risk factors for the disease may be multi-factorial with cigarette smoking, alcohol consumption and use of drugs playing an associative role.

## ACKNOWLEDGEMENT

We are grateful to the patients who have volunteered for current study and to the staff and members of the Biomedical Science Laboratory, Gauhati University, Guwahati for their help and co-operation.

**Table 4: Alcoholic and Smoking habits of Psoriatic patients and healthy controls**

| Case number | Smooking status | Alcoholic status | Incident of physical injury at the site of lesion (Koebner Phenomenon) | Healthy normal number | Smooking status | Alcoholic status | Alcoholic status | Incident of physical injury ( if any) (Koebner Phenomenon) |
|---|---|---|---|---|---|---|---|---|
| P1 | No | Ex-alcoholic | yes | N1 | No | no | no | No |
| P2 | No | yes | no | N2 | No | no | no | No |
| P3 | Yes | Ex-alcoholic | no | N3 | No | no | no | No |
| P4 | No | no | no | | | | | |
| P5 | Yes | yes | no | | | | | |
| P6 | Yes | yes | no | | | | | |

* This above table illustrate the various parameters including Alcoholic, Smoking and Koebner Phenomenon observed in Psoriatic patient and healthy control. Here all the total Seven Psoriatic patients were designated as: P1, P2, P3, P4, P5, P6 and P7. And the healthy controls were designated as: N1, N2 and N3.

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