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Study to Evaluate Pattern of Transforming Growth Factor-β1 Gene Polymorphism in Psoriasis Vulgaris Patients in Central Rajasthan's Population

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Abstract: ***Psoriasis is an immune-mediated inflammatory disease with a genetic component, characterized by keratinocyte hyperproliferation and angiogenesis. Transforming growth factor-β1 (TGF-β1), the predominant isoform in the skin, plays a dual role, inhibiting keratinocyte growth or promoting proliferation under inflammatory conditions. Elevated TGF-β1 levels have been linked to psoriasis severity, but the mechanisms behind this elevation remain unclear. Genetic polymorphisms in the TGF-β1 gene on chromosome 19 have been associated with various immune-mediated diseases, though their role in psoriasis is not fully understood. This cross-sectional study investigates the association of TGF-β1 gene polymorphism at codon 10 with psoriasis susceptibility in Central Rajasthan. Sixty-three psoriasis patients were recruited from dermatology clinics, with ethical approval and informed consent. Blood samples were analysed using PCR and restriction fragment length polymorphism (RFLP) techniques to identify genotypes (Leu/Leu [TT], Leu/Pro [TC], and Pro/Pro [CC]). The genotypic distribution showed 11.1% with CC, 85.7% with TC, and 0% with TT, indicating a significant predominance of the TC genotype. All patients with CC genotype had a positive family history.***

Keywords: Psoriasis, TGF-β1, gene polymorphism, codon 10, PCR, RFLP

1. Introduction

Psoriasis is an immune-mediated inflammatory disease with a genetic basis. Inflammatory cells and their secreted products such as cytokines, chemokines and growth factors ultimately lead to keratinocyte hyperproliferation, epidermal thickening, and angiogenesis with marked ectasia of blood vessels.[1]

Transforming growth factor-beta (TGF-beta) is a multipotent cytokine that regulates cell growth and differentiation. Transforming growth factor beta (TGF-b) is a multifunctional regulator of both cell growth and differentiation. TGF-b has five isoforms TGF-b1–5, however only TGF-b1, TGF-b2 and TGF-b3 have been found in mammals. TGFb1 is produced by many cells including activated inflammatory infiltrate cells and keratinocytes. TGF-b1 and TGF-b2 were found in the human epidermis whereas TGF-b3 is distributed in the dermis, mainly in the upper dermis [9–11].

. TGF-beta1 is the predominant isoform in the majority of tissues including the skin.2 The skin has been shown to be an important target tissue of TGF-beta1, and its receptors are detected in epidermal keratinocytes.3TGF-beta1 has a contradicting role in the pathogenesis of psoriasis. It has been demonstrated to inhibit the growth of keratinocytes, but occasionally it stimulates keratinocyte proliferation due to a secondary effect of the increased inflammatory cytokines and chemokines in psoriasis IL-1, IL-6, IL-8. TGF-beta1 also stimulates the growth of fibroblasts and induces angiogenesis and vasodilatation observed in early psoriasis.4–6Strong evidence was provided that psoriasis-like skin inflammation is closely related to overexpression of latent TGF-beta1 in the epidermis.3 Moreover, it mediates psoriasis-like lesions in mice.7 Increased TGF-beta1 in the epidermis and the serum has been found in psoriatic patients, which was closely correlated with disease severity.33-34 However, the mechanism of increased serum levels of TGF-beta1 in psoriasis remains unclear. In other diseases, TGF-beta1 polymorphism significantly affects serum levels of TGF-beta1.11, 12 Regulation of cytokine levels has been shown to be under genetic control. Genetic polymorphisms in the coding and promoter sequences of their genes affect the rate of cytokine expression. Association of allelic variations in specific cytokine genes has been reported in several diseases including T-cell-mediated diseases of the skin.[13]

The human TGF-beta1 gene is located on the long arm of chromosome 19. TGF-beta1gene has some polymorphisms; two in the promoter region at positions 800 G/A and 509 C/T and three in the coding sequence at positions 869 T/C, 915 G/C and 1628 C/A. TGF-beta1 gene polymorphism has been detected in many immune-mediated diseases such as systemic lupus erythematosus, 14 systemic sclerosis, 15 rheumatoid arthritis, 16, 17 Crohn's disease18 and asthma, 19 yet it remains to be determined whether these polymorphisms are linked with psoriasis.

This study aimed to assess the possible role TGF-beta1 gene polymorphism has in psoriasis and its relation to psoriasis susceptibility in a group of central Rajasthan's population.

2. Background

Increased transforming growth factor beta 1 in the epidermis and serum has been found in psoriatic patients. The mechanism for this increase remains unclear.

3. Objective

The aim of this project is to investigate the genetic polymorphism OF TGF beta 1 gene and its genetic distribution in psoriasis patients in central Rajasthan's population

To identify whether Tgf beta 1 can be considered as the basis of future gene therapy in the treatment of psoriasis.

4. Method

This cross-sectional observational study involved 63 subjects and 50 controls.

1) **Sample collection:** 5 ml of blood was drawn from each individual and collected in EDTA vials. Samples will be transported to the laboratory on ice and stored at 4°C until used.
2) **DNA isolation:** Genomic DNA was isolated using the Thermofisher whole blood DNA isolation kit (as per manufacturer protocol) with slight modification.
3) **Quality and quantity of genomic DNA:** The purity and concentration of the isolated genomic DNA was estimated using agarose gel electrophoresis and UV-absorption nanophotometer respectively. The absorbance at 260 and 280 nm wavelengths was used to measure the optical density (OD) of the DNA samples. A ratio between 1.4 and 1.9 is considered a relatively pure DNA sample as it did not show any effect on the PCR reaction (Sambrook and Russel, 2001).
4) **Synthesis of Primers:** Primer pairs were synthesized by IDT Pvt. Lt. India
5) **PCR amplification and Restriction digestion:** PCR amplification was done by using a Thermofisher PCR amplification master mix kit and PCR products will be subjected to restriction digestion with help of Pst1 fast restriction enzyme (as per manufacturer's protocol).
6) **Analysis of digested PCR products:** Digested PCR products were subjected to 3% Agarose

5. Results

The sample included 63 total patients-males 36 (57.14%) and 27 females (42.86%) with a mean age of approximately **42.17 years** between 20 to 73 years.50 healthy volunteers were taken as control 30 [60%] males and 20 [40%] females mean age ranging from 18-40 years.

The disease duration ranged between 4 months and 30 years, with a median of 5years.

25 patients [39.6%] had type 1 psoriasis [<40 years] and 38 [60.3%] patients had type 2 [>40 years] of psoriasis

2 patients had psoriatic arthritis and 12 patients had a positive family history of psoriasis

Genotyping of TGF-β1 codon 10 gene (Figure 1) is shown in Table 1
The genotypic distribution revealed the following pattern in central Rajasthan's population:

- **CC Genotype**: Present in 11.1% of patients. [7 patients]
- **TC Genotype**: Predominant at 85.7%. [56]
- **TT Genotype**: Absent (0%).

Table 1: Genotyping and allelic frequency of TGF-β1 gene in psoriasis patients

Genetics	Psoriasis	Control	P Value
GENOTYPE			
CC	11.1%	20%	0.09
TC	85.7%	78%	0.14
TT	0%	2%	

Table 2

Patients	Wild – type (CC)	Mutant (CT)
TOTAL	**7**	**56**
Sex		
Male	4	40 [71.43%]
Female	3	16 [28.57%]
Family history		
Positive	4 [57.14%]	8 [14.29%]
Negative	3 [42.86%]	48 [85.71%]
Onset of disease		
onset <40 years	3 [42%]	22 [39.2%]
Onset >40 years	4 [57.1%]	34 [60.7%]
Psoriatic arthritis		
Positive	1	1
Negative	6	55

Sex distribution, family history, ds onset and psoriatic arthrirtis frequencies are mentioned in table 2

TT genotype was found in none of the psoriasis patients, 2 controls showed TT genotype

Our findings differ from the study on Egyptian psoriasis patients which showed significantly higher risk of psoriasis with TT genotype

All patients having CC genotype had a positive family history

Otherwise, TGF-beta 1 gene mutation was not associated with age, sex, onset, duration, and severity of disease, and psoriatic arthritis

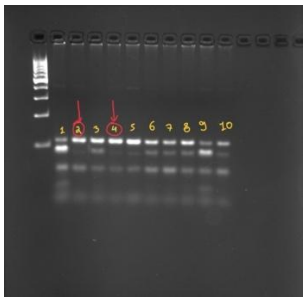

Figure 1: Pt 1 to 10

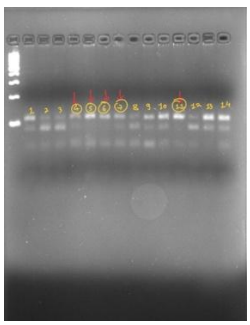

Figure 2: pt 11 to 24

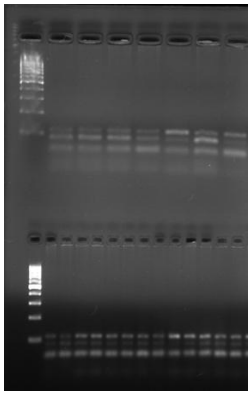

Figure 3: PT 25-45

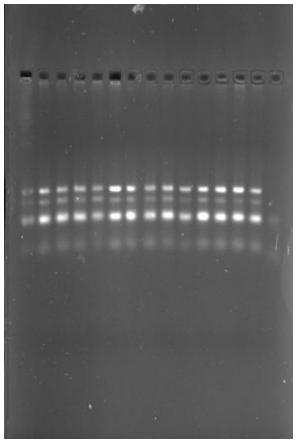

Figure 3: pt 46 to 63

6. Discussion

The expression of many cytokines is thought to be influenced by polymorphism in their gene loci and this may contribute to the development of inflammatory diseases.

TGF-b_1 polymorphisms are associated with asthma, Crohn's disease, rheumatoid arthritis, systemic sclerosis, liver cirrhosis, renal dysfunction after heart transplantation, liver graft acceptance and many other disorders [20, 23–27].

Single nucleotide polymorphism of the cytokine genes in psoriasis is the object of intensive researches and many of them disclosed interesting findings.

In this study, we did not find significant differences in genotypes between patients and control group. In 1996 Bonifati et al. [31] found increased serum concentrations of TGF-b_1 in psoriatic patients and strong correlation between TGF-b_1 concentration and severity of the disease, similar results obtained Nockowski et al. Leivo et al. [29] obtained very interesting results in immunohistochemical analysis of TGF-b receptors in the skin. Skin biopsies from healthy controls and non-lesional skin from psoriatic patients showed intense immunoreactivity for receptors in the epidermis, but lesional psoriatic skin lacked detectable immunoreactivity of them. This phenomenon suggests down-regulation of these structures in psoriasis.

Recently, it has been documented that yeast— Malassezia furfur up-regulate TGF-b_1 in the psoriatic skin [30].

Further studies concerning TGF-b_1 polymorphisms seem to be required to determine completely the molecular basis of the susceptibility to psoriasis in the context of increased levels of TGF-b_1 itself, down-regulated receptors for TGF-b_1 in the psoriatic lesions epidermis. Interestingly, previous studies found an association between rheumatoid arthritis and polymorphism of TGFbeta 1 gene at nucleotide T869C.

In conclusion, the present study demonstrated that the TGF-beta1 gene polymorphism of codon 10 at T869C nucleotide is not associated with susceptibility to psoriasis in Central rajasthan's population

Whether this polymorphism is associated with increased production of TGF-1 detected in psoriasis with a subsequent increase in keratinocyte proliferation, angiogenesis, and vasodilatation observed in psoriasis, is yet to be determined. Further studies are warranted to investigate the role of TGF-1 in psoriatic skin and whether it can be considered as the basis of future gene therapy in the treatment of psoriasis.

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Conflict of Interest: none

Patient consent on file: Consent for the publication of recognizable patient photographs or other identifiable material was obtained and included at the time of article submission to the journal. All patients gave consent with the understanding that this information may be publicly available

References

[1] Sabat R, Philipp S, Höflich C, et al. Immunopathogenesis of psoriasis. *Exp Dermatol*.2007; 16 (10): 779–798.
[2] Lawrence DA. Identification and activation of latent transforming growth factor beta. *Methods Enzymol*.1991; 198: 327–336.
[3] Han SW, Kim TY, Hwang PG, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol*.2005; 23 (11): 2493–2501.
[4] Bonifati C, Ameglio F. Cytokines in psoriasis. *Int J Dermatol*.1999; 38 (4): 241–251.
[5] Cutroneo KR. TGF-beta-induced fibrosis and SMAD signaling: oligo decoys as natural therapeutics for inhibition of tissue fibrosis and scarring. *Wound Repair Regen*.2007; 15 (Suppl.1): S54–S60.
[6] Han G, Williams CA, Salter K, Garl PJ, Li AG, Wang XJ. A role for TGFbeta signaling in the pathogenesis of psoriasis. *J Invest Dermatol*. 2010; 130 (2): 371–377.
[7] Zhang Y, Meng XM, Huang XR, Wang XJ, Yang L, Lan HY. Transforming growth factor-β1 mediates psoriasis-like lesions via a Smad3-dependent mechanism in mice. *Clin Exp Pharmacol Physiol*. 2014; 41 (11): 921–932.
[8] Flisiak I, Chodynicka B, Porebski P, Flisiak R. Association between psoriasis severity and transforming growth factor beta (1) and beta (2) in plasma and scales from psoriatic lesions. *Cytokine*.2002; 19 (3): 121–125.

[9] Kane CJ, Knapp AM, Mansbridge JN, Hanawalt PC. Transforming growth factor-beta 1 localization in normal and psoriatic epidermal keratinocytes in situ. J Cell Physiol 1990; 144: 144–50.
[10] Wang XJ, Greenhalgh DA, Bickenbach JR, Jiang A, Bundman DS, Krieg T, et al. Expression of a dominant-negative type II transforming growth factor beta (TGF-beta) receptor in the epidermis of transgenic mice blocks TGF-beta-mediated growth inhibition. Proc Natl Acad Sci USA 1997; 94: 2386–91.
[11] Wataya-Kaneda M, Hashimoto K, Kato M, Miyazono K, Yoshikawa K. Differential localization of TGF-beta-precursor isotypes in normal human skin. J Dermatol Sci 1994; 8: 38–44.
[12] Mao JH, Saunier EF, de Koning JP, et al. Genetic variants of TGF-β1 act as context-dependent modifiers of mouse skin tumor susceptibility. *Proc Natl Acad Sci USA*.2006; 103 (21): 8125–8130.
[13] Arkwright PD, Pravica V, Geraghty PJ, et al. End-organ dysfunction in cystic fibrosis: association with angiotensin I converting enzyme and cytokine gene polymorphisms. *Am J Respir Crit Care Med*.2003; 167 (3): 384–389.
[14] Lu LY, Cheng HH, Sung PK, Yeh JJ, Shiue YL, Chen A. Single nucleotide polymorphisms of transforming growth factor-beta1 gene in Taiwanese patients with systemic lupus erythematosus. *J Microbiol Immunol Infect*.2004; 37 (3): 145–152.
[15] Ohtsuka T, Yamakage A, Yamazaki S. The polymorphism of transforming growth factor-beta1 gene in Japanese patients with systemic sclerosis. *Br J Dermatol*.2002; 147 (3): 458–463.
[16] Sugiura Y, Niimi T, Sato S, et al. Transforming growth factor beta1 gene polymorphism in rheumatoid arthritis. *Ann Rheum Dis*.2002; 61 (9): 826–828.
[17] Zhou TB, Zhao HL, Fang SL, Drummen GP. Association of transforming growth factor-β1 T869C, G915C, and C509T gene polymorphisms with rheumatoid arthritis risk. *J Recept Signal Transduct Res*. 2014; 34 (6): 469–475.
[18] Cantor MJ, Nickerson P, Bernstein CN. The role of cytokine gene polymorphisms in determining disease susceptibility and phenotype in inflammatory bowel disease. *Am J Gastroenterol*.2005; 100 (5): 1134–1142.
[19] Chiang CH, Chuang CH, Liu SL, Shen HD. Genetic polymorphism of transforming growth factor β1 and tumor necrosis factor α is associated with asthma and modulates the severity of asthma. *Respir Care*. 2013; 58 (8): 1343–1350.
[20] Kim SY, Han SW, Kim GW, Lee JM, Kang YM. TGF-beta1 polymorphism determines the progression of joint damage in rheumatoid arthritis. Scand J Rheumatol 2004; 33: 389–94.
[21] Hutchinson IV, Turner D, Sankaran D, Awad M, Pravica V, Sinnott P. Cytokine genotypes in allograft rejection: guidelines for immunosuppression. Transplant Proc 1998; 30: 3991–2.
[22] SuthanthiranM, LiB, Song JO, Ding R, Sharma VK, Schwartz JE, et al. Transforming growth factor-beta 1 hyperexpression in African–American hypertensives: a novel mediator of hypertension and/or target organ damage. Proc Natl Acad Sci USA 2000; 97: 3479–84.
[23] Baan CC, Balk AH, Holweg CT, van Riemsdijk IC, Maat LP, Vantrimpont PJ, et al. Renal failure after clinical heart transplantation is associated with the TGF-beta 1 codon 10 gene polymorphism. J Heart Lung Transplant 2000; 19: 866–72.
[24] Gomez-Mateo J, Marin L, Lopez-Alvarez MR, Moya-Quiles MR, Miras M, Marin-Moreno I, et al. TGF-beta1 gene polymorphism in liver graft recipients. Transpl Immunol 2006; 17: 55–7.
[25] Osterreicher CH, Datz C, Stickel F, Hellerbrand C, Penz M, Hofer H, et al. TGF-beta1 codon 25 gene polymorphism is associated with cirrhosis in patients with hereditary hemochromatosis. Cytokine 2005; 31: 142–8.
[26] Schulte CM, Goebell H, Roher HD, Schulte KM. C-509T polymorphism in the TGFB1 gene promoter: impact on Crohn's disease susceptibility and clinical course?. Immunogenetics 2001; 53: 178–82.
[27] Pulleyn LJ, Newton R, Adcock IM, Barnes PJ. TGFbeta1 allele association with asthma severity. Hum Genet 2001; 109: 623–7
[28] Wataya-Kaneda M, Hashimoto K, Kato M, Miyazono K, Yoshikawa K. Differential localization of TGF-beta-precursor isotypes in psoriatic human skin. J Dermatol Sci 1996; 11: 183–8.
[29] Leivo T, Leivo I, Kariniemi AL, Keski-Oja J, Virtanen I. Down-regulation of transforming growth factor-beta receptors I and II is seen in lesional but not non-lesional psoriatic epidermis. Br J Dermatol 1998; 138: 57–62.
[30] Baroni A, Paoletti I, Ruocco E, Agozzino M, Tufano MA, Donnarumma G. Possible role of Malassezia furfur in psoriasis: modulation of TGF-beta1, integrin, and HSP70 expression in human keratinocytes and in the skin of psoriasis-affected patients. J Cutan Pathol 2004; 31: 35–42.
[31] Bonifati C, Carducci M, Mussi A, Pitarello A, D'Agosto G, Fazio M. The levels of transforming growth factor-b1 are increased in the serum of patients with psoriasis and correlate with disease severity. Eur J Dermatol 1996; 6: 486–90.
[32] Flisiak I, Zaniewski P, Chodynicka B. Plasma TGF-beta1, TIMP-1, MMP-1 and IL-18 as a combined biomarker of psoriasis activity. *Biomarkers*.2008; 13 (5): 549–556.
[33] Meki AR, Al-Shobaili H. Serum vascular endothelial growth factor, transforming growth factor beta 1, and nitric oxide levels in patients with psoriasis vulgaris: their correlation to disease severity. *J Clin Lab Anal*. 2014; 28 (6): 496–501.
[34] Akhurst RJ. TGF beta signaling in health and disease. *Nat Genet*. 2004; 36 (8): 790–792.