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ESTIMATION OF PEROXISOME PROLIFERATORS - ACTIVATED RECEPTOR γ GENE EXPRESSION IN INFLAMMATORY SKIN DISEASES: *ATOPIC DERMATITIS* AND *PSORIASIS*

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Abstract
Introduction: Peroxisome proliferators- activated receptors (PPARs) represent a major research target for the understanding and treatment of many skin diseases, such as benign epidermal tumors, psoriasis and atopic dermatitis.
Aim: Estimate and analyze the PPAR gamma expression and its pathological role in psoriasis and atopic dermatitis.
Materials and Methods: Fifteen patients with atopic dermatitis, fifteen patients with psoriasis and twenty apparently healthy subjects as controls,were included in the current study. We estimate the PPARgamma gene expression in the lesional skin of atopic and psoriatic patients and control, by quantitative real-time RT-PCR.
Results: Our data showed a significant decreased PPARgamma expression in lesional skin of atopic dermatitis patients and psoriatic patients (P value<0.001) compared to the control group, and the decrease was more marked in the psoriatic patients (P value<0.001).
Conclusion: Abberent PPAR γ expression has an important role in the pathogenesis of psoriasis and atopic dermatitis through the affection of cell proliferation, differentiation and inflammation.

Key words: PPAR γ; psoriasis; atopic dermatitis

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Introduction

The peroxisome proliferators - activated receptors (PPAR) belong to a subfamily of nuclear hormone receptors compromising three different isoforms of PPARs termed PPARα, PPARβ/δ and PPARγ. These subtypes are encoded by separate genes, exhibit different tissue distribution, functions and, to some extent, different ligand specificities. After ligand binding, PPARs can regulate gene expression by binding to peroxisome proliferator response elements (PPRE) in target genes as heterodimers with the retinoid X receptors (RXR) [1].
PPARs and corresponding ligands have been shown in skin and other organs to regulate important cellular functions, including cell proliferation and differentiation, as well as inflammatory responses [2].
PPARγ is the target ligand for thiazolidinediones (TZDs). Within the skin PPAR γ is present in sebaceous glands, inner root sheath epithelium, epidermis, and adipocytes. Also, melanocytes in benign nevi, primary melanomas, and melanoma metastases have all been shown to produce this protein. Staining for PPARγ in keratinocytes has been demonstrated in the nucleus and paranuclear region [3].
A gradual increase in expression of PPARγ from basal to granular layer has been observed in keratinocytes, and PPARγ ligands have been shown to induce the expression of genes associated with keratinocyte differentiation in vitro [2].
PPARγ plays a critical role in the regulation of genes that are involved in cellular proliferation, specific components of the T helper 2 (TH2) inflammatory pathway and maintenance of the skin barrier. This suggestion was supported by the observation that the PPARγ ligand ciglitazone inhibits allergic immune response by inhibiting TH2-driven IgE production and also production of (pro) inflammatory cytokines of the TH response in vitro and in vivo [4].

Based on the previous data showing the suggested important physiological role of PPARγ in cellular proliferation, differentiation and its possible association with inflammatory responses, the aim of the current study was to assess the tissue expression of PPAR gamma gene in psoriatic and atopic patients in an attempt to assess its suggested role in the aetiopathogenesis of these diseases which accordingly will introduce new future therapeutic strategies for both diseases.

Material and Methods

Fifty subjects were selected from the dermatology outpatient clinic, at kaser Alainy hospitals. Fifteen patients had atopic dermatitis and fifteen patients had psoriasis in addition to twenty age and sex matched, apparently healthy subjects as a control group. An informed consent was signed by each patient and ethical committee approval was fulfilled before the start of the study. Diagnosis was done on clinical basis and confirmed by skin biopsy.

The atopic patients were eight females and seven males.Their mean age was (21.07+10.6) years. The psoriatic patients were nine females and 6 males. Their mean age was (29.47+8.49) years.

Inclusion criteria:

1. Patients with childhood or adult atopic dermatitis.
2. Patients with any clinical variant of psoriasis.
3. Any age, both sexes.
4. Patients who consent to participate in the study.

Exclusion criteria:

1. Patients who received any systemic medications or phototherapy six months before the study nor topical medications three months prior to the study.
2. Patients with history of any systemic or dermatological diseases affecting the immune system.

Intervention:

All patients and controls were subjected to the following:

1. Informed consent.
2. Full history taking.
3. Full general and dermatological clinical examination.
4. Punch skin biopsy (4mm).
5. Histopathological examination for confirmation of the diagnosis.
6. PCR for the detection of PPAR gamma gene expression.

The history taken from the patients included:

- Personal history.
- History of the present condition.
- Onset of the disease.
- Duration of the disease.
- History of the treatment taken by the patients.
- Past medical history.
- Family history.

A full general examination was performed.

Assessment of disease severity was done by:

- PASI score for the psoriatic patients.

The biological severity of psoriasis at a given point in time is often quantified using the Psoriasis Area and Severity Index (PASI). This is a composite score incorporating a grading of erythema, indurations and scaling of plaques, multiplied by the clinical setting and although it has many shortcomings, it remains the gold standard tool for psoriasis assessment [5].

- Three-Item severity (TIS) score for the severity of atopic dermatitis.

It was developed as a simplified form of SCOR AD score. It is based on the evaluation of erythema, edema/papulation and excoriation, on a scale of 0-3. This score is particularly suitable in general practice, for routine clinical use and for screening purposes in clinical trials [6].

Skin biopsy:

A lesional punch skin biopsy of (4 mm) was taken from the patients and the healthy controls under local anesthetic (1% lidocaine). This procedure was done under aseptic precautions and the patients and controls were given topical antibiotic cream (Terramycin) and systemic antibiotic (Amoxil 500mg) as a treatment for the biopsy site.

Half of the biopsy taken from every patient was for histopathological study to confirm the diagnosis and the other half was kept frozen for PCR.

PCR technique

Detection of PPAR gamma gene expression using real time PCR (RT–PCR)

RNA extraction

Total RNA was isolated from skin tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA) according to manufacturers instruction. The purity (A260/A280 ratio) and the concentration of RNA were obtained using spectrophotometry (Gene Quant 1300, Uppsala, Sweden). RNA quality was confirmed by gel electrophoresis.

cDNA synthesis

First-strand cDNA was synthesized from 4 μg of total RNA using an Oligo(dT)12-18 primer and Superscript™ II RNase Reverse Transcriptase, This mixture was incubated at 42°C for 1h, the kit was supplied by SuperScript Choice System (Life Technologies, Breda, the Netherlands).

Real-time quantitative polymerase chain reaction (PCR)

Real-time PCR (RT-PCR) amplification was carried out using 10μL amplification mixtures containing Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA USA), equivalent to 8ng of reverse-transcribed RNA and 300nM primers, the sequences of PCR primer pairs used for each geneare shown in Table I. Reactions were run on an ABI PRISM 7900 HT detection system (Applied Biosystems) PCR reactions consisting of 95°C for 10min (1 cycle), 94°C for 15s, and 60°C for 1min (40 cycles),Data were analyzed with the ABI Prism sequence detection system software and quantified using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to housekeeping gene GAPDH (Tabl. I).

Statistical methods:

The data was coded and entered using the statistical package SPSS version 15.

The data was summarized using descriptive statistics: mean, standard deviation, median, minimal and maximum values for quantitative variables and number and percentage for qualitative values. Statistical differences between groups were tested using Chi Square test for qualitative variables, independent sample t test for quantitative normally distributed variables while Nonparametric Mann Whitney test was used for quantitative variables which aren't normally distributed. Correlations were done to test for linear relations between variables. P - Values less than or equal to 0.05 were considered statistically significant [7].

Primer	Sequence
PPAR gamma	Forward: 5' AAAGAAGCCGACACTAAACC 3'Reverse: 5' CTTCCATTACGGAGAGATCC 3' According to gene bank accession number :AB_565476.1
GAPDH	Forward 5' ACCACAGTCCATGCCATCAC 3' Reverse 5' TCCACCACCATGTTGCTGTA3' According to gene bank accession number :XM_005253678.1

Table I. Primer sequences used for RT-PCR.

Results

Demographic data:

This study included 50 subjects of whom 30 were patients and 20 healthy individuals who serve as controls. Patients were divided into two groups (Atopic and psoriatic groups).

Psoriatic group:

The psoriatic group included nine (60%) females and six (40%) male patients. The age of the psoriatic patients ranged between (20-55) with a mean value of (29.47+8.49).

The mean duration of the disease in the psoriatic group was (7.35+5.77) years. The extent of the disease was (33.33%+21.9) and the mean of PASI score was (9.15+6.29) (Tabl. II).

Twelve (80%) patients had psoriasis vulgaris, two (13.3%) had guttate psoriasis and one (6.7%) patient had palmo-planter psoriasis. Ten (70%) patients had(+ve)family history of psoriasis.

Atopic group:

The atopic group included eight (53.3%) females and seven (46.7%) males. The age of the atopic patients ranged between (10-40) years with a mean value of (21.07+10.6).

In the atopic group a positive family history of allergic disease (asthma, allergic rhinitis, food allergy or atopic dermatitis) was reported in all patients (100%).

The mean duration (in years) of the disease was (10.14+9.84) and the extent of the disease was (28.33%+24.03). Eight patients (53.3%) were of the adult type while seven patients (46.7%) were of the child hood type. The mean value of the disease severity was (6.6+1.29) by the TIS (Tabl. III).

Pt. No	age	sex	duration	extent	PASI	c. variant	f. history
1	26	M	10	10	4.2	PP.Ps	+
2	27	M	15	20	8.6	Ps.vulg	-
3	34	F	19	30	9.8	Ps.vulg	+
4	35	F	2	10	5.2	Ps.vulg	+
5	27	F	10	20	8	Ps.vulg	-
6	22	M	1	10	1.4	Ps.vulg	+
7	20	M	5	60	22.4	Ps.vulg	-
8	25	F	10	10	1.8	Ps.vulg	+
9	30	F	5	50	17	Ps.vulg	+
10	36	F	3m	50	9	guttate.ps	+
11	27	F	9	40	15.2	guttate.ps	-
12	26	F	2	70	17.4	Ps.vulg	+
13	22	M	3	60	8.7	Ps.vulg	+
14	30	F	15	50	4.9	Ps.vulg	+
15	55	M	4	10	3.4	Ps.vul g	-

Table II. Primer sequences used for RT-PCR.

M=male; F=female; f=family; c=clinical; vulg= vulgaris; PP=palmoplanter; m=month

Pt. No	age	sex	duration	extent	TIS	c. variant	f. history
1	12	M	7	20	9	ch.hood	+
2	10	F	9	10	8	ch.hood	+
3	14	F	14	30	8	ch.hood	+
4	14	F	10	30	7	adult.t	+
5	40	M	30	80	7	adult.t	+
6	15	M	7	20	7	ch.hood	+
7	15	F	14	20	6	adult.t	+
8	37	M	32	20	6	adult.t	+
9	16	F	1	30	6	ch.hood	+
10	10	M	2	20	6	ch.hood	+
11	16	F	16	10	5	adult.t	+
12	26	F	4	15	6	adult.t	+
13	40	M	5	90	5	adult.t	+
14	26	F	1	20	5	adult.t	+
15	25	M	2m	10	4	adult.t	+

Table III. Demographic data of atopic patients.

F=female; M=male; m=month; f=family; ch=child; t=type

Control group:
The control group included 11 (55%) females and nine (45%) males. The mean age was (27.50+7.5). All the control subjects had no family history of atopy nor psoriasis.

Analytic data:
Psoriatic group vs. control:
Statistical analysis of the PPARγ gene expression done by Mann--Whitney test revealed that the mean of expression of PPARγ was (0.15+0.05) in the psoriatic patients, and it was (1.20+0.43) in the controls. On comparing both groups (Ps & control) a statistically significant lower PPARγ expression was reported in the psoriatic patients (P.value<0.001) (Fig. 1).

Atopic group vs. control:
The mean value of PPAR γ expression in the atopic patients was (0.36+0.17) compared to (1.20+ 0.43) in the controls.

On comparing both groups there was an evident lower expression in the atopic patients which was statistically significant (P value <0.001) (Fig. 2).

On comparing both atopic and psoriatic groups:
Lower expression of PPARγ gene expression was observed in the psoriatic patients in comparison to the atopic patients with a significant statistical difference (P value <0.001) (Fig. 3).

No significant correlation between:
The mean level of PPARγ gene expression to all of the following parameters in both groups:
· Age of the patient.
· Duration of the disease.
· Extent of the disease.
· Severity of the disease.
· Clinical variant of the disease.

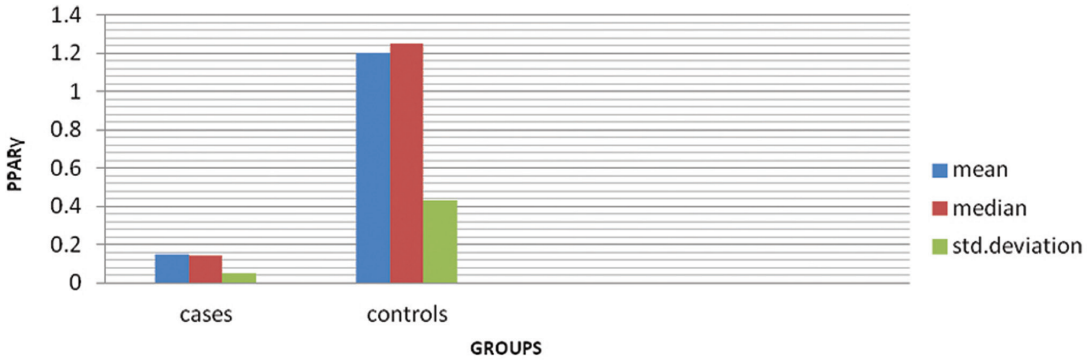

Figure 1. PPARγ gene expression in psoriasis and control.

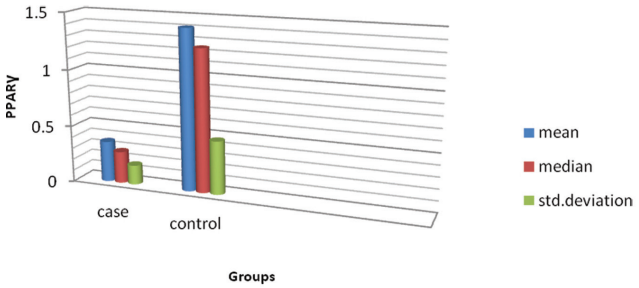

Figure 2. PPARγ gene expression in atopic and control.

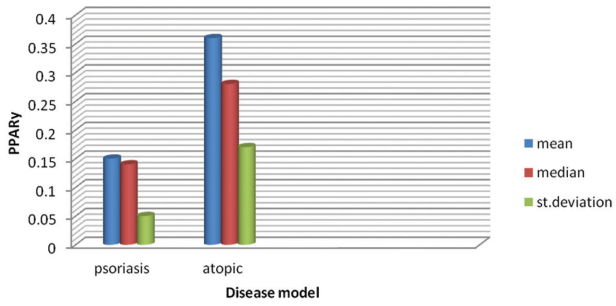

Figure 3. PPARγ expression in AD and psoriasis.

Discussion

The current study revealed reduced expression of PPARγ gene in both atopic and psoriatic patients with a more significant reduction in the psoriatic group.
In agreement with our results, Plager et al. (2007) [8] reported as well decreased PPAR activity and decreased gene expression of both (PPARα and γ) in AD patients. In addition, decreased expression of both PPARα and γ gene expression in psoriasis had been reported before by previous studies [9,10].
Based on those results, other studies investigated the role of PPARγ receptors activators in treatment of different inflammatory diseases. Exogenous ligands for PPARγ include several pharmaceutical products of which TZDs are the most selective ligand. TZDs are potent selective PPARγ agonists [11]. Some studies [2], found that activation of PPARγ receptor, attenuated the allergic immune response via mono- cytes and lymphocytes.
In addition others [12] reported that activation of PPARγ receptors, inhibited the maturation of bone marrow progenitor into connective tissue type mast cells thus giving a good model for therapeutic implications for mast cell-related diseases such as atopic or contact dermatitis.

On the same basis, a retrospective review done in 2005 [13], demonstrated the safety &efficacy of rosiglitazone, a PPAR agonist, in 6 cases of severe atopic dermatitis who were unresponsive to first and second line therapies. Moreover, Dahten et al. 2007 [4], stated that PPARγ ligand treatment inhibited not only systemic allergic immune response, but also local allergen-mediated dermatitis. Again it had been reported that activators of peroxisome proliferators-activated receptor (PPAR) α, β/δ, γ regulate epidermal protein and lipid production, leading to superior barrier function. Additionally, some of these activators exhibit potent anti-hyperplasic and anti-inflammatory activity in irritant contact dermatitis and acute allergic contact dermatitis. These results suggest that topical applications of certain activators/ligands of PPARα, β/δ, and γ could be useful for the treatment of AD in humans [14].

Regarding their use in psoriasis, a large ten-year case-control study identified an association between the use of TZDs (specific ligands for PPARγ) and the reduced risk of psoriasis. This association was not present with the use of other anti-diabetic drugs [1,15,16].

Studies in a mouse model of hyperproliferative skin disease have shown that topical administration of PPARγ ligands reduced epidermal hyperplasia and that the treatment had no effect on normal skin [17].

In an attempt to understand the pathogenic role played by the abberrent expression of PPARγ in both AD and psoriasis we reviewed the literature and searched for any previous studies incorporated in the same field.

Concerning AD, the epidermal barrier dysfunction together with the induced inflammatory processes constitute the main scenario for the disease pathogenesis. Activation of PPARγ receptor improves permeability barrier homeostasis by a number of mechanisms, including stimulating epidermal lipid synthesis, increasing lamellar body formation and secretion, and increasing the activity of enzymes required for the extra cellular processing of lipids in the stratum corneum, leading to the formation of lamellar membranes that mediate permeability barrier function [18].

Functional human PPARγ was originally cloned from the human bone marrow, and plays a role in the regulation and development of immune cells like mono-cytes and T-lymphocytes [19].

Several studies support the role of PPAR γ in inflammatory processes as its expression appears to be altered by (pro)-inflammatory cytokines. A different PPARγ expression profile due to the changes of the local inflammatory milieu may result in differential functional effects of the immune response [4]. In a review article published in 2010 [20], stated that peroxisome proliferator-activated receptor (PPAR) transcription factors thus act as connectors between the enzymatic mechanisms of the epidermal barrier and the abnormal immune and inflammatory responses that characterize atopic dermatitis.

The hallmarks of psoriasis are the abnormal differentiation and hyperproliferation of keratinocytes with inflammatory cell infiltration.

Ligand activation of PPARγ can also inhibit proliferation, promote differentiation and induce apoptosis in a variety of malignant and non-malignant tissues. As a reflection to their anti-proliferative property, PPARγ agonists inhibit VEGF-induced angiogenesis. An increasing body of evidence points to an interaction between vitamin D and PPAR-signaling pathways, where the anti-proliferative, differentiation regulatory effects of vitamin D compounds are at least in part mediated by transcriptional regulation done by PPARγ activity [21]. At the molecular level, PPARγ stimulation appears to function in a largely inhibitory fashion. PPARγ diminishes the activity of cytokines including signal transducer and activator of transcription (STAT), interleukin-1, nuclear factor-kappa-beta, and activator protein-1. It also decreases production of interleukin-1β, interleukin-6, and most importantly, TNFα. In general, there appears to be an antagonistic relationship between PPARγ and TNF-α, perhaps explaining its beneficial effects in psoriasis [22].

To the best of our knowledge, the current study was the first done comparing the level of PPARγ gene expression in atopic dermatitis versus psoriasis. We revealed a lower level of the gene expression in the psoriatic group with a significant statistical P value (< 0.001).

Those results point to the evident role played by PPARγ in keratinocyte proliferation and differentiation. Moreover this explains why PPARγ agonists, through their activation, could achieve a great success in psoriasis therapy as stated before by several studies [1,15-17]. Although this study revealed no correlation between the severity of the diseases (in both groups) and the level of PPARγ gene expression. Yet we suggest that the small sample size in each group might explain those findings and a larger sample size in further studies can elaborate more about PPARγ in relation to different clinical parameters in both diseases.

We deduced out of the results of the current study the important role for the PPARγ gene abnormal expression in the pathogenesis of both AD and psoriasis.

Increasing body of evidence out of this study indicates that PPARγ signaling pathways may represent interesting therapeutic targets for a broad variety of skin disorders, including inflammatory skin diseases such as psoriasis and atopic dermatitis.

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