Toll-Like Receptor 2 (TLR2) Gene Expression in Psoriasis Vulgaris and its Correlation with Vitamin D3 Serum Level before and after NB-UVB

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Abstract

Background: Psoriasis is a chronic, non-contagious skin disease characterized by red, inflamed cutaneous lesions covered with silvery-white scale. Both genetic and environmental factors are important in the etiology of the disease. The role of Toll like receptor 2 and vitamin D3 in the pathogenesis of psoriasis needs to be clarified.

Objective: The aim of this research is to estimate the level of Toll-like receptor 2 gene expression in skin tissue of psoriasis patients and Vitamin D3 in serum of psoriasis patients and compare the results to healthy control. We also used narrow band-UVB-311 as a treatment and evaluated the same parameters in psoriasis patients before and after treatment.

Patients and Methods: Fifteen patients of psoriasis and 15 age and sex matched healthy controls were enrolled in the study. TLR2 gene expression and Vitamin D3 serum levels were estimated and compared to healthy controls. Also these parameters were estimated after 36 sessions of NB-UVB.

Results: There was statistically significant increase in the levels of TLR2 in psoriatic patients skin tissue compared to control (p<0.001). There was statistically significant decrease in the level of Vitamin D3 in psoriasis patients serum compared to control. After using NB-UVB-311 as treatment, the level of TLR2 gene expression was significantly decreased (p<0.001) and the level of Vitamin D3 was significantly increased (p<0.001) in psoriasis patients.

Conclusion: These results provide some evidence regarding the role of Toll-like receptor 2 and Vitamin D3 in psoriasis and effect of NB-UVB as a modality of treatment.

Key Words: Toll like receptor 2 — Vitamin D3 — Narrow band UVB.

Introduction

PSORIASIS is a chronic relapsing skin disease mediated by elements of the innate and adaptive immune systems. Psoriatic skin is characterized by abnormal keratinocyte differentiation and proliferation. Signals derived from immune cells, such as proinflammatory cytokines, are able to stimulate keratinocytes proliferation, and the keratinocytes themselves may modulate immune cells through surface and secretory molecules. These molecules consist of toll-like receptors (TLR5), antimicrobial peptides (AMPs), and the active metabolite of vitamin D, 1,25(OH)2D3 III.

Toll-like receptors (TLRs) are recognition molecules for multiple pathogens, including bacteria, viruses, fungi, and parasites. TLR2 forms heterodimers with TLR1 and TLR6, which is the initial step in a cascade of events leading to significant innate immune responses, development of adaptive immunity to pathogens and protection from immune sequelae related to infection with these pathogens [2]. TLR2 expression has been detected in immune cells, endothelial, and epithelial cells. This ubiquity is consistent with the wide range of roles and functions of TLR2 [3,4].

Keratinocytes in the epidermis constitutively express TLR1,2 and 5. In psoriasis lesions, the expression of TLR1 and TLR2 on keratinocytes is further upregulated [5]. Keratinocytes in human psoriatic skin, activated by TLR2,3 and 4 ligands exhibited NF-KB nuclear translocation and release of TNF-a and IL-8 [6]. An immunohistochemistry-based profile of psoriatic skin also demonstrates the overexpression of TLR2 in epidermal and dermal dendritic cells (DCs) and the enhanced TLR2 expression in basal layer keratinocytes [7].

Vitamin D is produced in the skin from 7-DHC, the last precursor in cholesterol synthesis [7]. In adult human skin, approximately 50% of provitamin...
D3 (7-DHC) is found in the epidermis and the other half is found in the dermis. The skin occupies a central position within the vitamin D system. Epidermal keratinocytes also express the vitamin D hydroxylase enzymes 25-hydroxylase (CYP27A1) and 1a-hydroxylase (CYP27B1), enabling them to convert vitamin D3 into 25(OH)D and 1,25(OH)2D, the biologically active form of vitamin D3. UVB is mainly absorbed by epidermal components including keratinocytes, melanin and Langerhans' cells. Biological effects of UV radiation are generated through interaction with absorbing molecules called chromophores. In the case of UVB, the most important chromophores are proteins such as keratin, melanin, collagen and elastin, urocanic acid, DNA and provitamin D.

According to Feldman et al., with regard to efficacy, safety and cost effectiveness, UVB phototherapy appears to be the best first-line treatment for the control of generalized psoriasis. Data from Fischer & Alsins and Parrish & Jaenicke subsequently showed that wavelengths around 311nm provoke least erythema while being most effective for clearing psoriasis.

In the present controlled study we investigated the expression of TLR2 and Vitamin D3 in psoriatic patients in comparison to healthy controls. Also we investigated the value of narrow band UVB phototherapy in the treatment of psoriasis vulgaris and its possible effects on Toll-like receptor 2 and vitamin D3 levels in psoriatic patients by measuring the same parameters before and after treatment.

**Patients and Methods**

This study was done prospectively on fifteen psoriasis patients attending The Outpatient Clinic of Dermatology Department, Kasr Al-Aini Hospital, Cairo University. The patients were diagnosed clinically and histopathologically before entering the study. A total of fifteen age and sex matched healthy controls recruited from the general population attending the hospital were enrolled in this study. All patients and controls were included in the study after giving an informed consent.

All Patients were subjected to the following:

- Full history taking including Bio data (name, age, address, occupation), onset of the disease, duration and evolution of their disease, drug intake and family history of the disease.
- Dermatological examination to assess the site and extent of the disease. The clinical severity of psoriasis was graded according to the severity index (PASI) scoring system. The PASI score of all patients was determined by one dermatologist.
- Laboratory investigations including complete blood picture, erythrocyte sedimentation rate, liver and kidney function tests.
- All patients received NB-UVB sessions 3 times/week for 3 months.

**Estimation of vitamin D3 serum level:**

Blood samples were withdrawn and left to clot for 15min then centrifuged at 14,000rpm for 10min then the separated serum was kept frozen at 80c till analysis. Serum samples were examined for 25 OH D levels by Enzyme-linked immunosorbent assay (ELISA) by kit supplied by (Immundiagnostik USA) briefly, monoclonal antibody identify 25, OH vitamin D was used in the assay The samples were incubated with the detection antibody after the extraction step. Then Peroxidase-conjugated anti-mouse antibody was then added into microplate well, forming a complex of 25-hydroxy vitamin D-detection antibody-peroxidase conjugate. Tetramethylbenzidine (TMB) was used as a substrate, the colour density developed is proportional to vitamin D concentration. Finally, to terminate the reaction stop solution was added and the microplates were read by ELISA reader at 520nm.

**Detection of TLR2 gene expression in skin tissues by real time PCR:**

Ribonucleic acid (RNA) extraction and complementary deoxyribonucleic acid (cDNA) synthesis:

RNA was extracted from skin tissue homogenate using RNasy Purification Reagent (Qiagen, Valencia, CA) RNA amount was spectrophotometrically determined at 260/280nm. The first-strand cDNA was synthesized (Applied Biosystem) according to the manufacturer's instructions, briefly 5pg of total RNA extracted was added to (10pmol) oligo dt primer and AMV reverse transcriptase for 60min at 37°C.

**Real time quantitative PCR:**

Real-time RT-PCR was performed to detect TLR-2 gene expression. The 20111 reaction mixture contained 5.5111 DEPC-H20, 5.0111 cDNA, 2.0111 (50gM) of each primer For: AACTTACTGGGAA ATCCTTAC, Rev: AAAAAATCTCCAGCAGTAA AAT and Greens qPCRmix. The thermal cycle profile for PCR was as follows: 94°C for 3min, 40 cycles of PCR (94°C for 15sec; 55°C for 15sec; 72°C for 30sec). Data from real-time assays were calculated using the v1.7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative Ct method. All values were
normalized to the GAPDH gene with the following sequence For: GGATTGTGCTGTATTGGG, Rev: GGAAGATGGTGATGGGATT and reported as fold change over background levels detected in diseases group [17].

Statistical analysis:

Data were entered on Excel 2010. Statistical analysis was performed using the Statistical Package for Social Scientists (SPSS) version 17. Descriptive statistics were displayed as frequency distribution for categorical variables and measures of central tendency (the median) and dispersion (the range) for continuous variables. Comparison of vitamin D and TLR2 levels between cases and controls was done using the Mann-Whitney U test, while comparison of the levels of vitamin D, TLR2, and PASI levels before and after the intervention was done using the Wilcoxon Signed rank test. Comparison of the net changes and the percent changes in vitamin D, TLR2 and PASI levels between males and females was done using the Wilcoxon Signed rank test. The level of statistical significance is set at p-value < 0.05. p-value was estimated according to median value in Tables (2,3).

Results

Table (1) illustrates the clinical and dermatological characteristics of psoriatic patients comparable to sex and age matched control group. As seen, age and sex of patients were not statistically significantly different than controls (p>0.05). Duration of the disease ranged from 9m-35 years with mean duration 8.32±8.84. The psoriatic lesions were generalized in 14 patients and localized in 1 patient. The extent of disease ranged from 20-80% with mean of 53.33±17.99.

Before treatment the levels of TLR2 gene expression in psoriasis patients (median 1.090U/mg) were found to be significantly higher than the levels of TLR2 gene expression in normal skin of healthy controls (median 0.210U/mg). The difference was found to be statistically significant (p<0.000) (Table 2).

The levels of Vitamin D3 in the serum of psoriasis patients (Median=16.10U/mg) were found to be significantly lower than those of controls (Median=31.60U/mg). The difference was found to be statistically significant (p<0.000) (Table 2).

After treatment TLR2 gene median level decreased significantly from 1.090 to 0.6100 while the level of Vitamin D3 in serum of patients increased significantly from 16.10 to reach 22.100 with p-value=0.001.

This was associated with significant clinical improvement and significant decrease in PASI score from 22 to reach 4. p-value=0.001. The results are summarized in Table (3) & Fig. (1).

Table (1): Clinical and dermatological characteristics of the psoriatic patients and the control group.

<table>
<thead>
<tr>
<th></th>
<th>Patients (N=15)</th>
<th>Control (N=15)</th>
</tr>
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<tbody>
<tr>
<td>Age (Y)</td>
<td>36.800±17.15143</td>
<td>39.55±15.85</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>13/2</td>
<td>11/4</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>8.3167±8.83604</td>
<td></td>
</tr>
<tr>
<td>Range of disease</td>
<td>9m-35 Years</td>
<td></td>
</tr>
<tr>
<td>Distribution of the disease</td>
<td>14 Generalized/</td>
<td>one localized</td>
</tr>
<tr>
<td>Extent %</td>
<td>53.333±17.99471</td>
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</table>

Table (2): Levels of TLR2 gene expression and Vitamin D3 in psoriatic patients and healthy control.

<table>
<thead>
<tr>
<th></th>
<th>Patients (before)</th>
<th>Control (before)</th>
</tr>
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<tbody>
<tr>
<td>25(OH) Vit D (before)/U/mg</td>
<td>16.10</td>
<td>31.60</td>
</tr>
<tr>
<td>25(OH) Vit D (after)/U/mg</td>
<td>22.100</td>
<td></td>
</tr>
<tr>
<td>TLR2 (before)</td>
<td>1.090</td>
<td>0.210</td>
</tr>
<tr>
<td>TLR2 (after)</td>
<td>0.6100</td>
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Table (3): Estimation of TLR2, 25(OH) vitamin D levels and PASI score before and after treatment.

<table>
<thead>
<tr>
<th></th>
<th>25(OH) Vit D (before)</th>
<th>25(OH) Vit D (after)</th>
<th>TLR2 (before)</th>
<th>TLR2 (after)</th>
<th>Psoriasis area and severity index (before)</th>
<th>Psoriasis area and severity index (after)</th>
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<tbody>
<tr>
<td>Median</td>
<td>16.10</td>
<td>22.100</td>
<td>1.090</td>
<td>0.6100</td>
<td>21.0000</td>
<td>4.0000</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
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</table>
Discussion

Although the role of the TLRs in the pathogenesis of psoriasis remains to be clarified, TLR expression has been studied with conflicting results. Epidermal keratinocytes in normal skin constitutively expressed TLR1, TLR2 and TLR5, while TLR3 and TLR4 were barely detectable. TLR1 and TLR2 are expressed in entire epidermis, but more highly expressed in the basal keratinocytes. TLR5 is exclusively expressed in the basal cell layer [5].

In contrast, in lesional psoriatic skin, strong TLR1 staining is observed in the keratinocytes of the upper epidermis. TLR2 is highly expressed in the keratinocytes of the upper epidermis, but not in the basal layer, whereas TLR5 is down-regulated in the basal keratinocytes of patients with psoriasis. TLR3 and TLR4 are weakly expressed in healthy and psoriatic skin [1,5].

In the present study the level of TLR2 gene expression in skin tissue of psoriasis patients was estimated. Also, the serum level of Vitamin D3 was estimated in the sera of 15 psoriatic patients and compared to those of age and gender matched healthy controls. In our study, the levels of TLR2 gene expression in skin of psoriatic patients were found to be higher than its level in normal skin of controls (p<0.001) and this may assume a significant role of TLR2 in the pathogenesis of psoriasis.

Also, Begon et al., [6] compared TLR2 expression in psoriasis patients skin with normal control using immunostaining. They found a strong TLR2 over expression in the epidermis of lesional skin samples as compared to normal control.

Also our results were consistent with those obtained by Wu et al., [19], who found that incubated keratinocytes with mouse anti-K16 monoclonal antibodies showed increased TLR2 protein expression, but without change in the TLR4 protein expression. They suggested that anti-K16 autoantibodies promoted protein expression of TLR2 rather than TLR4 expression. Therefore, the modulation of TLR2 by anti-K16 autoantibodies, rather than TLR4, may be related to the chronic inflammation associated with psoriasis. Based on these findings and our results, TLR2 and TLR4 appeared to play different roles in the development of psoriatic lesions.

The higher levels of TLR2 gene expression observed in our study were in contrast with those obtained by Kim et al., [20] and Seung et al., [21] who found that the expression of TLR2 was lower in psoriatic lesions than healthy controls.

A study by Raby et al., [22] concluded that TLR over activation may lead to end organ damage and serious acute and chronic inflammatory conditions. TLR responses must therefore be tightly regulated to control disease outcomes.

Among 11 members of the TLR family, TLR2 and TLR4 have been identified as signaling receptors activated by bacterial wall components, such as lipopolysaccharide (LPS) from Gram negative bacteria and lipoteichoic acid (LTA) from Gram-positive bacteria. It has been shown that endogenous molecules such as HSP60 and fragmentation products of fibronectin can also trigger an inflammatory response via TLR2 and TLR4 [23]. TLR plays a major role in the initiation of protective immune responses. However, the extensive release of TLR-triggered proinflammatory mediators may harm the host as in cases of sepsis or chronic inflammatory disease [24].

Some studies demonstrate higher constitutive expression of TLR2 and TLR4 in blood monocytes derived from chronic inflammatory disease patients (Rheumatoid arthritis, inflammatory bowel disease) than that from healthy controls [25,26]. As TLRs in monocytes are instrumental in both launching innate immune responses and influencing adaptive immunity, the regulation of TLR expression in chronic inflammatory diseases could be an important therapeutic target for the disease activity [27].
Vitamin D is produced by keratinocytes and regulates keratinocyte differentiation. The biological effects of 1,25(OH)2D3 are mediated through the vitamin D receptor (VDR) [28]. The metabolite, 1,25(OH)2D3, inhibits keratinocyte proliferation and induces terminal differentiation of the keratinocytes. Vitamin D also modulates the immune system in a variety of ways [29-31].

In the present study a significant low level of Vitamin D3 in serum of psoriasis patients compared to normal control (p<0.000) was detected.

This was also suggested by Morimoto et al., [32] who stated that serum vitamin D levels in psoriasis are inversely related to disease severity. Our results were also comparable to those obtained by Kim et al., [20], who found a significant negative correlation between TLR2 and vitamin D receptor expression in psoriatic skin.

However our results differed from those of Milde et al., [31], who reported that VDR expression in eight patients with psoriasis and 10 normal volunteers showed increased expression of VDR in the psoriatic lesional skin. However, they did not evaluate the serum vitamin D levels of patients [33].

VDR has been shown to be upregulated by 1,25-(OH)2D3 in human keratinocytes [34]. The immunomodulatory effect of 1,25(OH)2D3 through down-regulation of the expressions of TLR2 and TLR4 was demonstrated in human monocytes in vitro model [35].

In a study carried by Do et al., [27] on the modulation of TNFα by 1,25(OH)2D3 revealed opposing effects between peripheral monocytes and monocyte cell line: TNFα production was inhibited by 1,25(OH)2D3 in mature peripheral blood monocytes, whereas they increased TNFα production in LPS/LTA-stimulated THP-1 cell line (a human myelomonocytic cell line). As Hakim and Bar-Shavit [36] noticed, the differentiation/maturation status of the cells seems to play a role in determining their response to 1,25(OH)2D3. The hormone increases TNFα production in immature cells (cell lines and bone marrow cells), whereas it decreases the cytokine production in more mature cells (peripheral blood monocytes and peritoneal macrophages).

Information about vitamin D status in patients with psoriasis and the effect of phototherapy on vitamin D status in this group is sparse. Phototherapy is an excellent option for patients with generalized psoriasis because of its superior systemic safety profile in comparison to systemic or biological agents [37]. Low-dose NBUVB treatment gives a significant increase of the vitamin D status in patients with psoriasis, atopic eczema and other skin disorders with low initial levels of 25(OH)D [38].

In our study, after treatment with NBUVB a significant clinical improvement was achieved. This was manifested by the significant decrease in the PAST score of the patients and was associated with significant decrease of the TLR2 gene expression and a significant increase in serum vitamin D level. This proposes a mechanism of action of NBUVB in the treatment of psoriasis through reduction of TLR2 level and increase serum Vitamin D. This mechanism of action was also proposed by Milliken et al., [39] who reported that narrowband UVB increased T-regulatory cell (Treg cell) numbers in human peripheral blood. His result was supported by the demonstration [40] of a relative increase in dermal Fox P31 cells in patients with psoriasis exposed to controlled natural sunlight. In contrast, a study of 9 patients with atopic dermatitis treated with narrowband UV-B failed to show a significant change in Fox P31 cells [41] in lesional skin. This data suggest that the effects of narrowband UV-B on Treg cells were mediated via the restoration of 25(OH)D levels, a view supported by the emerging evidence of the roles of vitamin D in immune function.

Oral supplementation with vitamin D and an analogue alfacalcidol increased Treg cell number in 2 human studies [42,43] and function but not numbers in another, 15 but again the effects we describe may be more prominent due to low baseline levels. Vitamin D can promote a Treg-cell response in vitro [44,45] and immunization of mice through skin pretreated with the analogue calcipotriol led to the generation of Fox P31 antigen-specific Treg cells, mimicking the effect of UV-induced tolerance [46]. Furthermore, knockout experiments indicate that UV-induced tolerance in the mouse is dependent on vitamin D receptor [46]. One question is whether the local production of vitamin D in the skin has more potent, or different, immune effects from dietary supplementation. Formal comparison of the 2 approaches is now required, but there is already evidence of important local properties. Thus, topical 1,25(OH)2D calcitriol and analogues are effective treatment for psoriasis, and priming of epidermal or dermal dendritic cells with vitamin D induces Fox P31 Treg cells or IL-10-secreting Trl regulatory cells, respectively.
In conclusion, UVB improved PASI score, increased serum Vitamin D3 and reduced TLR2 gene expression. A role for the innate immune system in driving the autoimmune T Background cell cascade in psoriasis has been proposed. Toll-like receptors (TLR)-2 and -4 play a role in inflammation, atherosclerosis, and their specific role in psoriasis remains unclear.

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References


