Original Article

Measurement of vitamin D and cathelicidin (LL-37) levels in patients of psoriasis with co-morbidities

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ABSTRACT

Background: During the last decade, a lot of co-morbidities (diabetes, obesity, heart disease, etc.) have been described to be associated with psoriasis, but the exact link at the molecular level is not well-known. Researchers have shown molecular level changes in vitamin D pathway and its relationship to cathelicidin. **Aims:** To estimate the levels of cathelicidin (LL-37), and vitamin D in psoriasis patients with co-morbidities, and compare them with matched healthy controls. **Methods:** One hundred consecutive patients with stable plaque psoriasis (psoriasis area and severity index ≥10) with no systemic treatment in the past 3 months were investigated for the serum levels of vitamin D and LL-37, and compared with equal number of matched healthy volunteers. **Results:** The serum vitamin D levels were significantly lower in patients. Furthermore, the levels of serum LL-37were significantly high. **Conclusion:** Our study showed that the low serum levels of vitamin D, and higher blood levels of cathelicidin could form a molecular level clue in the pathogenesis of psoriasis patients, who are more likely to develop co-morbidities.

Key words: Antimicrobial peptides, cathelicidin (LL-37), psoriasis, serum vitamin D

INTRODUCTION

Psoriasis is a common chronic inflammatory skin disease, with over 2-3% of the world population suffering from psoriasis.[1] Although, the exact pathogenesis of this disease is still not completely understood, it is characterized by dysregulation of cutaneous innate immunity.[2] Various cytokines, chemokines, antimicrobial peptides (AMP) are found in high-levels in psoriatic plaques.[3] Psoriasis is not just one disease, it is turning out to be a syndrome having significant associations with many chronic diseases. Increasingly large numbers of studies have been published, regarding the co-morbidities associated with psoriasis.[4-7] Furthermore, lot of research is going on at molecular level to fight chronic conditions such as heart disease, diabetes, obesity, and metabolic syndrome. And, some of the molecular level changes seen in these conditions have also been observed in patients of psoriasis.[8] Hence, it would be interesting to find a link between psoriasis and co-morbidities at molecular level. Recently vitamin D pathway has been extensively studied, and vitamin D receptors (VDR) have been found to play a significant role in diabetes mellitus, heart disease, malignancies etc.[9] and hence, we decided to study the interplay of vitamin D levels, and AMP cathelicidin (LL-37) in patients with psoriasis associated with co-morbidities, and compared them with age and sex matched healthy controls.

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METHODS

One hundred consecutive eligible patients (71 male and 29 female) of age above 18 years were enrolled in this study. They had at least 10% body surface area affected by stable plaque psoriasis (Group A), having an associated co-morbid condition like diabetes,

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obesity, heart disease, hypertension, and/or metabolic syndrome. These patients were seen in Dermatology Clinic of Farwaniya Hospital between January 2010 and March 2012 [Table 1]. This work was approved by the Ethics Committee of Farwaniya Hospital. Equal number of healthy gender and age matched subjects were also recruited as controls (Group B). All these subjects were included in the study after obtaining informed voluntary consent. They were of Fitzpatrick skin types III to V and, none of them had received any form of systemic therapy for psoriasis or vitamin D supplementation for at least 3 months prior to the study. The psoriasis disease severity was evaluated and was graded according to the area affected by psoriasis area and severity index (PASI) scoring system. The PASI score of all patients was determined by one dermatologist. Data was collected from all the cases, which included, age, sex, gender, weight, height, body mass index (BMI), waist circumference, blood pressure (BP), smoking habit, age at onset of psoriasis, severity of psoriasis, presence and distribution of psoriatic arthropathy and concomitant systemic diseases (co-morbidities). To determine waist circumference, we located the upper hip bone and placed a measuring tape at the level of the uppermost part of the hipbone around the abdomen (ensuring that the tape measure was horizontal).

All investigations including, blood sugar, lipid profile, liver function test, and kidney function test were carried out for all the patients and controls. The reference values used for defining the co-morbidities were as follows: Hypertension (BP ≥140/90 mm of Hg); diabetes mellitus (fasting blood sugar >125 mg%); dyslipidemia (triglyceride ≥150 mg%, and/or low-density lipoprotein cholesterol ≥160 mg%, and/or high-density lipoprotein (HDL) cholesterol<40 mg% in males and <50 mg% in females); obesity (BMI>30 kg/m^2). Metabolic syndrome was diagnosed in the presence of three or more criteria of the National Cholesterol Education Program's Adult treatment Panel III (ATP III): hypertriglyceridemia ≥150 mg/dL (>1.7 mmol/l); HDL cholesterol<40 mg/dL (<1.0 mmol/l) in men or <50 mg/dL (<1.3 mmol/l) in women; BP>135/85 mm of Hg; fasting plasma glucose >100 mg/dL); waist circumference>102 cm in men or >88 cm in women.[10]

Blood samples were taken from all the subjects for estimation of levels of vitamin D and human cathelicidin peptide, LL-37. Vitamin D was measured using competitive enzyme-immunoassay technique with a selected monoclonal antibody recognizing 25-hydroxy (OH) vitamin D. Standards; non-specific binding, controls, and patient samples which were assayed for 25 (OH) vitamin D were incubated after the extraction step with the detection antibody. The pre-incubated solution was then transferred to a microplate coated with 25 (OH) vitamin D. During this incubation step, 25 (OH) vitamin D in the sample and a fixed amount of 25 (OH) vitamin D bound to the microtiter well competed for the binding of the detection antibodies. Then a peroxidase-conjugated anti-mouse antibody was added into each microplate well and a complex of 25(OH) vitamin D-detection antibody–peroxidase conjugate was formed. Tetramethylbenzidine was used as a peroxidase substrate. Finally, an acidic stop solution was added to terminate the reaction.

The cathelicidin, LL-37 was measured in serum by solid phase enzyme linked immunoassay (ELISA) using

Table 1: Demographic characteristics in patients and controls

Baseline characteristic	Psoriasis with co-morbidities	Controls	*P* value
Total number of patients	100	100	
Male/female	71/29	67/33	
Age (years), median	42 years (range: 18-66 years)	39 years (range: 18-64 years)	
BMI (mean±SD)	36.98 ± 5.08	26.17±(3.45)	
Mean duration of psoriasis in years (range)	12.2 (0.3-31.3)	NA	
Smoking, *n* (%)	30 (30)	33 (33)	
Skin type, *n* (%)			
III	42 (42)	44 (44)	
IV	57 (57)	56 (2·5)	
V	1 (1)	0 (2·5)	
Mean LL-37(range)	18.16 (9.1-35.9)	7.92 (0.91-11.2)	<0.001
Serum 25(OH) D_3 mean±SD	31.5 ± 14.41	53.5±19.6	<0.005
Baseline PASI, median, range	7.1 (4.2-15.4)	NA	

BMI: Body mass index, LL: Cathelicidin, 25(OH): 25-hydroxy, PASI: Psoriasis area and severity index, NA: not applicable

a commercial assay with aminimum concentration, which could be measured (limit of detection [LOD] of 0.14 ng/mL), and measurable concentration range of 0.14-100 ng/ml. (Hycult Biotechnology, Uden, The Netherlands).

The generated results were analyzed by SPSS (SPSS 17.0, SPSS Inc., Chicago, IL). Data reported as medians (range, minimum to maximum). Comparison of laboratory parameters between and within the two groups (*t*-tests and ANOVA) was carried out to identify the biochemical inter relationship between the patients and normal controls.

RESULTS

The median age was 42 years (range:18-66 years) in the psoriasis with co-morbidities group, and 39 years (range: 18-64 years) in the healthy control group.The median duration of psoriasis was 12.2 years (range: 0.3-31.3 years). Both the groups were similar in distribution for gender and age. Co-morbid conditions that existed in our patients were hypertension, diabetes, cardiovascular disease, and metabolic syndrome as given in Table 2.

The mean ± SD serum 25(OH) vitamin D concentration was 29·53 nmol/L ± 9·38 nmol/L in patients, and 53.5 nmol/L± 19.6 nmol/L in healthy controls. The difference was statistically significant ($P < 0.0001$). Eighty seven (87%) patients with psoriasis, and 56 (56%) healthy controls had low vitamin D levels (serum levels of 25 [OH] vitamin D < 50 nmol/L). Of these subjects, 12 with psoriasis and nine healthy controls had vitamin D deficiency (serum levels of 25 (OH) vitamin D <25 nmol/L) Table 3.

Table 2: Details of the co-morbidities (refer to definition in methods) in patient group

Co-morbid condition	Number (male/female)
Diabetes	35 (18/17)
Obesity	38 (20/18)
Hypertension	26 (17/9)
Heart disease	12 (8/6)
Metabolic syndrome	23 (15/3)

Table 3: Serum 25 hydroxy vitamin D concentrations in patients and control group

25-hydroxyvitamin D levels	Patients (*n*)	Controls (*n*)	*P* value
<25 nmol/l	12	9	
25-50 nmol/l	87	56	<0.005
>50 nmol/l	1	35	<0.001

Serum LL-37 levels were above the LOD of ELISA kit in all psoriasis samples (sensitivity 0.14 ng/ml). Analysis of cathelicidin levels revealed significantly elevated levels of cathelicidin in psoriasis patients compared with the healthy control group. The median serum LL-37 levels were significantly higher in psoriasis patients versus healthy controls (18.16 ng/mlvs. 7.92 ng/ml). We tested the association of BMI with LL-37 and it showed no statistically significant relationship ($P = 0.18$).

DISCUSSION

A significant proportion of research carried out on psoriasis in the last decade has focused on co-morbidities and other conditions associated with it. Psoriasis has been linked to obesity, diabetes, heart disease, metabolic syndrome, hypertension, and various other conditions, and the list keeps on increasing. While the link has been more or less established beyond doubt, their origin is less known.[11] Various studies have shown that the individuals with severe psoriasis have an increased risk of heart attack, and this risk is independent of other major risk-factors like hypertension, diabetes, obesity, dyslipidemia, and smoking, which is also common in psoriasis.[12] Futhermore, recent studies[13] have suggested that people with severe psoriasis have a 50% higher mortality risk, and they die 3-6 years younger than those without psoriasis. These observational studies although, are helpful in generating a hypotheses, but are limited by the fact that they are unable to generate results that allow researchers to differentiate clearly between association and causality. The chances of observing a causal relation increase when there is a clear biological explanation for the association, the association is confirmed in multiple studies, and there is a dose–response relationship and a clear temporal relationship between exposure and outcome. The possible link between psoriasis and many of the associated diseases may be the presence of chronic inflammation and, in particular, elevated levels of the multifunctional cytokine tumor necrosis factor-α. It has been hypothesized that elevated levels of tumor necrosis factor-α are a biological explanation for few of the observed associations, which have shown chronic inflammation.[14] However, several other factors may play important roles and confound this association.

Recently, vitamin D has been shown to be playing an essential role in many conditions, apart from its well established link in the calcium metabolism and bone health. Recent largest to date study carried out in Denmark[15] showed that low-levels of vitamin D are associated with a markedly higher risk of heart attack and early death. In this study, the authors observed 40% higher risk of ischemic heart disease, 64% higher risk of heart attack, 57% higher risk of early death, and no less than 81% higher risk of death from heart disease even after adjustment for several factors that can influence vitamin D levels, and the risk of disease and death. Similarly, there are studies available in the literature, which have suggested a similar inverse relationship with obesity,[16] diabetes,[17] metabolic syndrome,[18] and hypertension.[19]

On the other hand, studies[8,20] carried out on psoriasis patients have also shown deficiency of serum concentration of 25 (OH) vitamin D in these patients, and the evidence is growing. Furthermore, epidermal keratinocytes produce and secrete AMP that subsequently forms a biological guard on the skin surface. Dysfunction of these peptides has been involved in the pathogenesis of many inflammatory skin diseases.[21] Around 30 cathelicidin members have been identified in mammals, and one cathelicidin, named human cationic antibacterial protein of 18kDa, has been identified in humans. Its mature antibacterial peptide, LL-37, was shown to be expressed by keratinocytes in inflamed skin.[22] The cathelicidin, LL-37 is overexpressed in inflamed skin in psoriasis.[21,22] Recently, the vitamin D pathway was identified as a regulator of cathelicidin expression in man.[23] Cathelicidin expression is directly regulated through vitamin D_3, in which epigenetic changes such as histone acetylation can be activated or coactivators of the vitamin D_3 could be targeted.[24-27] In co-activator pathway 1, 25 D_3 binds with VDR which subsequently heterodimerizes with the retinoid X receptor. This complex enhances the secretion of cathelicidin.[28-32] Schauberand Gallo[27] demonstrated that 1a, 25 $(OH)_2D_3$ directly induced cationic AMP gene expression in a variety of tissue and cell type.The recent identification of the cationic AMP cathelicidin as a vitamin D target gene[25] and of cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1) gene and VDR upregulation in monocytes as the link between toll-like receptor-2 (TLR-2) activation on the one hand and cathelicidin production and intracellular mycobacteria killing on the other hand, a previously unknown and unexpected link between innate immunity and the vitamin D system has been created.[20] Lande *et al*.[33] showed that in patients of psoriasis, cathelicidin (LL-37) initiates an autoimmune response by activating TLR signaling in plasmacytoid dendritic cells (pDCs) of skin. It forms condensed aggregates by binding directly to DNA in pDCs, which are then presented to TLR-9 receptors. These activated pDCs secrete large amount of interferon α to lead to a T cell mediated immune response in psoriatic skin.

Our psoriasis group patients showed reduced level of circulating 25 (OH) vitamin D, with over-expression of cathelicidin LL-37 level, in contrast with the healthy control group. These findings show the existence of inverse relationship between vitamin D and LL-37 in psoriasis patients with associated co-morbidities. The limitation of our study was that it was not a blinded study, and some of the confounding factors like amount of sun exposure, or clothing habits could have an influence on the results.

CONCLUSION

From the results of our study and also keeping in mind the findings of various studies carried out in the past, it seems that vitamin D and not cathelicidin, may be the main factor in the pathogenesis of psoriasis. The levels of AMP cathelicidin, LL-37seems to be controlled by vitamin D. However, much more needs to be carried out to prove this hypothesis. It would be interesting to measure the cathelicidin levels in vitamin D deficient individuals without any chronic illness, associated co-morbid condition, or psoriasis.

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