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Assessment of angiotensin converting enzyme gene polymorphism in patients with psoriasis

Nagwa I. Ashry[1], Fadia M. Attia[2], Amal H. Ahmed[1], Hesham A. Nada[1] and Ahmed I. Maaty[3]

[1]Dermatology, Venerology & Andrology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.
[2]Clinical Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.
[3]Physical Medicine, Rheumatology, & Rehabilitation, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

Corresponding author: Ahmed I. El-Sayed Maaty, Physical Medicine, Rheumatology & Rehabilitation Department, Faculty of Medicine, Suez Canal University, Ismailia, Ismailia Campus, Ring Road, Post-office No. 411522, Egypt.
Email: ahmed_maaty@med.suez.edu.eg.

Abstract

Psoriatic patients had diversity of clinical presentations and complications. Psoriasis can have significant interference with the patient's quality of life, recovery, and outcome. Some evidences suggest that the angiotensin converting enzyme (ACE) is present in the skin of psoriatic patients. This study intended to assess the patterns of ACE insertion/deletion (ACE ID) polymorphism and the levels of serum ACE among psoriatic patients in comparison to normal controls. The study included two groups: 20 patients with psoriasis and 20 apparently healthy adults with negative family history of psoriasis as a control group. Psoriasis area and severity index (PASI) was used to measure of severity of psoriasis. In both groups, ACE ID gene polymorphism was assessed by quantitative real-time polymerase reaction and serum ACE levels was evaluated using an enzyme-linked immunosorbent assay. ACE ID genotype was significantly higher among the psoriatic group in comparison to the control group (40.0% versus 15.0%, respectively, p=0.016). D allele was significantly higher among the psoriatic group than the control group (25.0% versus 7.5%, respectively, p=0.034). ACE ID genotype carried significantly higher risk in psoriatic group versus control group (OR=3.8). The D allele carried higher risk in psoriatic group versus control group (OR=4.1). ACE serum levels were significantly higher among the psoriatic group compared to the control group (87.4±7.03 versus 2.3±0.7, respectively; p<0.001). We concluded that ACE ID gene polymorphism may be considered as a risk factor for developing psoriasis.

Keywords: ACE polymorphism, psoriasis, ACE serum levels

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Introduction

Psoriasis is a chronic, common, multifactorial, inflammatory skin disorder typically characterized by erythematous papules and plaques with a silver scale, although other presentations occur. It involves hyperproliferation of the keratinocytes in the epidermis, with an increase in the epidermal cell turnover rate.[1] Approximately 2-3% of people are affected by psoriasis worldwide during the last decade.[2] Psoriasis can begin at any age.

Nearly 10-15% of new cases begin in children younger than 10 years. The median age at onset is 28 years. Psoriasis appears to be slightly more prevalent among women than among men; however, men are thought to be more likely to experience the ocular disease.[3] The severity cases ranged from mild form that treated in the outpatient setting to life-threatening condition that required intensive inpatient management. Psoriasis can have a significant effect on quality of life.[1] The disease most commonly manifests on the skin of the elbows, knees, scalp, lumbosacral areas, intergluteal clefts, and glans penis. Mease et al., 2013[4] found that psoriatic arthritis (PsA) affects 30% of patients with psoriasis in 7 European and North American countries. Environmental, genetic, and immunologic factors appear to play a role in pathogenesis of psoriasis and PsA.[5]

Angiotensin has also been associated with a pro-inflammatory effect. The angiotensin-converting enzyme (ACE) insertion/deletion (ID) gene polymorphism has been associated with several inflammatory disorders. The ACE D allele carries with it an increased risk of hypertension, pre-eclampsia, coronary artery disease, diabetic nephropathy, and type 2 diabetes mellitus.[6-10] On the positive side, the D allele appears to offer protection against schizophrenia and chronic periodontitis and confers greater upper-body strength in old age.[11]

Some evidence suggest that the ACE is present in skin.[12] The real value of the determination of ACE activity as a clinical-biochemistry test for the diagnosis of psoriasis has not been attained.[12] There are major debates about the association between ACE and psoriasis/PsA. In one direction, Huskić et al., 2008[12] found that determination of tissue ACE activity can be used in the differential diagnostic of indistinct clinical forms of psoriasis. Al-Awadhi et al., 2007[13] investigated the frequency of ACE gene ID polymorphism in adults with PsA. The frequency of ACE genotype was significantly higher in patients with late disease onset than in those with early onset. In the opposite direction, Song et al., 2015[14] performed a meta-analysis and demonstrated that the ACE ID polymorphism is not associated with susceptibility to psoriasis in Europeans. In the same line, Al-Awadhi et al., 2007[13] did not find a significant difference between the distribution of the ACE genotype in PsA patients and the general population in Kuwait.

In our daily practice, we encounter many psoriatic patients with different clinical presentations and complications. Psoriasis can have significant interference with the patient's quality of life, recovery, and outcome. Therefore, this study was done to determine the association between ACE ID polymorphism and psoriasis.

Patients and Methods

Participants

This study was conducted as a case-control study to determine the patterns of ACE gene polymorphism and serum ACE levels in psoriatic patients and normal controls in Ismailia, Egypt. This study was carried out at the Dermatology, Venerology and Andrology Department, Suez Canal University Hospital. The studied population included 20 patients with plaque psoriasis (psoriatic group) aged at least 18 years of both genders. The patients with other dermatological diseases, autoimmune disorders, or receiving ACE inhibitors were excluded. We also recruited 20 adults who were age and gender matched to the psoriatic group with no clinical evidence or family history of psoriasis as a control group. They were enrolled from Suez Canal University Hospital Staffs (employees, secretaries, and workers).

Ethical consideration

The study protocol was reviewed and approved by the Ethical Committee at the Faculty of Medicine, Suez Canal University (March 2017). An informed consent was obtained from each participant (patients and controls).

Methods

All participants were subjected to full medical history, general examinations to exclude any other general or chronic disease that may affects the results. Dermatological examinations were performed to all patients to determine the site, distribution, number, and approximate surface area of the lesions.

Psoriasis Area and Severity Index (PASI)

PASI is a valid and reliable tool for the measurement of severity of psoriasis. PASI combines the assessment of the severity of lesions and the area affected into a single score in the range 0 (no disease) to 72 (maximal disease). The body is divided into four sections (head (H) (10% of a person's skin); arms (A) (20%); trunk (T) (30%); legs (L) (40%)). Each of these areas is scored by itself, and then the four scores are combined into the final PASI. For each section, the percent of area of skin involved, is estimated, and then transformed into a grade from 0 to 6, where, grade 0: 0% of involved area, grade 1: <10% of involved area, grade 2: 10–29% of involved area, grade 3: 30–49% of involved area, grade 4: 50–69% of involved area, grade 5: 70–89% of involved area, and grade 6: 90–100% of involved area. Within each area, the severity was estimated by three clinical signs: erythema (redness), induration (thickness) and desquamation (scaling). Severity parameters were measured on a scale of 0 to 4, from none to maximum. The sum of all three severity parameters was then calculated for each section of skin, multiplied by the area score for that area and multiplied by weight of respective section (0.1 for head, 0.2 for arms, 0.3 for body and 0.4 for legs). A PASI score of ≤10 suggests mild psoriasis, 10-20 moderate psoriasis and >20 severe psoriasis.[15]

ACE ID genotyping determination

Genomic DNA was isolated from peripheral blood, and quantitative real-time (qRT) PCR was performed for the ACE gene ID allele. The amplification mixtures, at a final volume of 10 µl, included 25 ng genomic DNA, 5 µl 2×Maxima SYBR Green qRT-PCR Master Mix (Fermentas Life Sciences, Burlington Ontario, Canada), and forward (F) and reverse (R) primers at a final concentration of 100 nM. The following primer sequences (5'-3'), synthesized by Eurofins MWG Operon (Huntsville, Alabama, USA), were used: ACE1F, CATCCTTTCTCCCATTTCTCT'; ACE2InsF, TGGGATTACAGGCGTGATACA; and ACE3R, 'GCTGGAATAAAATTGGCGAA. The qRT-PCRs were performed using an ABI 7500 qRT-PCR System (Applied Biosystems, Foster City, California, USA). The cycle conditions included incubation at 50°C for 2 min, initial denaturation at 95°C for 10 min, and 40 cycles at 95°C for 15 s. After the amplification was completed, a melting curve analysis was performed by cooling the reaction to 60°C and then heating slowly to 95°C (Applied Biosystems). The melting curve analyses were performed to assay the ACE ID polymorphism. In addition, the amplified PCR product was separated on 2% agarose gel electrophoresis and visualized using UV light and ethidium bromide staining. PCR products of 57 base pairs (bp) and 75 bp, corresponding to the two melting peaks at 72.9°C and 74°C, represented the I and D alleles, respectively.[16] A set of PCR products at 57 bp and 363 bp for the two melting peaks at 72.9°C and 86.2°C indicated the I allele, and a PCR product at 75 bp for a melting peak at 74°C indicated the D allele.

Serum ACE levels

Serum ACE levels was evaluated using enzyme-linked immunosorbent assay (ELISA) using an ACE kit (Shanghai Koran Biotech Co., Ltd., China), according to the manufacturer's instructions. The assay range is 1-300 ng/ml and, its sensitivity up to 0.53 ng/ml. Normal adults' levels of ACE level are less than 40 microgram/Liter (µg/L).

Statistical analysis

Data collected throughout history, basic clinical examination and laboratory investigations was coded, entered, and analyzed using Microsoft Excel software. Statistical analysis was performed by using Statistical Package of Social Science (SPSS) software, version 20.0 (IBM SPSS, Inc., Chicago, Illinois). Results were expressed as the mean ± standard deviation for quantitative variables or frequency and percentages for qualitative variables. Testing difference between means was carried out by t-test. Analysis of allele frequencies (number of copies of a specific allele divided by the total number of alleles in the group) and carriage rates (number of individuals with at least one copy of allele D divided by the total number of individuals within the group) was carried out using Chi-squared test for testing the

differences in patterns of ACE ID polymorphism between psoriatic patients and control adults. A *P* value <0.05 was considered as statistical significance.

Results

The age and gender of the psoriatic group was successfully matched to the control group. There was no significant difference between the mean age of the psoriatic and control groups (37.3±14.7 years and 39.1±7.6 years, respectively) (*P*>0.05). There was no significant difference in the female/male ratio between the psoriatic and control groups (female/male= 45.0%/55.0% and 55.0%/45.0%, respectively; *P*>0.05). Regarding disease severity in psoriatic group, according to PASI about 50.0% of the psoriatic patients had mild disease, 10.0% moderate disease, and 40.0% severe disease. ACE serum levels were significantly higher among the psoriatic group compared to the control group (87.4±7.03 µg/dl versus 2.3±0.7 µg/dl, respectively; *P*<0.05) (Table 1).

Table 1. Demographic, clinical and laboratory findings of both studied groups.

Variables	Psoriasis group (n=20)	Control group (n=20)	*P*-value
Age (years)			
Mean ±SD	37.3±14.7	39.1±7.6	NS*
Range	9-60	26-55	
Gender, n (%)			
Male	11 (55.0%)	9 (45.0%)	NS$
Female	9 (45.0%)	11 (55.0%)	
PASI			
Mild	10 (50.0%)	-	
Moderate	2 (10.0%)	-	-
Severe	8 (40.0%)	-	
ACE levels (µg/dl)			
Mean ±SD	87.4±7.03	2.3±0.7	
Range	40-300	1.4-3.9	0.0001*

SD=standard deviation, , PASI=Psoriasis area and severity index, ACE=Angiotensin converting enzyme, **P* value>0.05 is not significant (NS). *t= t-test, $ χ^2= Pearson chi-square test.

ACE ID genotypes were significantly higher among the psoriatic group in comparison to the control group (40.0% versus 15.0%, respectively, *P*=0.016).). The II genotypes were significantly higher among the control group in comparison to the psoriatic group (85.0% versus 55.0%, respectively; *P*<0.05). The D allele was significantly higher among the psoriatic group compared to the control group (25.0% versus 7.5%, respectively; *P*<0.05) (Table 2).

Table 2. Angiotensin converting enzyme (ACE) genotypes and allele frequencies in both studied groups.

Variables	Psoriasis group (n=20) No. (%)	Control group (n=20) No. (%)	*P*-value
Genotype frequencies			
DD	1 (5.0%)	0 (0.0%)	
ID	8 (40.0%)	3 (15.0%)	0.016*
II	11 (55.0%)	17 (85.0%)	
Allele frequencies			
D	10 (25.0%)	3 (7.5%)	0.034*
I	30 (75.0%)	37 (92.5%)	

*Significant p-value <0.05, *χ^2=Pearson Chi-square test.*

Table (3) shows that ID genotypes carry significantly higher risk in psoriatic group versus the control group (OR=3.8; *P*<0.05), indicating approximately 4-folds risk for developing psoriasis. II genotypes showed protective effect in control group compared to the psoriatic group (OR=0.22; *P*<0.05). The D allele carried higher risk (4-folds risk) for developing psoriasis compared to the control group (OR=4.1; *P*<0.05), as shown in Table 4. The DD genotype was significantly higher among male patients in comparison to female patients (9.1% versus 0.0%, respectively; *P*<0.05).

Table 3. Potential risk of ACE genotypes and allele frequencies.

Variables	OR	95%CI	*P*-value
Genotype frequencies			
DD	-	-	-
ID	3.8	0.8-17.3	0.049*
II	0.22	0.05-0.10	0.038*
Allele frequencies			
D/ I	4.1	1.0-16.2	0.034*

Significant *P*-value <0.05, *χ^2= Pearson Chi-square test, OR=Odds ratio, CI=Confidence interval, ACE=Angiotensin converting enzyme.

Table 4. Association between ACE genotype frequencies, alleles, and gender.

Variables	Male (n=11) No. (%)	Female (n=9) No. (%)	*P*-value
Genotype frequencies			
DD	1 (9.1%)	0 (0.0%)	0.0032*
ID	4 (46.4%)	4 (44.4%)	NS
II	6 (54.5%)	5 (55.6%)	NS
Genotype alleles			
D	6 (27.3%)	4 (22.2%)	NS
I	16 (72.7%)	14 (77.8%)	

P value>0.05 is not significant (NS)., *χ^2= Pearson Chi-square test, ACE=Angiotensin converting enzyme.*

Discussion

Our study indicated that ID genotypes were significantly higher among the psoriatic group in comparison to the control group (P<0.05). D allele was significantly higher among the psoriatic group compared to the control group (P<0.05). Also, ID genotypes and D allele carried significantly higher risk for developing psoriasis compared to the control group (P<0.05). Likewise, Ahmetov et al., (2013),[17] suggested that approximately 50% of the inter-individual variability of plasma ACE is attributable to ID polymorphism. Plasma and tissue ACE concentrations were found to be related to the D allele of ID polymorphism, with DD genotypes having the highest and II genotypes having the lowest ACE activity.

In a meta-analysis study, Song et al., (2015),[14] reported combined evidence on the associations between the ACE ID polymorphism and susceptibility to psoriasis. The meta-analysis showed significant associations of the DD+ID genotype with psoriasis (OR 0.753, 95% CI 0.601–0.921, P = 0.006). The study also demonstrates that the ACE ID polymorphism is associated with susceptibility to rheumatoid arthritis, especially in Arab population. In contrast to these findings, Domínguez-Vías et al., (2016),[18] investigated the association between ACE polymorphisms and susceptibility to psoriasis in white and Asian populations, but the results were inconsistent. In addition, Burden, and Kirby (2016)[19] emphasized that ACE II carriers have a higher risk for psoriasis than ID and DD carriers in Turkish populations.

Our study observed a significant association between ACE genotype frequencies in psoriatic group and gender. DD genotype was significantly higher among male patients in comparison to female patients (P<0.05). Interestingly, Munir et al. (2016),[20] reported that genotype II and allele I frequencies were significantly higher in male Pakistani patients with psoriasis than in the male control group, with no such association in the female group. These gender-based differences suggested that there may be differences in the renin–angiotensin system of men and women, and that the mechanism might involve the role of sex hormones.[21] Overall differences in our results compared with some of the previous studies may be due to several factors, such as racial/ geographical differences, the number of male and female patients under study and the genetic heterogeneity and multifactorial etiology of psoriasis.[13]

Our research showed that ACE serum levels were significantly higher among the psoriatic group compared to the control group (P<0.001). In the same line, Huskić et al. (2008),[12] reported elevated serum activity of ACE in psoriasis.

In conclusion, our findings suggested that ACE ID gene polymorphism may be considered as a risk of developing psoriasis.

Author Contributions

NIA, AHA, and HAN designed and conducted the study, including participants' recruitment and data collection. NIA and FMA conceived and planned the interventions and investigations. AIM performed data management and statistical analysis. NIA and AIM contributed to interpretation of data. NIA and AIM prepared the manuscript draft with important intellectual input from AHA, FMA, and HAN. All authors revised and approved the final manuscript. NIA and AIM had complete access to the study data.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Ethical Committee at the Faculty of Medicine, Suez Canal University (March 2017).

Informed consent

An informed consent was obtained from each participant (patients and controls).

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