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Detection of Cyclophilin D in Psoriasis and its Correlation with Disease Severity

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Abstract:

The aim of the work to detect the level of Cyclophilin-D in psoriasis and its correlation with disease severity. Samples included 84 persons divided into 2 groups (42) patients having psoriasis and (42) apparently healthy age and sex-matched individuals. All individuals included in the study were subjected to full history, full clinical examination, 3 cm of blood had been taken from patients of psoriasis and matched controls and centrifuged for serum separation which kept frozen at -80c till analysis of Cyclophilin-D by ELISA technique.

Keywords: Cyclophilin D, Psoriasis

1. Introduction:

Psoriasis is a complex, chronic, multifactorial, inflammatory disease that involves hyperproliferation of the keratinocytes in the epidermis, with an increase in the epidermal cell turnover rate.

Environmental, genetic and immunologic factors appear to play a role. The disease most commonly manifests on the skin of the elbows, knees, scalp, lumbosacral areas, intergluteal clefts and glans penis [1]

Psoriasis has a tendency to wax and wane with flares related to systemic or environmental factors, including life stress events and infection. It impacts quality of life and potentially long-term survival. There should be a higher clinical suspicion for depression in the patient with psoriasis [2]

Psoriasis is a long-lasting autoimmune disease characterized by patches of abnormal skin [3] These skin patches are typically red, dry, itchy, and scaly. On people with darker skin the patches may be purple in color [4]

PASI score is a tool used to measure the severity and extent of psoriasis (Psoriasis Area and Severity Index). It takes a few minutes and experience to calculate it accurately [5] Cyclophilins are a family of proteins that bind to cyclosporine. These proteins have peptidyl prolyl isomerase activity, which catalyzes the isomerization of peptide bonds from *trans* form to *cis* form at proline residues and facilitates protein folding [6]

Cyclophilin D is a unique isoform that is imported into the mitochondrial matrix. Mitochondria have an important role in regulating both apoptotic and necrotic cell death through the formation of the permeability transition pore. The opening of this pore is regulated by the mitochondrial protein Cyclophilin-D [7]

Cyclophilin-D, which is located in the matrix of mitochondria, is only a modulatory, but may or may not be a structural component of the mitochondrial permeability transition pore. The pore opening raises the permeability of the mitochondrial inner membrane, allows influx of cytosolic molecules into the mitochondrial matrix, increases the matrix volume and disrupts the mitochondrial outer membrane. As a result, the mitochondria fall into a functional disorder, so the opening of the pore plays an important role in cell death. Cyclophilin D is thought to regulate the opening of the pore because cyclosporine A, which binds to CyP-D, inhibits the pore opening [8] Overexpression of Cyclophilin has been linked to inflammatory diseases [9] Cyclophilin inhibitors, such as Cyclosporin, are being developed to treat neurodegenerative diseases [10]

2. Patients And Methods:

This study was a case control study conducted at dermatology department in Beni-Suef University hospital. Population of the study was enrolled from (October 2018 to February 2019) at the outpatient clinic in

the hospital according to the following inclusion and exclusion criteria:

2.1 Inclusion criteria:

1. All psoriatic patients regardless of their age and sex.
2. Healthy control group will be age and sex matched with our patient. **Exclusion criteria**:

1.Patient suffering from cutaneous tumors.

2.Patients with other autoimmune diseases.

Sample size:

The present study included 84 persons divided into 2 groups as the following:

Group 1: Comprising 42 patients having psoriasis. Diagnosis of psoriasis was confirmed based on clinical signs and symptoms of the disease. Cases were recruited from outpatient clinic of the dermatology department of Beni-suef University Hospital.

Group 2: Comprising 42 apparently healthy age and sex-matched individuals as controls, giving no personal or family history of psoriasis. Control subjects were recruited from among medical students, health care personnel and patients presenting at the dermatological outpatient clinic. They were selected not to have any autoimmune illness and on no interfering medications.

2.2 All individuals included in the study were subjected to the following:

1. Informed Consent.
2. Full history and full clinical examination.
3. Psoriatic patients had been classified according to severity via PASI score.
4. Three cm of blood had been taken from patients of psoriasis and matched controls.
5. Blood sample had been centrifuged for serum separation which kept frozen at -80c till analysis of Cyclophilin D by ELISA technique.

Statistical methodology:

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 25. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non parametric Mann-Whitney test .

3. Results:

This study was a case control study on forty-two patients with Psoriasis and forty-two apparently healthy age and sex- matched individuals as controls. The average patient age was 46.13 ±13.9 (SD) years. Studied Psoriasis cases were distributed as 31% of them were females and 69% were males.

Table (1): Sex Distribution of the Studied Population; (N= 84):

		N (%)		TOTAL	p-value
		Cases N= 42	Controls N= 42		
Sex	Male	29 (69.0)	26 (61.9)	55 (65.5)	0.323
	Female	13 (31.0)	16 (38.1)	29 (34.5)	

Table (2): Age Distribution of the Studied Population; (N= 84):

-97wqa	Mean ±SD	Minimum	Maximum	Range	p-value
Cases	46.13 ±13.9	18	75	14	0.579
Controls	47.70 ±6.7	32	58	26	

As illustrated in table (3); the Psoriasis Areas and Severity Index (PASI) was range from (0.9) to (37) with a mean of 9.34 ±7.4.

Table (3): Psoriasis Areas and Severity Index among the Studied Psoriasis Patients; (N= 42):

PASI	
Mean ±SD	9.34 ±7.4
Range	0.90
Minimum	36.10
Maximum	37.00

Table (4) illustrates the disease course among studied psoriasis patients where it was in the form of Remission & Exacerbation in (52.4%) of cases and (23.8%) were with progressive course.

Table (4): Disease Course among Studied Psoriasis Patients; (N= 42):

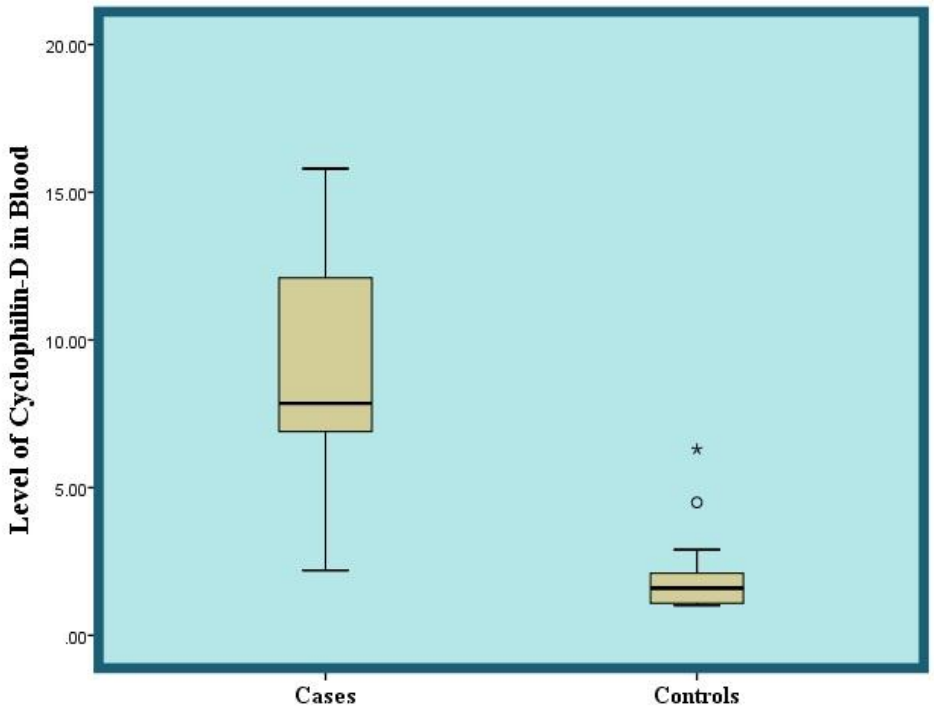
Disease Course	N	(%)
Stationary	7	16.7
Regressive	3	7.1
Remission &Exacerbation	22	52.4
Progressive	10	23.8

Table (5) demonstrates that the level of Cyclophilin-D in blood of psoriasis patients was significantly higher than in healthy controls (p-value =0.001); where the mean levels were (8.39 vs. 1.58) in cases and controls respectively.

Table (5): Comparison of the level of Cyclophilin-D in blood of psoriasis cases and in Controls:

	Cases N= 42	Controls N= 42	*p-value*
Mean ±SD	8.39 ±4.2	1.85 ±1.1	0.001*
Range	13.60	5.29	
Minimum	2.20	1.01	
Maximum	15.80	6.30	

**** P-value ≤ 0.05 is considered significant by (Mann–Whitney U test).***



Comparison of the level of Cyclophilin-D in blood of psoriasis cases and in Controls

Pairwise comparison to detect which pair of means had a statistically significant difference reveled that; patients with Remission &Exacerbation course had significantly higher level as compared with stationary course (p-value= 0.026), also patients with Progressive course had significantly higher level as compared with stationary course (p-value= 0.007), no statistically significant differences detected regarding other subgroups.

Table (6): Relation between the level of Cyclophilin-D in blood of psoriasis cases and Disease Course; (N= 42):

	Mean ±SD	*P-value*
Stationary	4.60 ±2.8	0.042*[1]
Regressive	9.77 ±1.6	
Remission &Exacerbation	8.59 ±4.7	0.026*[2]
Progressive	10.21 ±2.8	0.007*[3]

p-value ≤ 0.05 is considered significant by Non-Parametric Kruskal Wallis Test.

[1]Overall p-value,

[2]Statisticall difference between Stationary and Remission &Exacerbation,

[3]Statisticall difference between Stationary and Progressive,

4. Discussion:

Psoriasis represents a significant public heath challenge, affecting approximately 125 million people globally [11]

It is an immune-mediated, chronic inflammatory disease of genetic basis, which affects mainly the skin, although it has systemic pathological effects. Psoriasis exists on a fairly broad spectrum in terms of clinical manifestations [12]

Of all the psoriasis studies that have been performed, genetic studies published in the last two decades have arguably provided the most robust insights into the critical pathogenic mechanisms in psoriasis. These have demonstrated that psoriasis is a complex genetic disorder involving multiple risk variants involved in various biological processes such as inflammation (cytokine and cytokine receptors), antigen presentation, epidermal biology, cell signaling, and transcriptional regulation [13] In normal skin the ratio of proliferating to

non-proliferating keratinocytes, is around 60% [14] whereas in psoriasis it is almost 100%, and the mean cell cycle time is reduced from 311 to 36 h in psoriatic lesions [15]

It has been suggested that this keratinocyte hyper-proliferation is not restricted to the basal cell compartment containing the stem cells, but may also involve supra-basal cells [16] Apoptosis is a process of programmed cell death that maintains homeostasis of the skin [17] Apoptotic cell death regulates keratinocyte proliferation and formation of stratum corneum [18] Dysfunctional apoptosis has an important role in the development of several skin diseases including psoriasis. Psoriatic keratinocytes possess an enhanced ability to resist apoptosis, which might be one of the key pathogenetic mechanisms in psoriasis [18] Cyclophilins have been identified as cellular binding proteins for the immunosuppressive drug cyclosporin A (CsA) [20] and are constitutively expressed in most tissues [21] Cyclophilins have peptidyl prolyl cis-trans isomerase (PPIase) activity, which catalyzes protein folding in cells. Several classical isoforms of cyclophilins including CypA, CypB, CypC and CypD (also known as PPIA, PPIB, PPIC and PPID, respectively) have been identified and found to reside in their distinctive cellular locales, seemingly providing the compartment-specific functions. CypD is an integral component of the

mitochondrial permeability transition complex, and plays a crucial role in apoptosis [22] CypA is the most abundant CsA binding cytosolic protein. CypC localizes both in the cytosol and ER lumen, whereas CypB is detected mainly in the ER lumen [23]Although there has been some speculation that CypB plays a role in protein folding in the ER, there is currently no direct information supporting the notion that it provides a significant role in response to ER stress. In the present study, we have demonstrated that CypB performs vital functions to protect cells against ER stress [24] The mitochondrial permeability transition pore (mPTP) is involved in the mitochondrial pathway of apoptosis [25] Cyclophilin D, a pore component, has catalytic activity as a peptidyl prolyl cis, trans-isomerase (PPIase), which is essential to the pore opening. It has been reported that cyclophilin D overexpression suppresses apoptosis in many cells [26]

We performed this study to detect the level of Cyclophilin D in blood of 40 patients with psoriasis and comparing it to 40 normal control persons to detect the possible role of Cyclophilin D in the pathogenesis of the psoriasis disease and correlate it with disease severity.

This present case-control study included sixty individuals from dermatology outpatient clinic at Beni-Suef University hospital during the period (from October 2018 to March 2019). The individuals were divided into two groups matched in age and sex distribution as 40 individuals in each group; the average Psoriasis patients' age was 46.13 ±13.9 (SD) ranging from 18 to 75 years with no statistically significant difference between cases and control groups.

In this study; psoriasis disease was evaluated by the Psoriasis Areas and Severity Index (PASI), onset of psoriasis and course of the disease. Majority of the studied psoriatic cases had negative family history of the disease which was different than many other studies in this regard where positive family history is reported in psoriasis disease [27] Exposure to cold was the most frequent predisposing factor among the studied population where nearly half patients reported it, followed by exposure to stress.

Regarding the serum levels of Cyclophilin-D in blood of studied individuals It turns out that psoriasis patients had significantly higher level of CyP-D as compared with healthy controls

(p-value =0.001); where the mean levels were (8.39 vs. 1.58) in cases and controls respectively. The level of Cyclophilin-D in blood of psoriasis cases was significantly lowest in patients with stationary disease course as compared with other disease courses. Patients with progressive course had significantly highest CyP-D level followed by Remission &Exacerbation course.

To compare the results of this study with previous studies, we found an old study comparing cyclophilin content of normal and psoriatic epidermis; where they reported no statistically significant difference in the cyclophilin content of epidermal samples of psoriatic and normal skin [28] however this study was different than ours where we examined the cyclophilin in blood sample.

There are many previous studies that have indicated that the CyP-D is associated with pathology of many other diseases; however we could not find many of the published research on CyP-D and its relation to psoriasis skin disorder and this was among the limitations of this study. There are still a lot of gaps in the knowledge base that need to be filled and therefore irrespective of the precise underlying mechanism of action, our data underscore the difference of CyP-D in blood of psoriatic and non-psoriatic patients; based on these results, the next steps would be to build a stronger overall evidence base.

5. Conclusion:

Our findings reveled that Cyclophilin-D in blood of psoriasis patients was significantly higher than in healthy controls.

Further studies are warranted to better understand the molecular mechanisms by which Cyclophilin-D can play a role in pathogenesis of psoriasis diseases.

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