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Original article

Estimation of the Serum Level of Poly Adenosine Diphosphate Ribose Polymerase-1 in Psoriasis and its Correlation with Disease Severity

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Abstract

Background: Psoriasis is a complicated inflammatory condition of chronic nature with several etiologies. In addition to an increase in the turnover rate of epidermal cells, psoriasis is characterized by hyperproliferation of keratinocytes in the epidermis. **Objective:** The objective of the current study was to determine the level of PARP-1 in psoriasis patients compared to healthy controls and its relationship to the severity of the disease. Blood samples from 40 patients with fully developed psoriasis were collected, and each patient's PASI (Psoriasis Areas and Severity Index) score was determined. **Patients and methods:** Blood samples from 40 healthy volunteers who had no history of psoriasis were also collected, and those blood samples were analyzed using ELISA (enzyme-linked immunosorbent assay). **Results:** This study provides that the serum level of poly ADP ribose polymerase-1 was significantly higher in patients compared with the controls with no correlation to age, sex or family history (P-value = 0.020). Additionally, the research found no association between the PASI score and the blood level of PARP-1 in psoriatic individuals (r = 0.156, p-value = 0.337).

Conclusion: We came to the conclusion that PARP-1 could be involved in the pathogenesis of psoriasis and PARP inhibitors might be an effective adjunct to current anti-psoriatic therapies.

1. Introduction:

Psoriasis is a skin disease that develops following chronic inflammatory signaling and keratinocyte hyperproliferation and according to recent estimates, 30-65 million individuals worldwide have psoriasis with substantial differences across ethnicities [1]. Common classifications of psoriasis include age of occurrence, severity, and anatomical site (e.g., nail, scalp, genital). Additionally, the condition may be divided into a number of clinical subtypes, such as plaque, guttate, erythrodermic, and pustular psoriasis, Scaly plaques that mostly affect the scalp, knees, and elbows define plaque psoriasis. Given that it makes about 90% of cases, chronic plaque psoriasis is the phenotype that has been investigated the most [2]. Psoriasis is a condition that often co-occurs with other medical conditions such as gastrointestinal problems, autoimmune illnesses and metabolic disorders [3]. Given that it impairs the quality of life, it is strongly linked to psychiatric problems. The disease's underlying mechanisms haven't been thoroughly investigated. The variables that cause illness include immunological processes, environmental factors, hereditary factors and the development of new blood vessels [4]. Adenosine diphosphate - ribosyl transferases (ARTs), often referred to poly (ADP-ribose) polymerases (PARPs), are a class of proteins that are essential to several biological processes. Using nicotinamide adenine dinucleotide (NAD+) as a donor, the PARP superfamily catalyzes either mono-ADP-ribose (MAR) or poly ADP-ribose (PAR) to target proteins; these reactions are also known as MARylation or PARylation, respectively [5]. A rising corpus of research over the last ten years has shown that PARP1 may be crucial in controlling the expression of genes linked to the illness (such as chemokines, pro-inflammatory mediators and metabolic related factors) [6]. Numerous studies have been done on the relationship between inflammation and PARP activity. The expression of proinflammatory cytokines, proinflammatory enzymes and other proinflammatory gene products is regulated by PARP1 [7]. Being a transcriptional coactivator/corepressor is one of PARP1's key roles in the control of the inflammatory process. Through the regulation of significant immune-related transcriptional factors such as NF-kB (Nuclear factor kappa-light-chain-enhancer of activated B cells) or p53, PARP1 modifies the inflammatory response [8]. Several publications have well reviewed the potential of PARP1 to auto-modify itself or PARylate histones and other chromatin-associated proteins to change the chromatin structure, resulting to the fine-tuning of gene expression under circumstances

of genotoxic stress signaling [9]. The repair of damaged DNA and maintenance of genomic stability are caused by mild genomic stress, which activates PARP1. The excessive PARylation that results from severe DNA damage (such as traumatic brain injury and ischemia/reperfusion injury) causes cell death in numerous tissues. Parthanatos is the name for caspase-independent, PARP1-dependent cell death. Parthanatos has been linked to a number of illnesses, including diabetes, Parkinson's disease, heart disease, and stroke [10] It is distinct from other cell death processes like apoptosis, necroptosis, or autophagic cell death. The excessive activation of PARP-1 that results in an increase in PAR polymers, which serve as a signal for cell death, is the underlying molecular process of parthanatos. Macrophage migration inhibitory factor (MIF) attaches to AIF when PAR connects to it, which induces AIF to move from the mitochondria into the cytosol. Once there, the AIF/MIF complex penetrates the nucleus, where MIF causes extensive DNA breakage and chromatin condensation [11].

2. Patients and Methods:

2.1. Study type and sampling:

This prospective case control study was conducted in the period between March 2021 and January 2022. The study included 80 participants divided into two groups, the case group included 40 cases diagnosed with psoriasis, and the control group included 40 healthy persons with matched age and gender.

Inclusion criteria: All psoriatic patients regardless of their age and sex. Apparently healthy control group will be age and sex matched with our patients.

Exclusion criteria: Patients receiving phototherapy, patients suffering from cutaneous tumors, patients using drugs that interfere with the parameter to be estimated (for example: PARP-1 inhibitors as olaparib and veliparib) and patients with other autoimmune diseases.

The sample size was calculated using G.Power program *(t tests - Means: Difference between two independent means (two groups) The following criteria were set; Effect size was 0.64 with a confidence level 95% ,margin error 5% , allocation ratio was 1 to 1 and an actual power was 80% the estimated study participants were 40 subjects at each group.*

2.2. Data Collection Methods:

Patients as well as healthy controls were subjected to: Complete history taking, Complete general and dermatological examination. Psoriatic patients were categorized based on severity via PASI score: the 3 characters of psoriatic plaques (redness, scaling and thickness) are given a number from (0 - 4), 4 being the worst score. The degree of affection of every area of the body is scored from (0 - 6), the scores give a range from (0 - 72), if the score <10, it indicates mild disease,

while >10 indicates moderate to severe affection.

Sample collection and storage:

Under all aseptic precautions, blood samples (3 ml blood) were drawn from peripheral veins and collected in tripotassium (K3) EDTA tubes. Samples were then subjected for centrifugation, serum was separated and kept frozen at -20°C till analysis of PARP 1 by ELISA (enzyme-linked immunosorbent assay) technique. We used a double-antibody sandwich enzyme-linked immunosorbent assay. Capture antibody was pre-coated onto 96 well plates and the biotin conjugated antibody was used as a detection antibody. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently and washed with a wash buffer. HRP-Streptavidin (Horseradish peroxidase) was added to form an immune complex and unbound conjugates were washed away with a wash buffer. TMB (Tetramethylbenzidine) substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding an acidic stop solution. The density of yellow is proportional to the target amount of sample captured in the plate. Then the concentration of the PARP 1 enzyme is calculated. Specimens were experimented as soon as possible after collection according to the relevant literature. We added PBS (Phosphate buffered saline) PH 7.4 , homogenized by hand or grinders, then centrifugation 20 minutes at the speed of 2000-3000 r.p.m. We used a dilution puffer to make the diluted target protein concentration fall in the optimal detection range of the ELISA.

Assay procedure:

We brought all samples and reagents to room temperature before using.

1- First we put standard, test samples (diluted with sample dilution buffer), control (blank) wells on the pre-coated plate respectively, and then we recorded their positions. We washed the plate 2 times before adding standard, sample and control (blank) wells.

2- We added 100μl of properly diluted sample into test sample wells.

3- Incubation: we sealed the plate with a cover and incubated at 37°C for 90 minutes.

4- Wash: we removed the cover, discarded the plate content, and washed the plate 2 times with Wash Buffer.

5- Biotin-labeled antibody: we added 100μl of biotin-labeled antibody working solution into above wells (standard, test sample and blank wells) and incubated at 37°C for 60 minutes.

6- Wash: we removed the cover, washed the plate 3 times with wash buffer and let the wash buffer stay in the wells for 1-2 minute each time.

7- HRP-Streptavidin Conjugate (SABC): we added 100μl of SABC working solution into each well, covered the plate and incubated at 37°C for 30 minutes.

8- Wash: we washed the plate 5 times with wash buffer.

9- TMB Substrate: we added 90µl TMB Substrate into each well, covered the plate and incubated at 37°C in the dark within 10-20 minutes. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes).

10- Stop: we added 50µl stop solution into each well. The color turned yellow immediately. The adding order of stop solution should be the same as the TMB substrate solution.

11- OD Measurement: we read the O.D. absorbance at 450 nm in Microplate Reader immediately after adding the stop solution. *The sensitivity* of this assay was defined as the lowest protein concentration that could be differentiated from zero. It was calculated by sub tracing two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration. Assay range: 75pg/ml-6000pg/ml. This assay has excellent specificity for detection of PARP1. No significant cross-reactivity or interference between PARP1 and analogues was observed.

* Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between PARP1 and all the analogues, therefore, cross reaction may still exist.

2.3. Data Analysis:

• Data were collected, tabulated, and statistically analyzed using statistical package of Social Science (SPSS) version 26:

● Descriptive statistics: in which quantitative data were presented in the form of mean (X-), standard deviation (SD), range, and qualitative data were presented in the form of numbers and percentages.

● Analytical statistics: used to find out the possible association between studied factors and the targeted disease.

The used tests of significance included:

● T-test: is a test of significance used for comparison between two groups normally distributed having quantitative variables.

● Spearman's correlation (r): is a test used to measure the association between quantitative and qualitative ordinal variables.

2.4. Ethical Consideration:

All the individuals included in the study had been informed about the procedures regarding the study and informed of their rights to refuse participation or withdraw from the study without having to give reasons. Participants were guaranteed anonymity and all information provided would be treated with confidentiality. Before the interviews, a written consent and statement of voluntary participation of the participants was obtained after they were informed about the purpose of the research and how it will to be conducted. The required administrative regulations were fulfilled. The ethical approval of the faculty of medicine, Beni-Suef University research ethical committee (REC) was obtained prior to the beginning of the work.

3. Results:

The current study included 40 psoriasis patients all presented to dermatology department at Beni-Suef University hospital 19 females and 21 males' patients, their age ranged from 18 to 75 years, the average age was 46.25 ±10.63. Additionally, 40 healthy controls were collected, they were age and sex matched to the psoriasis cases. Regarding psoriasis patients as shown in figure (1) the disease duration was ranged from 1 month to 260 months (21 years) with an average disease duration of (109.90 ±87.90) months. Majority of the studied psoriasis cases as showed in table (1 had gradual onset of the disease (38) cases (95%); while only 2 cases (5%) had a sudden onset of psoriasis. more than half the studied psoriasis cases had a Remission & Exacerbation psoriasis course (21 cases, 52.5%), ten cases had a progressive psoriasis course, six cases had stationary course and only three cases had a regressive psoriasis course.

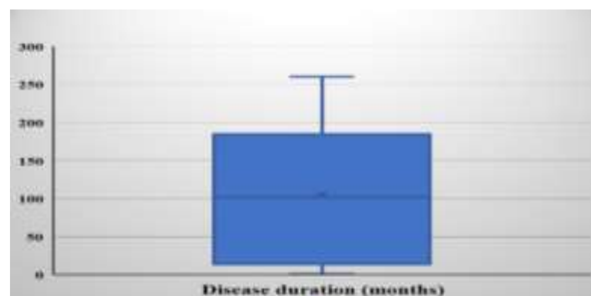

Figure 1 Disease duration (months) among studied psoriasis .cases

Table (1): psoriasis description among studied Psoriasis Cases:

psoriasis description		Frequency	Percent
Onset	Gradual	38	95.0
	sudden	2	5.0
Disease Course	Remission &Exacerbation	21	52.5
	Progressive	10	25.0
	Regressive	3	7.50
	Stationary	6	15.0
Family History	No	34	85.0
	Yes	6	15.0
History of previous treatment	No	18	45.0
	Yes	22	55.0

Table (2): Relation between Serum level of PARP-1 and patients' variables; (N= 40):

	PARP-1					
patients' variables	N	Minimum	Maximum	Mean	Std. Deviation	p.value
Gender						
Female	19	7.02	28.56	14.97	6.15	0.767
Male	21	3.42	149.0	17.12	6.71	
Onset of the disease						
Gradual	38	3.42	149.0	16.29	3.7	0.229
Sudden	2	10.29	14.49	12.39	2.96	
Course of the disease						
Remission &Exacerbation	21	4.44	149.00	14.32	6.65	0.861
Progressive	10	3.96	23.88	12.88	2.23	
Regressive	3	9.54	21.30	14.04	3.66	
Stationary	6	3.42	20.70	12.05	2.30	
Family History						
No	34	3.42	149.00	16.62	4.16	0.818
Yes	6	6.90	23.88	13.14	2.40	
History of previous treatment						
No	18	3.42	28.56	13.02	1.63	0.439
Yes	22	3.96	149.00	18.62	6.33	

Table (1) showed that only six cases had a positive family history of psoriasis, while (85%) reported negative family history. Regarding previous treatment 22 (55%) cases had a positive history of previous treatment for psoriasis, while 18 (45%) cases reported no history of previous treatment for psoriasis. Our results revealed that severity index (PASI) score was ranged from 0.90 to 23.8 with an average PASI score of (6.82 ±5.82). As demonstrated in **figure (2);** serum level of PARP-1 was significantly higher in psoriasis patients as compared with healthy controls; the mean expression values were (16.12 vs. 7.58) in psoriasis cases and healthy controls respectively with a statistically significant p-value=0.020.

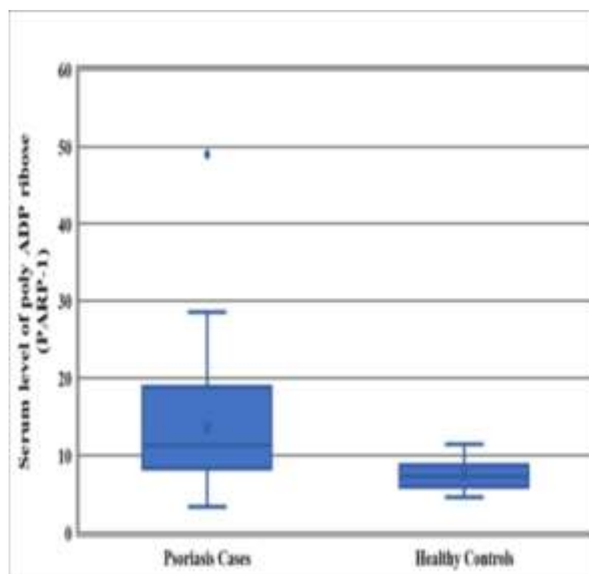

Figure 2 Serum level of PARP-1 in psoriasis patients as compared with healthy controls.

Table (2) showed the relation between Serum level of PARP-1 and patients' variables which revealed that Serum level of PARP-1 had no statistically significant difference when it compared with patients' variables. Also, there were non-statistically significant linear correlation between serum level of PARP-1 and

Patients' Age in studied psoriasis patients; (r= -0.043, p= 0.705) or Severity Index (PASI) score in studied psoriasis patients; (r= 0.156, p=0.337).

4. Discussion:

The aim of the present study was to find out the role of poly ADP ribose polymerase 1 (PARP1) in psoriasis and its relation to severity. Blood samples were taken from the patients and also from controls for assessment of the poly ADP ribose polymerase 1 (PARP1) by ELISA.

The present study showed that the mean value of poly ADP ribose polymerase 1 (PARP1) was significantly higher in patients compared with the controls with no significant correlation to age, sex or disease severity.

Martínez-Morcillo et al (2021) showed strong increase of mRNA levels of PARP1 in psoriasis lesional skin (12). Transcription levels of the genes encoding several PAR hydrolases, namely MACROD1 (Macro domain with ADP ribose hydrolase), MACROD2, and TARG1 (Terminal ADP ribose glycohydrolase) were lower in psoriasis lesional skin. Moreover, there is an increase of PARylation in lesional skin from psoriasis patients. The study also found that inhibition of PARP reduced the transcription levels of DEFB4 (Defensin beta 4) and S100A8, an inflammation markers associated with psoriasis. These results confirmed that PARP1 inhibitors can reduce inflammation in human psoriasis models.

In his thesis, Martínez-Morcillo (2019) demonstrated that there is PAR accumulation in the nucleus of epidermal keratinocytes from psoriatic lesions. In addition, psoriasis and atopic dermatitis samples transcriptomic data revealed increased mRNA levels of PARP 1 in lesional tissue compared with healthy skin. In atopic dermatitis, the expression of PARP1 is positively correlated with IL13(13).

Ghonim et al (2018) showed that PARP1 inhibition by gene knockout or pharmacologically by Olaparib provided a robust reduction in the manifestation of all traits of the atopic dermatitis including Redness, thickness, scratching and lichenification as assessed by the Eczema Area and Severity Index (EASI). The protective effects of PARP inhibition may be mediated by a differential regulation of Th1/Th2 cytokines production (14). PARP-1 inhibition may alter the function and ability of T cells to produce cytokines.

Brunyánszki et al (2010) (15) demonstrated that genetic ablation of PARP-1 reduced inflammatory cells immigration and neutrophil infiltration in an oxazolone-induced contact hypersensitivity (CHS) reaction. In addition, expression of cytokines and chemokines (inflammatory protein-1a and-2, IL-1b, tumor necrosis factor-α and monocyte chemotactic protein-1) was markedly reduced. Namazi (2003) reasoned that nicotinamide (16), which is PARP1 inhibitor, could be a useful addition to anti-psoriatic weapon. Inhibition of PARP1 plays a role in decreasing the expression of inflammatory cytokines, chemokines, adhesion molecules, and inflammatory media.

On the other hand, in a study done by Kiss et al (2020) assessed the expression of PARP1 in the skin of psoriatic lesions and control dry skin in human samples from similar anatomical locations, they found that PARP1 expression decreased in human psoriatic lesions but did not reach the level of significance (17). They also assessed the expression of cytokines relevant for psoriasis (IL6, IL1β and IL8). IMQ treatment increased the mRNA expression of these cytokines, moreover, when applied in combination with PARP inhibitors cytokine expression was further exacerbated. The expression of IL17 and IL23 followed the same pattern; however, readouts were not significant. We concluded that assessment of PARP-1 in the psoriatic patient's skin may give different results from its estimation in the serum.

As previously described, PARP1 is known to play a significant role in some acute and chronic inflammatory diseases such as neurological disorders (Parkinson's disease), sepsis, arthritis, colitis, diabetes and myocardial infarction. In this line, it has been hypothesized that cell death might be responsible for immune cell recruitment. Prevention of cell death by PARP1 inhibitors may decrease inflammation. Moreover, this reduction decreases tissue damage and proinflammatory cytokines generation, limiting immune cell recruitment (Kunze et al. 2019) (18).Collectively, our results point to the role of PARP1 and PAR metabolism in psoriasis. Further studies would be necessary to prove that and to definitively demonstrate the role of PARP1 as a new potential therapeutic target to treat psoriasis.

5. Conclusion and Recommendations:

Our study revealed that the expression level of poly ADP ribose polymerase 1 (PARP1) was significantly higher in the serum of psoriatic patients in comparison with the controls. According to these findings, PARP1 is suggested to play an essential role in the mechanisms that are responsible for development of the disease.

Further studies and large group of subjects are essential to confirm the role of PARP1 in the pathogenesis of psoriasis.

Conflicts of Interests

The authors 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 was approved by the Research Ethical Committee, Faculty of Medicine, Beni-Suef University.

Approval No: FMBSUREC/01122019/Othman

Informed consent

A signed consent form was obtained from each study participant.

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