Evaluation of protein carbonyl levels as an indicator of protein oxidation in psoriasis patients

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Abstract

Background: Psoriasis is a persistent, non-infectious skin condition that impacts people of all ages and has no sex preference, which is caused by environmental stressors involving skin cells, immunocytes, and several biological signalling molecules. Recent studies propose that the underlying mechanisms of psoriasis may involve an elevated production of (ROS) and indicate that individuals with psoriasis exhibit elevated levels of reactive oxygen species (ROS) in their skin. Protein carbonylation is a widely adopted biomarker for indicating protein oxidation. The objective of this study was to examine serum protein carbonylation levels as a marker of protein oxidation in psoriasis patients, evaluating the impact of age, body mass index (BMI), and waist circumference (WC) on these levels.

Methods: Conducted as a case-control investigation, this study enlisted 98 psoriasis patients and 81 healthy individuals, matched in terms of age and sex as controls. Blood samples, obtained through venipuncture (5 ml), were analyzed for protein carbonyl, random blood sugar, lipid profile, liver enzymes, and complete blood count (CBC).

Results: The study found a significant difference in the mean value of serum protein carbonyl among patients with psoriasis as compared to the control group (3.82 ± 2.24 ng/ml vs. 3.28 ± 2.54 ng/ml) respectively ($P<0.05$).

Conclusion: The study observed a non-significant negative correlation between levels of protein carbonyl and both age and the duration of psoriasis. Additionally, there was no significant negative correlation found between protein carbonyl levels and waist circumference, indicating that central obesity may render individuals more susceptible to protein oxidation, even when their BMI is found within the normal range.

Keywords: Psoriasis, Protein carbonyl, and Protein oxidation

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Introduction

Psoriasis is a long-term skin condition that is not infectious and can develop in people of all ages and genders (1). the prevalence of psoriasis can range from 0% to 11.8% in various populations (2). Although psoriasis can impact individuals across all age groups, it is more frequently observed in people who are under 35 years old (3). Psoriasis is thought to result from a blend of genetic, epigenetic, and environmental factors. These factors contribute to abnormal interactions between immune cells, cytokines (proteins involved in cell signalling), and skin cells, leading to the development of psoriasis (4). Protein oxidation, a significant category of post-translational modifications, occurs due to interactions between amino acid residues of proteins

and reactive oxygen species (ROS) or reactive nitrogen species (RNS) (5). Protein oxidative alterations are categorized into irreversible and reversible forms, both selectively induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (5). Recent studies propose that the underlying mechanisms of psoriasis may involve an elevated production of (ROS) and a compromised antioxidant system's ability to function effectively (6). Moreover, a recent study has indicated that individuals with psoriasis exhibit elevated levels of reactive oxygen species (ROS) in their skin (7). The diverse array of posttranslational modifications that can occur on proteins, which involve various reaction sites, can significantly impact protein structure, net charge, folding, and hydrophobicity/hydrophilicity. Such changes have implications for the functional roles of proteins, whether they serve as enzymes, receptors, carriers, or structural proteins (8).

When (ROS) attacks proteins, specific amino acid residues can undergo modification, leading to the creation of protein carbonyl groups. Additionally, myeloperoxidase in activated neutrophils produces hypochlorous acid, chloraminated oxidants, and chloramines, which generate advanced oxidation protein products (AOPPs) that contain cross-linked dityrosine. Both the formation of carbonyl groups by ROS and the presence of AOPPs are considered early indicators of oxidative stress and are used to assess protein damage caused by oxidation (9).

Utilizing protein carbonyl (PC) as a diagnostic tool for oxidative protein damage offers several advantages compared to measuring other oxidation products. This is primarily due to the early production and relative stability of PC (10). Increased levels of PC have been documented in various conditions, such as psoriasis (11). Protein carbonyls in healthy men and women rise with age (12).

The objective of this study was to examine serum protein carbonylation levels as a marker of protein oxidation in psoriasis patients, evaluating the impact of age, body mass index (BMI), and waist circumference (WC) on these levels.

Materials and Methods

Study Population

This study involved a case-control design and was conducted at Basrah College of Medicine; Department of Biochemistry in Al-Basrah, Iraq, from December 2022 to September 2023. The study comprised 179 participants were divided into two primary groups; 98 psoriasis patients as cases and 81 healthy individuals as control who were matched in terms of both age and sex with cases. Patients of psoriasis visit the dermatology clinic in Al-Basrah Teaching Hospital for medical consultation or routine check-ups. Exclusion criteria comprised diabetic mellitus (DM), liver diseases, renal diseases, tumors and congestive heart failure. Each participant completed a thorough questionnaire comprising demographic data (gender, age, type of treatment, whether topical, systemic (MTX / Biologic), or phototherapy, and duration of psoriasis).
During the morning, the researchers measured the body weight, height, and waist circumference (WC) of each participant.

Sample Collection

Five millilitres of venous blood were collected via venipuncture and separated into two sections: 2 ml were placed in an anticoagulant tube (K3EDTA) to be utilized in the analysis of complete blood count (CBC). The remaining blood was placed in a gel tube without any anticoagulant and containing a gel and clot stimulator. After centrifugation, the serum was transferred into two separate Eppendorf tubes. One of these tubes was utilized for routine biochemical tests such as Random Blood Sugar (RBS), Blood Urea, Total Cholesterol(TC), High-Density Lipoprotein-Cholesterol (HDL-C), Triglyceride(TG), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Total Bilirubin (TSB), and Direct Bilirubin. The other Eppendorf tube was stored in a deep freezer at a

temperature of -30°C for the following estimation of PC.

Laboratory Investigation

RBS, B. urea, total cholesterol (TC), high-density lipoprotein-cholesterol(HDL-C), triglyceride (TG), aspartate Transferase (AST), Alanine Transaminase (ALT), and total serum bilirubin (TSB) were measured utilizing automated colorimetric methods with kits supplied by Roche diagnostics, Germany, while low-density lipoprotein-cholesterol (LDL-C) was carried out utilizing Friedewald's equation. Complete Blood Count (CBC) was measured by Sysmex XT-1800i automatic hematological analyzer provided by Kokusan, Japan. To quantify the serum levels of Protein Carbonyl (PC), a sandwich enzyme-linked immunosorbent assay (ELISA) kit was employed, following the manufacturer's instructions (Sunlong, China, REF SL1473Hu). The absorbance was evaluated at 450 nm, and a standard curve was created using known dilutions of PC. Subsequently, the obtained results were measured against this standard curve, featuring a detection range spanning from 0.8 to 50 ng/ml. The inter-assay precision was below 12%, and intra-assay precisions were under 10%.

Statistical Analysis

In the current investigation, statistical analysis was conducted using the Statistical Package for Social Science (SPSS) program version 23. Descriptive statistics, including Mean ± Standard Deviation (SD) and percentages, were employed for data presentation. The Independent t-test was utilized to compare continuous data between two groups, while the Chi-square (χ^2) test was used for analyzing categorical data. Pearson correlation was applied to assess the correlation coefficient (r-value). A significance level of less than 0.05 was considered as the threshold for significance.

3. Results

(Table 1) displays the demographic, clinical, and biochemical data of the participants. No statistically significant differences were observed between the patients and the control group in terms of gender, age, BMI, WC, RBS, TC, TG, HDL, LDL, TSB, WBCs, and platelets ($P > 0.05$). Slightly over half of the participants were male, accounting for 59.2% among patients and 56.8% among controls, respectively. A significant difference was observed between psoriasis patients and the control group concerning B.urea, ALT, AST, and hemoglobin ($P < 0.05$). The average serum PC level was significant higher in psoriasis patients in comparison to the control group (3.31 ± 1.92 vs. 2.57 ± 1.52 ng/ml; $P < 0.05$).

Table 1: presents the demographic, clinical, and biochemical data of the study participants.

Variables		Controls n=81	Cases n=98	*P* value *
Age (years)		32.62 ± 10.64	34.62 ± 12.71	NS ⁂
Gender	Male	46 (56.8%)	58 (59.2%)	NS ⁂
	Female	35 (43.2%)	40 (40.8%)	
BMI(kg/m2)		28.65 ± 6.05	29.19 ± 3.10	NS ⁂
Duration of disease (years)		----	10.44 ± 8.56	-----
Random blood sugar(mg/dL)		103.20 ± 12.01	101.90 ± 16.87	NS ⁂
Urea (mg/dl)		26.40 ± 4.77	23.05 ± 6.29	<0.05 ⁂
Serum total cholesterol(mg/dL)		175.24 ± 38.31	180.62 ± 42.76	NS ⁂
Triglyceride (mg/dL)		141.23 ± 74.80	145.57 ± 67.48	NS ⁂
HDL-C (mg/dL)		50.77 ± 12.81	54.14 ± 20.15	NS ⁂
LDL-C (mg/dL)		96.22 ± 39.27	96.88 ± 37.29	NS ⁂
AST (U/L)		22.35 ± 7.09	25.84 ± 11.33	<0.05 ⁂
ALT (U/L)		20.10 ± 8.59	25.28 ± 12.65	<0.05 ⁂
TSB (mg/dl)		0.681 ± 0.17	0.76 ± 0.34	NS ⁂
PC(ng/ml)		3.28 ± 2.54	3.82 ± 2.24	<0.05 ⁂
HGB (mg/dl)		14.13 ± 1.67	12.97 ± 1.77	<0.05 ⁂
WBC (10^3/µL)		7.38 ± 2.23	7.52 ± 2.66	NS ⁂
Platelets (10^3/µL)		297.23 ± 53.65	321.32 ± 39.08	NS ⁂

*P value of significance between cases and controls ⁂ Student t-test

Examining the influence of age on serum PC levels, participants were divided into four age groups (refer to Table 2). The research revealed fluctuations in the mean PC levels corresponding to participants' age, without any notable distinction between the cases and the control group($p>0.05$) between ages (10 - 40 and ≥56) years. Furthermore, the study showed significant differences between cases and controls with ages between (41 - 55) years ($p<0.05$) concerning the distribution of the mean of PC levels according to the four age categories.

Table 2: The distribution of PC in the study population based on age.

Age (Years)	PC (Mean±SD)(ng⁄ml)		P value*
	Control	Patients	
10-25	3.84 ± 2.79	2.97 ± 2.14	NS
26-40	3.84 ± 2.17	3.88 ± 2.81	NS
41-55	3.84 ± 1.57	2.72 ± 2.46	<0.05
≥ 56	3.05 ± 1.65	3.13 ± 2.37	NS

*P value of significance between cases and controls ⁂ Student t-test

The study showed that the mean values of PC levels were higher in controls with obese (4.13 ± 2.02 ng⁄ml) than in obese patients (3.05 ± 2.04 ng⁄ml) with significant differences ($P<0.05$).In addition, the study showed that the mean of PC levels was no significant difference in patients with normal weight and overweight (3.66 ± 3.03 and 3.18± 2.24 ng/ml) respectively when compared with controls with normal weight and overweight (2.35 ± 2.26 and 3.80 ± 2.35ng/ml) respectively ($p>0.05$).

Regarding PC levels, the mean values of PC were higher in patients with healthy controls, but no significant difference ($P >0.05$) in males with central obesity ≤102cm and >102cm and females with central obesity ≤88, however, the mean values of PC were higher in patients of psoriasis than in control groups, with significant differences in female with WC more than 88cm ($p<0.05$).

Table 3: The distribution of PC within the study population categorized by BMI and waist circumference (WC).

BMI(Kg/m^2)		PC (Mean±SD)(ng⁄ml)		P value*
		Control	Patients	
Normal weight 18.5-24.9		2.35 ± 2.26	3.66 ± 3.03	NS
Overweight 25-29.9		3.80 ± 2.35	3.18± 2.24	NS
Obese ≥30		4.13 ± 2.02	3.05 ± 2.04	<0.05
Waist circumference (cm)		**PC(ng⁄ml) (Mean±SD)**		**P value**
		Controls n=81	**Patients n=98**	
Male	**≤102**	3.25 ± 2.29	3.75 ± 2.73	NS
	>102	3.82 ± 1.81	3.08 ± 2.62	NS
Female	**≤88**	1.98 ± 1.60	3.45 ± 3.32	NS
	>88	4.22 ± 2.60	2.90 ± 1.71	<0.05

*P value of significance between cases and controls ⁂ Student t-test

The study found that serum of PC, the study showed a negative correlation but not significantly between PC with age, duration of psoriasis, BMI and WC in all studied populations (Table 4).

Table 4: Pearson correlation of PC levels with other variables of the study

Variables		PC (ng/ml)
Age	Correlation Coefficient	-0.150
	P value	0.14
Gender	Correlation Coefficient	0.075
	P value	0.46
Duration of psoriasis	Correlation Coefficient	-0.146
	P value	0.151
BMI	Correlation Coefficient	-0.029
	P value	0.77
WC	Correlation Coefficient	-0.153
	P value	0.13

* Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

Psoriasis, a skin disease caused by immune system dysfunction, is linked to various other conditions such as psoriatic arthritis, cardiometabolic diseases, and mental health issues. This makes it a global health concern and a major economic burden (13).

Protein carbonyl (PC) measurement is a frequently utilised biomarker to determine the degree of oxidative damage to proteins (14,15). Protein carbonyl (PC) is a reliable indicator of oxidative damage because it forms early during oxidative stress, is more stable than other markers, and has a longer half-life in the blood. These qualities increase its dependability as a most widely used biomarker for protein (15). The findings of the study demonstrated a positive correlation between protein carbonyl levels and psoriasis, Significantly elevated protein carbonyl levels were observed in psoriasis patients compared to the control group($P<0.05$). These results were in concordance with the findings of other studies (10,16–18). Oxidative stress is characterized by an atypical elevate in levels of reactive oxygen and nitrogen species. This condition can arise from either excessive production or a decline in the capacity to remove them through antioxidant defence mechanisms (19). The skin, acting as a protective barrier, is constantly exposed to environmental factors and thus becomes a notable contributor to free radicals. In small amounts, these free radicals serve an important purpose by defending against microorganisms and supporting cell differentiation (20). Increased levels of free radicals can cause oxidative stress, where reactive oxygen and nitrogen species can cause DNA alterations, degradation of cell proteins, lipid peroxidation, cell death (apoptosis), tissue damage, changes in the response of T-helper cells, and the production of interleukin-17 (IL-17) (21). These components play a vital role in the onset and enhancement of psoriasis, underscoring their paramount importance. This aligns with the theory that oxidative stress could be a pivotal factor contributing to the fundamental mechanisms of this persistent condition(22).

In conclusion, the results of the present study revealed that the serum PC levels were significant among psoriasis patients compared to the controls. Moreover, the study observed a non-significant negative correlation between protein carbonyl levels and both age and the duration of psoriasis.

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تقييم مستويات كربونيل البروتين كمؤشر لأكسدة البروتين في مرضى الصدفية

الخلفية: الصدفية حالة جلدية مزمنة غير معدية تصيب الأشخاص من جميع الأعمار ولا تفرق بين الجنسين. تحدث نتيجة عوامل ضغط بيئية تتعلق بخلايا الجلد والخلايا المناعية وعدة جزيئات إشارات بيولوجية. تقترح الدراسات الحديثة أن الآليات الكامنة وراء الصدفية قد تنطوي على إنتاج مرتفع لـ (ROS) وتشير إلى أن الأفراد المصابين بالصدفية لديهم مستويات عالية من أنواع الأكسجين التفاعلية (ROS) في جلدهم. يعد تكوين بروتين الكربونيل مؤشرا حيويا يُعتمد عليه على نطاق واسع للإشارة إلى أكسدة البروتين. هدفت هذه الدراسة إلى فحص مستويات تكوين بروتين المصل كمؤشر لأكسدة البروتين في مرضى الصدفية، وتقييم تأثير العمر ومؤشر كتلة الجسم (BMI) ومحيط الخصر (WC) على هذه المستويات.

الطرق: أُجريت الدراسة على شكل تحقيق حالة-ش شاهد، وشملت ٩٨ مريضًا بالصدفية و ٨١ فردًا صحيًا مطابقين لهم من حيث العمر والجنس كمجموعة ضابطة. تم تحليل عينات الدم، التي تم الحصول عليها عن طريق الوريد (٥ مل)، من أجل بروتين الكربونيل، وسكر الدم العشوائي، ومستويات الدهون، وإنزيمات الكبد، وعدد الدم الكامل (CBC).

النتائج: وجدت الدراسة اختلافًا كبيرًا في القيمة المتوسطة لبروتين الكربونيل في المصل بين مرضى الصدفية مقارنة بمجموعة الضبط (٣,٨٢ ± ٢,٢٤ نانوجرام / مل مقابل ٣,٢٨ ± ٢,٥٤ نانوجرام / مل) على التوالي (p <٠,٠٥).

الخاتمة: لاحظت الدراسة ارتباطًا سلبيًا غير ذي دلالة إحصائية بين مستويات بروتين الكربونيل وكل من العمر ومدة الإصابة بالصدفية. بالإضافة إلى ذلك، لم يُعثر على ارتباط سلبي ملحوظ بين مستويات بروتين الكربونيل ومحيط الخصر، مما يشير إلى أن السمنة المركزية قد تجعل الأفراد أكثر عرضة لأكسدة البروتين، حتى عندما يكون مؤشر كتلة جسمهم ضمن المعدل الطبيعي.

الكلمات المفتاحيه: الصدفية، بروتين الكربونيل، وأكسدة البروتين